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HOPE

How Oncogenetics Predicts & Educates

2018-1-RO01-KA202-049189

Promoters of advanced oncogenetics openline training and multimedia raise awareness on multidisciplinary assessment of patients and their families at risk of hereditary or familial cancer

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FOREWORD

Every day, worldwide, millions of people fight a battle against cancer, undergoing devastating therapies that hugely impact on their Quality of Life. Over the next 2 decades, the number of new cases is expected to rise by about 70% (WHO, 2017). Oncogenetics can contribute to change this future scenario, addressing those people for which prevention is still possible and the disease's onset avoidable.

This medical discipline, in progressive expansion, is focused on understanding and monitoring the genetic predispositions of persons at risk, because of mutations or family histories of cancer.

Through guided professional decisions, the implementation of evidence-based prevention strategies will lead to considerable improvements in clinical decisions and outcomes. In order to move towards this predictive system, it is essential to raise awareness of those vulnerable people and to empower them.

On this basis, it will be possible to facilitate an appropriate checking and an early detection of prodromes, as well as the modification of harmful habits and behaviours.

The focus of the project HOPE - How Oncogenetics Predicts & Educates (Promoters of advanced oncogenetics open online training and multimedia raise awareness on multidisciplinary assessment of patients and their families at risk of hereditary or familial cancer), funded by the Erasmus+ Programme in the field of Strategic Partnerships for vocational education and training (Ref no.2018-1-RO01-KA202-049189) is to raise awareness about the importance of the existence and expansion of oncogenetics as a discipline depending on advances in understanding genes associated with inherited susceptibility to common adult malignancies.

Aiming to further develop the field of oncogenetics, the book we introduce represents the printed equivalent of the *Training Guide for Advanced High-Specialized Intervention in Oncogenetics* to be found on the dedicated HOPE - ONCOGENETICS project platform (https://hope.projects.umfiasi.ro/open-online-course/). Using different media, both materials provide training related to the multidisciplinary assessment of patients and their families at risk of hereditary cancer.

More specifically, the topics covered in the book have been developed by 20 medical doctors from the University of Medicine and Pharmacy "Grigore T. Popa" Iaşi, all specialists in various medical fields who regularly detect, diagnose and monitor people with hereditary cancer risk. The final edit, however, brings together contributions of specialists from Centre Jean Perrin, Clermont-Ferrand, France - the promoter of oncogenetics in Europe - and the partner universities: Medical University Plovdiv, Bulgaria, University of Szeged, Hungary and EuroEd Foundation Iaşi, Romania. Our special gratitude goes to the specialists of Centre Jean Perrin who vouched for the validity of the data included.

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Oncogenetics equals HOPE because it can save lives!

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CONTENTS

PART I

I.1. Descriptive epidemiology of cancers

Learning objectives

- introductory data on the general definition of epidemiology, cancer epidemiology and descriptive or analytical evaluation methods for understanding epidemiological phenomena;
- presentation of global morbidity and mortality data for various types of cancer;
- distribution of different types of cancer according to age, gender, geographical region and the level of economic development of different countries worldwide;
- current status of age-standardized rates for the incidence and mortality of breast, ovarian and colorectal cancers in various regions of the world.

Introduction

"**Epidemiology** is the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to control of health problems."

(*Last,* 1995)

Cancer epidemiology is the study of the distribution, determinants, and frequency of malignant disease in specific populations in order to define causative factors (including preventable/ avoidable causes and inherited tumour susceptibility) and to formulate preventive strategies for the disease control.

The **epidemiologic assessment**:

- provides the medical specialists with a quantification of cancer risk; - outlines the basis for screening methods used for high-risk groups; - determines the efficacy of any preventive intervention.

Source: Hennekens CH & Buring JE (1987)

Descriptive epidemiology

Describes the difference in occurrence of a particular cancer between different groups (age, gender, race, country, time period) and **generates** the hypothesis for analytical epidemiology.

Descriptive epidemiology:

- incidence;
- prevalence;
- mortality.

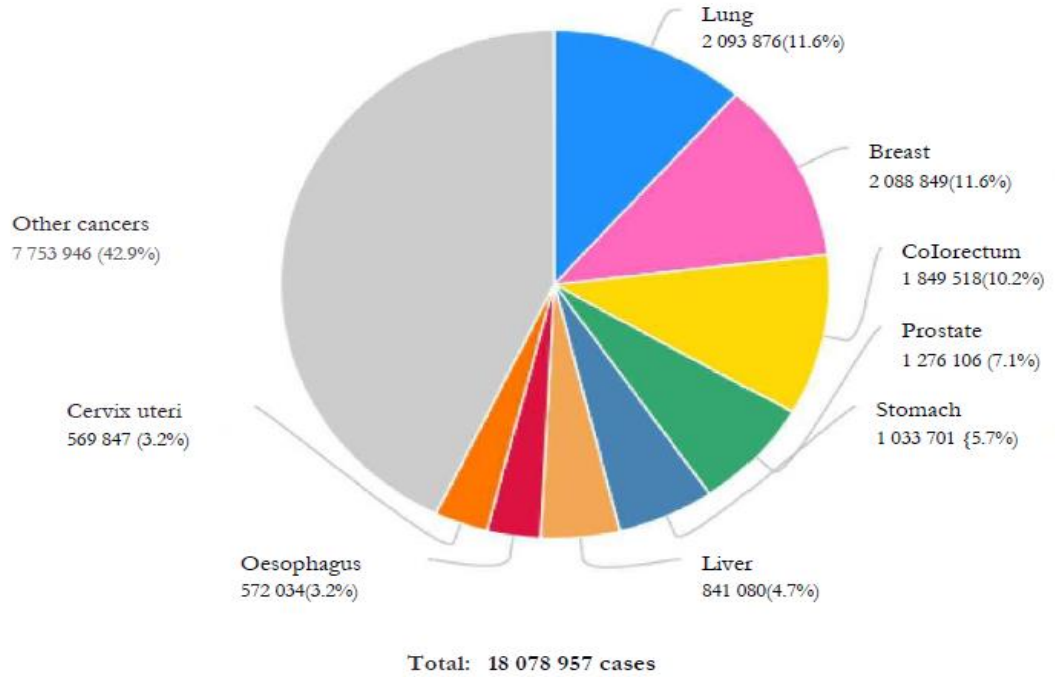
Incidence = the number of new cases arising in a specific period and population. Often given as an absolute number of cases per year or as a standardized rate per 100,000.

Mortality = the number of deaths occurring in a specific period and population. Often given as an absolute number of deaths per year or as a standardized rate per 100,000.

Prevalence = the number of persons in a defined population diagnosed during a fixed time in the past with that type of cancer, and who are still alive at the end of a given year.

Usually given as a number and proportion per 100,000 persons

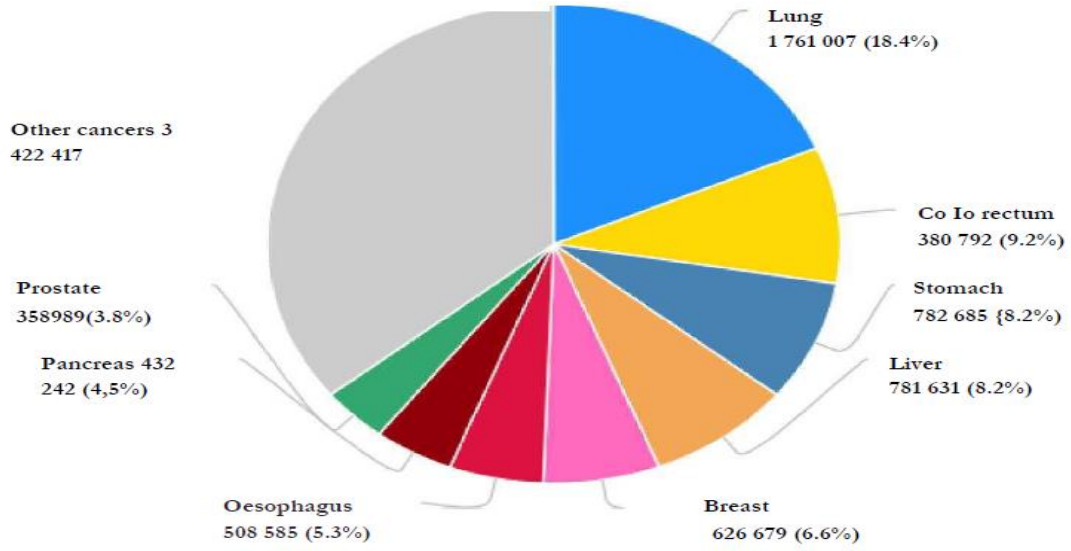
Number of new cancer cases in 2018 (both genders, all ages, worldwide)



Source: International Agency for Research on Cancer. GLOBOCAN 2018.

Data shows that in 2018 the number of new cases of breast and colon cancer in both genders and all age groups was among the highest, along with lung cancer.

Number of deaths in 2018 (both genders, all ages, worldwide)



Source: International Agency for Research on Cancer. GLOBOCAN (2018)

In 2018, deaths reported worldwide in the population of both sexes and at all ages were the highest for lung cancer (18.4%) followed by colon cancer (9.2%). Breast cancer death rate was 6.6% of total cancer deaths.

Cancer incidence and mortality statistics worldwide and by region

By geographical region:

• the incidence of cancers in both sexes was the highest in East Asia (5,622,367 cases out of 18,078,957 cases worldwide), in Central and Eastern Europe, reaching 1,260,057 cases

• cancer mortality registered in both sexes was increased in East Asia (3,456,734 deaths out of a total of 9,555,027 deaths worldwide).

By degree of population development:

• the highest incidence was in highly developed countries (8,054,578 cases)

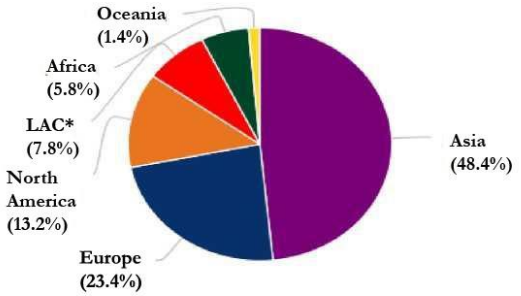
• the highest level of mortality was registered in the high developed countries (2,425,680 deaths).

	Incidence						Mortality					
	Both sexes		Males		Females		Both sexes		Males		Females	
	New cases	Cum. risk 0-74 (%)	New cases	Cum. risk 0-74 (%)	New cases	Cum. risk 0-74 (%)	Deaths	Cum. risk 0-74 (%)	Deaths	Cum. risk 0-74 (%)	Deaths	Cum. risk 0-74 (%)
Eastern Africa	332 177	13.47	129 476	11.56	202 701	15.20	230 968	10.21	94 731	8.89	136 237	11.40
Middle Africa	95 735	10.86	41 407	10.62	54 328	11.23	68 763	8.30	30 852	8.01	37 911	8.66
Northern Africa	283 219	14.27	134 627	14.69	148 592	13.94	178 754	9.39	96 874	10.64	81 880	8.22
Southern Africa	114 582	19.93	50 814	22.35	63 768	18.54	61 670	11.33	30 106	13.54	31 564	9.84
Western Africa	229 459	11.42	90 232	10.10	139 227	12.72	153 332	8.26	63 968	7.36	89 364	9.14
Caribbean	111 933	20.23	57 728	22.41	54 205	18.28	63 075	10.42	34 354	11.69	28 721	9.29
Central America	256 782	14.70	115 751	14.53	141 031	14.90	119 168	6.86	57 609	6.83	61 559	6.89
South America	1 044 017	20.56	509 014	22.40	535 003	19.12	490 515	9.51	253 762	10.79	236 753	8.43
North America	2 378 785	33.13	1 274 306	36.25	1 104 479	30.31	698 266	9.64	367 738	10.82	330 528	8.58
Eastern Asia	5 622 367	21.54	3 108 655	24.23	2 513 712	18.91	3 456 734	12.88	2 136 217	16.36	1 320 517	9.35
South-Eastern Asia	989 191	15.29	478 093	16.33	511 098	14.52	631 190	10.14	345 482	11.95	285 708	8.57
South-Central Asia	1 739 497	10.26	859 799	10.57	879 698	10.02	1 167 183	7.22	619 488	7.84	547 695	6.62
Western Asia	399 877	17.51	210 004	19.94	189 873	15.55	221 957	10.17	130 276	12.75	91 681	7.81
Central and Eastern Europe	1 240 057	24.95	612 026	29.43	628 031	21.98	699 446	13.84	385 301	18.83	314 145	10.16
Western Europe	1 370 332	31.24	752 802	34.94	617 530	27.73	548 355	10.99	307 423	13.28	240 932	8.82
Southern Europe	933 181	27.55	516 339	31.78	416 842	23.72	422 054	10.60	246 579	13.53	175 475	7.91
Northern Europe	686 092	30.44	366 351	33.07	319 741	28.01	273 623	10.32	146 289	11.58	127 334	9.14
Australia and New Zealand	233 773	41.53	140 821	49.06	92 952	33.28	59 247	9.39	33 374	10.75	25 873	8.07
Melanesia	15 379	20.05	6 840	20.76	8 539	19.78	9 257	13.07	4 375	13.94	4 882	12.48
Polynesia	1 539	23.68	805	26.56	734	21.05	838	13.08	472	15.52	366	10.77
Micronesia	983	18.93	528	21.29	455	16.48	632	11.99	370	14.56	262	9.44
Low HDI	672 218	11.79	270 241	10.52	401 977	13.02	464 569	8.80	196 682	7.92	267 887	9.66
Medium HDI	2 828 475	11.94	1 364 853	12.30	1 463 622	11.67	1 861 723	8.21	984 221	9.08	877 502	7.42
High HDI	6 515 063	19.97	3 476 436	22.24	3 038 627	17.88	4 020 422	12.36	2 425 680	15.46	1 594 742	9.34
Very high HDI	8 054 578	29.05	4 340 394	32.72	3 714 184	25.88	3 204 212	10.52	1 776 814	12.81	1 427 398	8.47
World	18 078 957	20.20	9 456 418	22.41	8 622 539	18.25	9 555 027	10.63	5 385 640	12.71	4 169 387	8.70

Source: International Agency for Research on Cancer. GLOBOCAN 2018.

Cancer incidence (both genders)

In 2018, 48.4% of new cases of cancer registered in Asia, followed by Europe with 23.4% of the total cases worldwide.



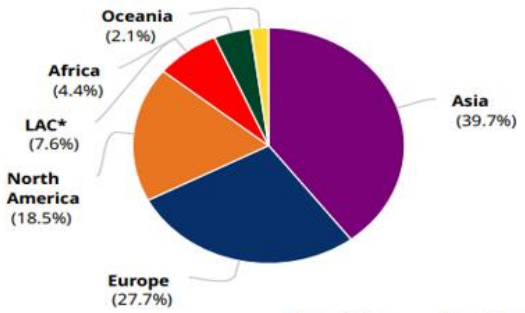
Population	Number
Asia	8 750 932
Europe	4 229 662
North Amarica	2 378 785
*Latin Amarica and the Caribbean	1 412 732
Africa	1 055 172
Oceania	351 674
Total	18 078 957

Source. International Agency for Research on Cancer. GLOBOCAN 2018.

5-year prevalence (both genders)

The prevalence of cancers estimated over a 5-year period revealed that the highest rates were in Asia (39.7% of the world total) and in Europe it was 27.7%, far from North America (18.5%) and other regions (e.g. Africa with 4.4%).

Even though in Europe the percentage of deaths was about half that of Asia, its value was far from other regions such as North America and Africa, with 7.3% respectively.

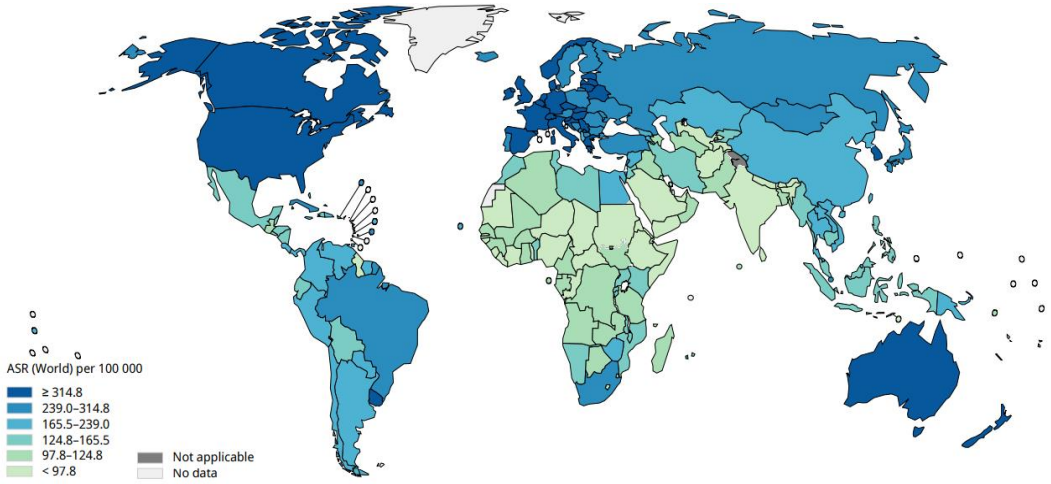


Population	Number
Asia	17 387 570
Europe	12 132 287
North America	8 132 437
*Latin America and the Caribbean	3 336 468
Africa	1 930 912
Oceania	921 628
Total	43 841 302

Source: International Agency tor Research on cancer GLOBACAN 2018.

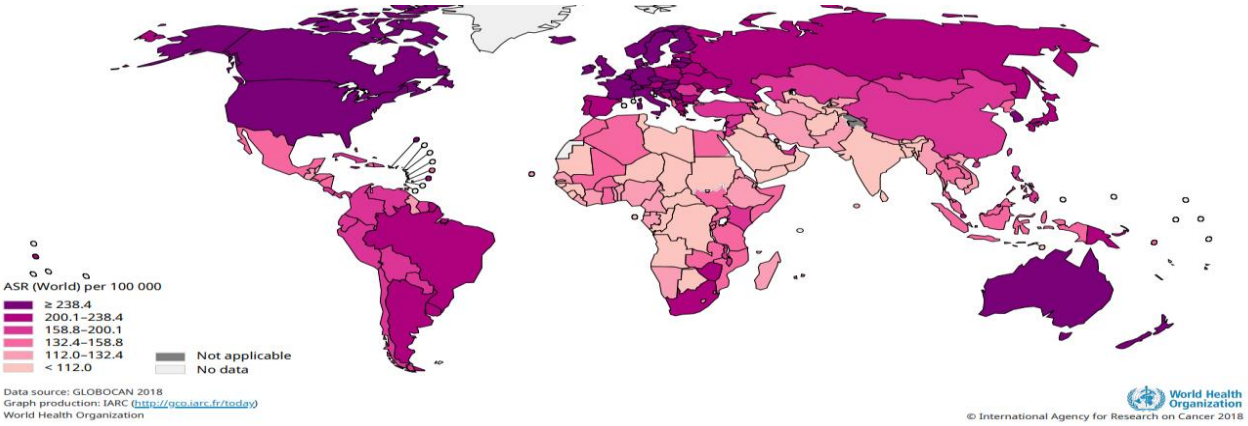
Age standardized (world) incidence rates (all cancers, males, all ages)

The standardized incidence rates of all cancers in men, by age record the highest values of more than 314.8 cases per 100,000 inhabitants, in North America, Western Europe and Australia.



Source: International Agency for Research on Cancer. GLOBOCAN 2018.

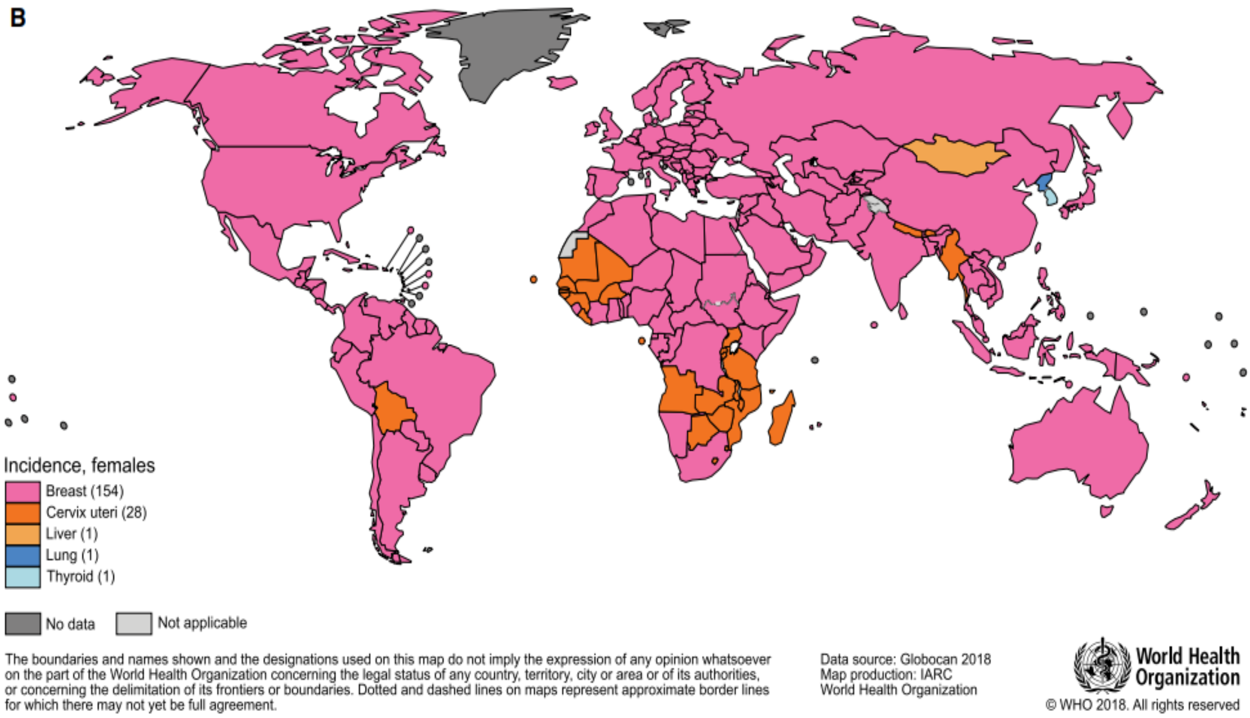
Age standardized (world) incidence rates (all cancers, females, all ages)



Source: International Agency for Research on Cancer. GLOBOCAN 2018.

In women, the situation is similar, thus the highest incidence rates of cancer, at all ages (over 238.4 cases per 100,000 inhabitants) have been recorded in North America, Western and Northern Europe, as well as in Australia.

Most Common Type of Cancer Incidence in 2018, per Country Among Women



Source: Bray F. et al. (2018)

The incidence of breast cancer is significantly increased in all continents, as compared to other cancers.

Incidence, Mortality and Prevalence by cancer site (2018)

Of all types of cancer, the incidence, prevalence and mortality data reported worldwide for a 5-year period ranks breast cancer among the first places after lung cancer.

Colorectal cancer follows at a small difference in the hierarchy of cancers, by location.

Although ovarian cancer ranks 19th in incidence, it is ranked 15th in mortality among all cancers which may suggest late detection, at an advanced stage of evolution.

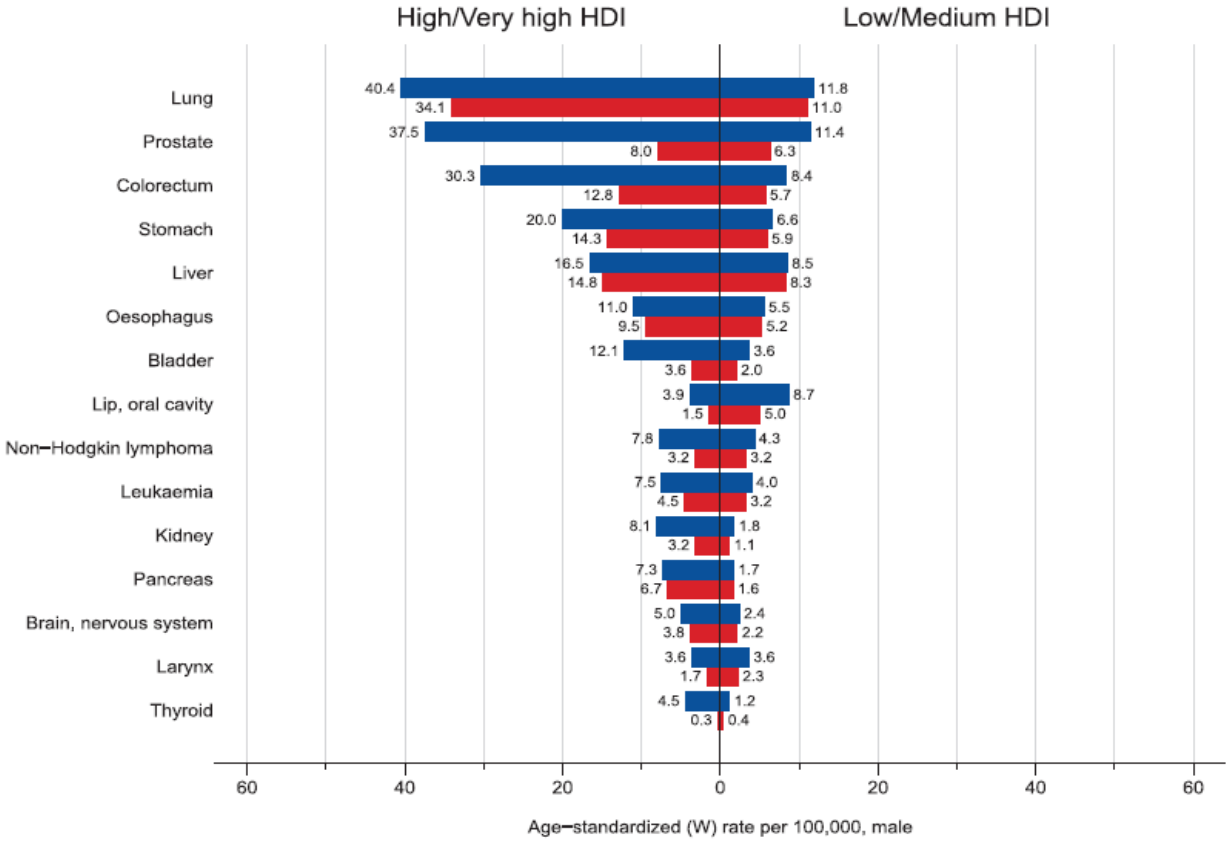
	New cases				Deaths				5-year prevalence (all ages)	
Cancer	Number	Rank	(%)	Cum.risk	Number	Rank	(%)	Cum.risk	Number	Prop.
Lung	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	27.91
Breast	2 088 849	2	11.6	5.03	626 679	4	6.6	1.41	6 875 099	[illegible]
Prostate	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	96.73
Colon	1 096 601	4	6.1	1.31	551 269	5	5.8	0.54	2 785 583	[illegible]
Stomach	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	20.83
Liver	841 080	6	4.7	1.08	781 631	3	8.2	0.98	675 210	8.85
Rectum	704 376	7	3.9	0.91	310 394	10	3.2	0.35	1 876 453	24.58
Oesophagus	572 034	8	3.2	0.78	508 585	6	5.3	0.67	547 104	7.17
Cervix uteri	569 847	9	3.2	1.36	311 365	9	3.3	0.77	1 474 265	38.98
Thyroid	567 233	10	3.1	0.68	41 071	25	0.43	0.05	1 997 846	26.17
Bladder	549 393	11	3.0	0.65	199 922	14	2.1	0.18	1 648 482	21.60
Non-Hodgkin lymphoma	509 590	12	2.8	0.61	248 724	12	2.6	0.27	1 353 273	17.73
Pancreas	458 918	13	2.5	0.55	432 242	7	4.5	0.50	282 574	3.70
Leukaemia	437 033	14	2.4	0.48	309 006	11	3.2	0.33	1 174 433	15.39
Kidney	403 262	15	2.2	0.52	175 098	17	1.8	0.20	1 025 730	13.44
Corpus uteri	382 069	16	2.1	1.01	89 929	21	0.94	0.21	1 283 348	33.93
Lip, oral cavity	354 864	17	2.0	0.46	177 384	16	1.9	0.23	913 514	11.97
Brain, nervous system	296 851	18	1.6	0.36	241 037	13	2.5	0.30	771 110	10.10
Ovary	[illegible]	19	1.6	0.72	184 799	15	1.9	0.45	[illegible]	[illegible]
Melanoma of skin	287 723	20	1.6	0.35	60 712	23	0.64	0.07	965 623	[illegible]
Gallbladder	219 420	21	1.2	[illegible]	[illegible]	[illegible]	[illegible]	0.18	233 820	3.06
Larynx	177 422	22	0.98	0.25	94 771	20	0.99	0.13	488 900	6.41
Multiple myeloma	159 985	23	0.88	0.20	106 105	19	1.1	0.12	376 005	4.93
Nasopharynx	129 079	24	0.71	0.16	72 987	22	0.76	0.10	362 219	4.75
Oropharynx	92 887	25	0.51	0.13	51 005	24	0.53	0.07	280 508	3.68
Hypopharynx	80 608	26	0.45	0.11	34 984	26	0.37	0.05	119 130	1.56
Hodgkin lymphoma	79 990	27	0.44	0.08	26 167	27	0.27	0.03	275 947	3.62
Testis	71 105	28	0.39	0.14	9 507	34	0.10	0.02	284 073	7.38
Salivary glands	52 799	29	0.29	0.06	22 176	29	0.23	0.03	123 460	1.62
Anus	48 541	30	0.27	0.06	19 129	31	0.20	0.02	127 599	1.67
Vulva	44 235	31	0.24	0.09	15 222	32	0.16	0.03	132 269	3.50
Kaposi sarcoma	41 799	32	0.23	0.04	19 902	30	0.21	0.02	88 379	1.16
Penis	34 475	33	0.19	0.09	15 138	33	0.16	0.04	93 850	2.44
Mesothelioma	30 443	34	0.17	0.04	25 576	28	0.27	0.03	31 250	0.41
Vagina	17 600	35	0.10	0.04	8 062	35	0.08	0.02	43 877	1.16
All cancer sites	18 078 957	-	-	20.20	9 555 027	-	-	10.63	43 841 302	574.38

Source: International Agency for Research on Cancer

Incidence and Mortality Age-Standardized Rates in High/Very-High Human Development Index (HDI) Regions Versus Low/Medium HDI Regions Among Men (2018)

For most cancers with different locations, in men, the highest level of standardized incidence and mortality is reported in countries with very high and high levels of development.

This aspect should be highlighted in the case of colon cancer with a standardized incidence rate of 30.3 per 100,000 men, respectively mortality of 12.6 per 100,000 in the countries with very high and high level of development, as compared to 8.4 to 5.7 per 100,000 men respectively in medium and poor developed countries.



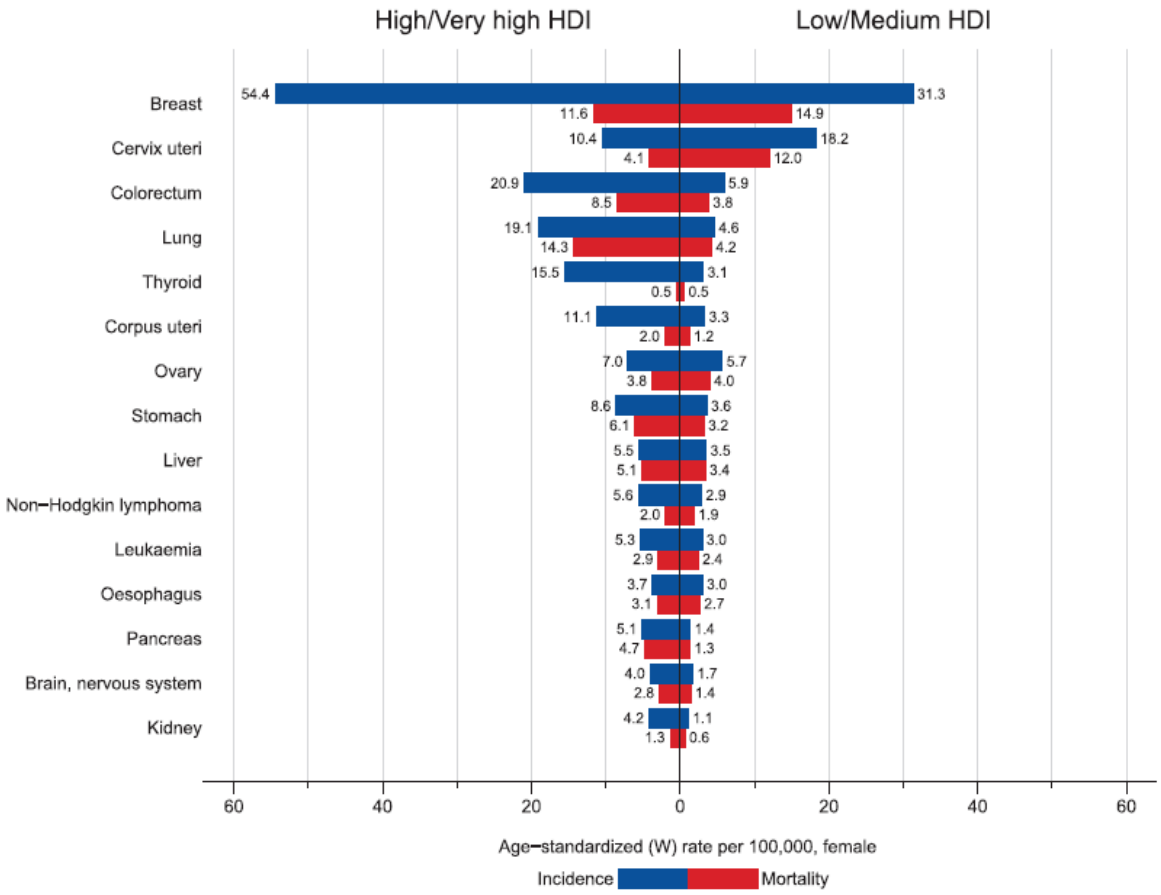
Source: Bray F. et al.

Incidence and Mortality Age-Standardized Rates in High/Very-High Human Development Index (HDI) Regions Versus Low/Medium HDI Regions Among Women (2018)

For most cancers with different locations in women, the highest level of standardized incidence is reported in countries with high/very high levels of development.

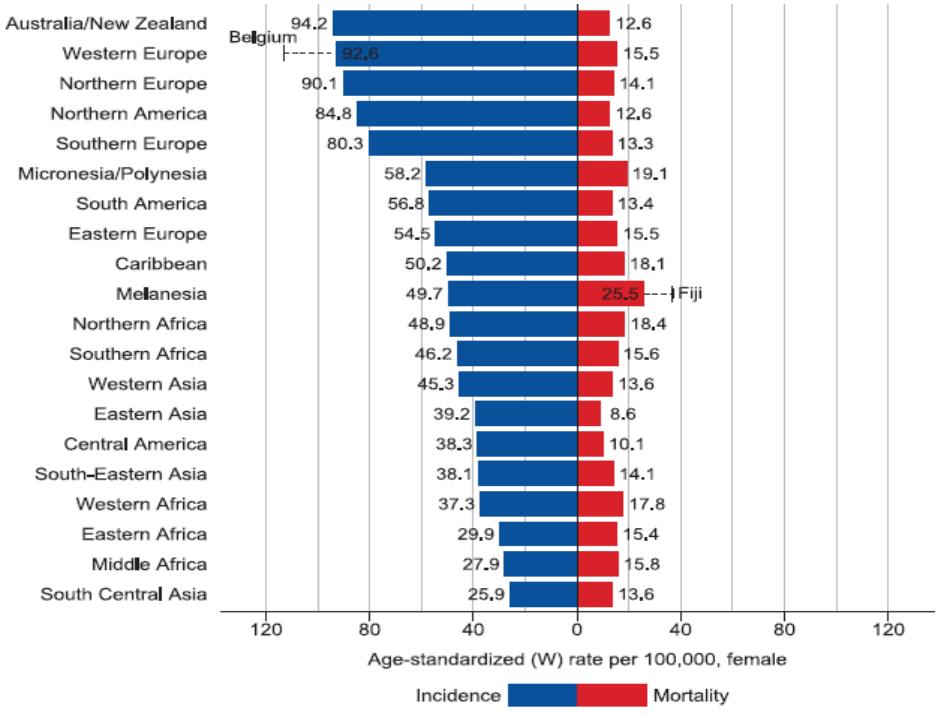
This particular aspect is noteworthy for breast cancer with a standardized incidence rate of 54.4 per 100,000 women in high/very high developed countries, but with a mortality rate of only 11.6 per 100,000 women.

The phenomenon correlated with the degree of development of the areas can be explained by the efficiency of the measures that allow the survival of these categories of patients for longer periods of time.



Source: Bray F. et al.

Region-Specific Incidence and Mortality Age-Standardized Rates for Female Breast Cancer in 2018



Source: Bray F. et al. (2018)

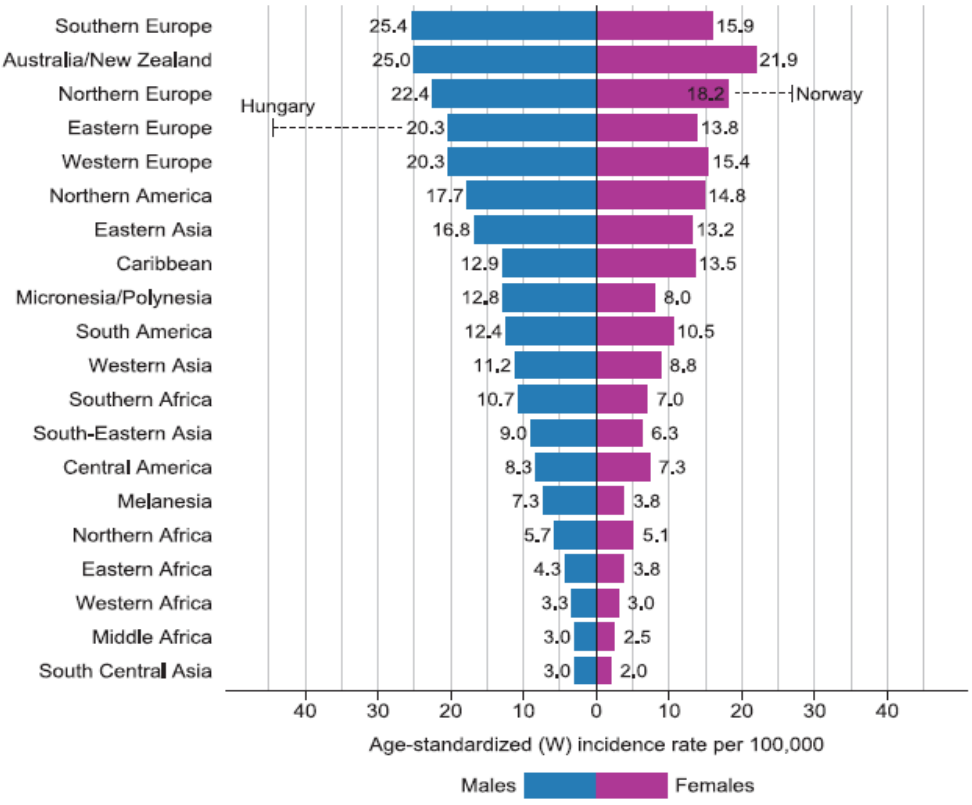
By regions, the highest standardized incidence rates for breast cancer are those reported in Australia and New Zealand followed by Western and Northern Europe.

Significant differences are observed for the standardized incidence rates between the different areas of Europe, respectively those of the West or North and those of the South.

This North-South gradient is also observed for other continents (Americas, Asia, Africa).

Region-Specific Incidence Age-Standardized Rates by Sex for Colon Cancer in 2018

Colon cancer has data of higher incidence standardized by sex higher in men than in women, noting on the first 4 places with high values in Southern Europe, New Zealand, Northern Europe and Eastern Europe.



Source: Bray F. et al. (2018)

Take Home Message

1. Epidemiology provides information on the determinants of health status (incidence, prevalence, mortality), useful for establishing priorities in health strategies at the level of each country and worldwide.

2. The latest data (2018) for reporting the number of cases and deaths due to cancer shows that in both sexes and at different age categories breast cancer and colon cancer rank 2 and 3 respectively worldwide, which places them among the main health priorities related to oncological pathology.

3. By geographical region, East Asia totals the highest number of cases (incidence and prevalence rates estimated over a 5-year period) as well as deaths from all cancer causes.

4. The age-standardized incidence of all types of cancer in both male and female cancers has the highest rates in continents such as North America, Western Europe and Australia.

5. Global incidence, prevalence and mortality data place breast cancer among the first places after lung cancer. Colorectal cancer follows a relatively small difference in the hierarchy of cancers by location and ovarian cancer although it is ranked 19th as the incidence is ranked 15th as mortality due to the particularities related to the survival of those diagnosed.

6. For breast cancer in women and colon cancer in men, significant differences are observed for the standardized incidence rates between the different areas of Europe, respectively those of the

West or North and those of the South. This North-South gradient is also observed in other continents.

7. By level of development of the regions, in most cancers in men and women, the highest incidence and mortality rates are recorded in countries with very high and high standards. In men, colorectal cancer is the 3rd place in the very high and high developed countries, while in women the 1st place breast cancer has a higher mortality in the very high and high developed countries but an increased incidence in those poor and medium developed.

8. Addressing the epidemiological phenomena from the point of view of the analysis of the individual risk factors (analytical epidemiology) but also of those that are dependent on behavioural or environmental factors can provide explanations regarding the manifestation and spread of various types of cancer (descriptive epidemiology) depending on the distribution by age groups or by gender of the populations in various geographical areas, with various degrees of development.

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1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA CANCER J CLIN* 2018; 0:1—31.doi:10.3322/caac.21492. Available at: https://g8fip1kplyr33r3krz5b97d1-wpengine.netdna-ssl.com/wp-content/uploads/2018/09/caac 21492 Final Embargoed.pdf.

2. dos Santos Silva I. Cancer epidemiology: principles and methods. International Agency for Research on Cancer. Lyon, France, 1999. Available at: https://publications.iarc.fr/Non- Series - Publications/Other-Non-Series-Publications/Cancer-Epidemiology-Principles-And-Methods -1999.

3. ***International Agency for Research on Cancer. GLOBOCAN 2018. http://gco.iarc.fr./today/data/factsheets/cancers/39-All-cancers-fact-sheet.pdf

Learning objectives

- General risk factors for cancers
- Risk factors for hereditary cancers
- Risk factors for breast cancer
- Risk factors for ovarian cancer
- Risk factors for colorectal cancer
- Protective factors for cancers

Introduction

Any factor that increases the likelihood of an event to occur - the cancer in our situation - is called ***risk factor,*** and the factors that decrease the chance of developing this event are called ***protective factors***.

Also, some of these risk factors can be avoided, while the action of others cannot be influenced, thus the risk factors are divided into:

- modifiable risk factors (smoking, diet, number of births etc.) and unchangeable (genetic factors, family history, age, sex etc.).

According to IARC and World Cancer Research Fund (WCRF)/American Institute for Cancer Research (AICR), the risk (RF) or protective (PF) factors for cancer can be divided into the following categories depending on the type of evidence identified in the specialty literature:

- *factors that increase the risk* (sufficient or convincing evidence);
- *factors that could increase the risk* (limited or probable evidence);
- *factors that reduce the risk* (sufficient or convincing evidence);
- *factors that could increase the risk* (limited or probable evidence).

Factors known to increase the risk of cancer:

- smoking
- infections
- radiation
- immunosuppressive drugs
- etc.

Factors that could increase the risk of cancer:

- diet
- alcohol consumption
- physical activity
- obesity
- carcinogens in the environment
- etc.

General risk factors for cancers

Tobacco

- Smokeless tobacco, environmental tobacco smoke

Alcohol

Diet

- High animal - fat intake; aflatoxins; deficiencies in vitamins A and C and beta-

Radiation

- Ionizing and ultraviolet radiation, radon and its by-products

Infection

- Bacterial (*Helicobacter pylori*)
- Parasites (*Schistosoma haemotabium*, *Clonarchis sinensis*)

carotenes

Occupational exposures

- Aromatic amines, arsenic, asbestos, nickel, pesticides, polycyclic hydrocarbons, vinyl chloride, wood dusts, others

Medications

- Viral (Epstein-Barr virus, hepatitis B and C viruses, HIV, HPV, human T-lymphotropic virus type 1)

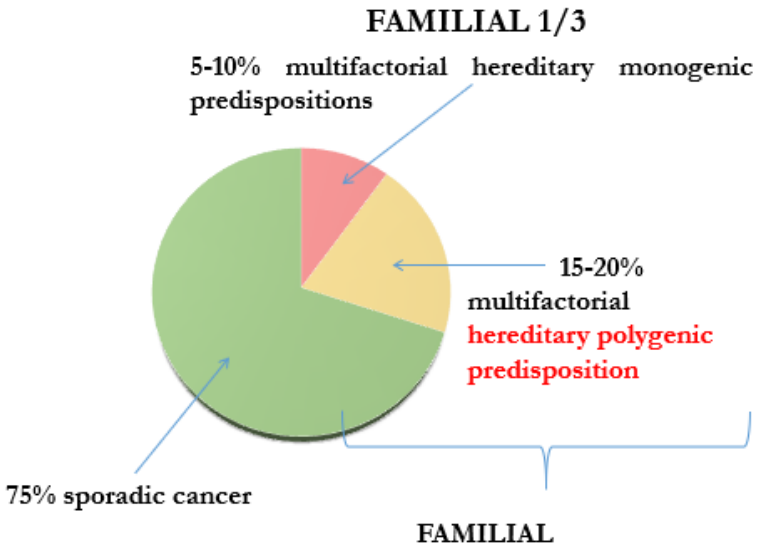
Family history

Genetic susceptibility

SPORADIC 2/3

Sporadic multifactorial: 70 - 80%

Lifestyle: 66%
Age
Smoking
Reproductive life and the endocrine hormones
Diet: 30-60%
Sedentariness/obesity: 10-30% ?
Environment: 2% ?
Stress
Ionizing radiation
Toxic chemicals (air, water, diet)
Infections: 16-25 % (2/3 virus, 1/3 bacteria, parasites)
Immune deficiencies



General risk factors for cancers – Tobacco/ Smoking

Smoking is a factor that increases the risk of many cancers, being the main cause for the following neoplasms: lung, oesophageal, oral cavity, bladder cancer, kidney cancer, gastric, pancreatic cancer, cervical cancer and acute myeloid leukaemia.

The relationship between smoking and lung cancer varies depending on the duration and intensity of exposure to smoking, if there is or not smoking cessation, type of cigarettes smoked, histological type of lung cancer, and population characteristics.

After 10 years of abstinence, the risk for lung cancer decreases by 30-50% compared with those who continue to smoke, and the risk for oral and oesophageal cancer halves 5 years after smoking cessation.

Also, those who quit smoking may lower their risk of developing cervical, gastric or bladder cancer.

The risk for colorectal cancer is higher in both active smokers (17-21%) and former smokers (17-25%), compared to non-smokers.

The association is stronger in men compared to women and is more important for rectal neoplasm compared to colon cancer.

The relative risk identified in the literature for smoking-colorectal cancer association is 1.18 (smokers versus non-smokers).

The IARC classifies smoking as a factor that may increase the risk for breast cancer or as a probable cause for this neoplasia, due to limited scientific evidence.

Current smokers have increased rate of breast cancer recurrence and mortality by 41% and 60% respectively.

The literature highlights a 12% higher risk in active smokers compared to non-smokers and 9% higher in former smokers, partially explained by the fact that smoking is associated with high levels of sex hormones.

Former smokers who exposed to 20-34.9 pack-years of cigarette had a 22% increased risk of recurrence in breast cancer.

Source: *** PDQ database of National Cancer Institute (2018)

General risk factors for cancers – Infections

Some viruses or bacteria may increase the risk of developing cancers (usually in developing countries) - Infectious agents cause about 15% of all cancer cases.

HPV infection increases the risk for cervical, oropharyngeal, vaginal, anal, and penile cancer, depending on the type of HPV involved.

HPV types 16 and 18 are responsible for approximately 70% of all cervical cancers and nearly 50% of vaginal, vulvar and penile cancers.

Source: *** PDQ database of National Cancer Institute (2018)

Infection with viral hepatitis B and C viruses (HBV and HCV) = proven factor in increasing the risk for liver cancer, according to IARC and a probable cause for cholangiocarcinoma.

HBV infection is responsible for 50-90% of cases of hepatocellular carcinoma in areas with high endemicity.

Investigating the relationship between HBV infection and liver cancer shows that the relative risk varies between 9.6 and 71, with values reaching 161 in the case of HCV co-infection.

Other types of association pathogenic agent – cancer:

- Epstein-Barr virus and Burkitt lymphoma
- *Helicobacter pylori* and gastric cancer

Source:

*** IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Biological agents.

*** PDQ database of National Cancer Institute. *Cancer Prevention Overview* – November 2019

General risk factors for cancers - Radiations

Exposure to radiation is a well-known cause of cancer. Two types of radiation are associated with increased risk for cancer, namely:

- solar ultraviolet radiation (the leading cause of non-melanoma skin cancers) and
- ionizing radiation, which include medical radiation from cancer diagnostic tests (x-ray, CT, fluoroscopy or nuclear medicine) and radon that is found in the house atmosphere (higher concentration in houses and at the ground floor of the blocks).

There is a documented link between exposure to ionizing radiation and increased risk of leukemia, thyroid and breast cancer, as well as melanoma, lung, gastric, colon, esophageal, bladder, and ovarian cancers.

Source: *** National Cancer Institute. *Cancer Prevention PDQ Overview.*

General risk factors for cancers - Other factors

Many other factors have been associated with increased risk for different types of cancer:

- *diet rich in fats, proteins, calories and red meat* (colorectal cancer);
- *alcohol* (oral, esophageal, breast, colorectal and hepatocellular cancer);
- *obesity* (associated with an increased risk of developing postmenopausal breast cancer, colorectal, endometrial, esophageal, renal and pancreatic cancers);
- *carcinogens in the environment* (passive smoking, external air pollution and exposure to asbestos, which increase the risk for lung cancer; increased concentrations of arsenic in drinking water, associated with an increased risk of skin, bladder or lung cancer);
- *immunosuppressive medication*, commonly used in organ transplant patients.

Source: *** National Cancer Institute. *Cancer Prevention PDQ Overview.*

Risk factors for hereditary cancers

Hereditary cancers

	Genetic predispositions	The most important associated genes
1	Breast-ovarian syndrome	BRCA1, BRCA2, PALB2 Genes with moderate penetrance (CHEK2, RAD51C, RAD51D and ATM)
2	Lynch syndrome	MLH, MSH2, MSH6, PMS2 and EPCAM
3	Familial pituitary adenoma	AIP
4	Ataxia-telangiectasia syndrome	ATM, MRE11A
5	Hereditary diffuse gastric cancer	CDH1
6	Hereditary renal papillary carcinoma	FH, MET
7	Hyperparathyroidism	CDC73, CASR
8	Cowden syndrome	PTEN
9	Fancony syndrome	FANC
10	Von Hippel-Lindau syndrome	VHL
11	Familial malignant melanoma	CDKN24, MITF, BAP1, POT1, CDK4
12	Endocrine neoplasms	MEN1, RET, CDKN1B
13	Neurofibromatosis	NF1, NF2, LZTR1, SMARCB1, SPRED1, SMARCE1
14	Hereditary pheochromocytoma	SDH, TMEM127, MAX, EPAS1
15	Familial adenomatous polyposis	APC, MUTYH, POLE, POLD1, NTHL1
16	Retinoblastoma	RB1
17	Birt-Hogg-Dubé syndrome	FLCN
18	Bloom syndrome	BLM
19	Carney-Stratakis syndrome	PRKAR1A
20	Gorlin syndrome	PTCH1, PTCH2, SUFU
21	Li-Fraumeni syndrome	TP53
22	Peutz-Jeghers syndrome	STK11
23	Familial juvenile polyposis syndrome	BMPR1A, SMAD4
24	Werner syndrome	WRN
25	Seroderma Pigmentosum	XP

Source: *** National Cancer Institute. Cancer Genetics PDQ Overview.

Risk factors for breast cancer

Among the risk factors for ***breast cancer*** are:

- *age* (80% of cases being diagnosed after the age of 50)

• Women have a lifetime risk of developing breast cancer that is approximately 100 times the risk for men.

• The short-term risk of breast cancer in a 70-year-old woman is about 10 times that of a 30-year-old woman.

- *endogenous hormones* (increased levels of oestradiol, estriol, androsterone and testosterone or IGF-1 - insulin-like growth factor 1)

- *reproductive factors* (nulliparity and small number of births; age over 35 years at first birth; early menarche - under 12 years of age; late menopause - over 52 years)

Sources:

1. Anothaisintawee T. et al. (2013).
2. *** National Cancer Institute. Breast Cancer Prevention PDQ.
Matei M and Azoicai D. (2015)

Among the risk factors for ***breast cancer*** are:

- the use of *combined oral contraceptives* (OC) (oestrogen-progestin)

• among women who were current or recent users of any hormonal contraception: RR = 1.20 (95% CI, 1.14–1.26)

• RR increase with duration of use:

- RR = 1.09 (95% CI, 0.96–1.23) for less than 1 year of use
- RR = 1.38 (95% CI, 1.26–1.51) for use longer than 10 years

- hormone replacement therapy (HRT)

• during years 1–4 of current use:

- RR = 1.60 (95% CI, 1.52–1.69) for oestrogen – progestagen HRT
- RR = 1.17 (95% CI, 1.10–1.26) for oestrogen-only HRT

• during years 5–14 current use:

- RR = 2.08 (95% CI, 2.02–2.15) for oestrogen-progestagen HRT
- RR = 1.33 (95% CI, 1.28–1.37) for oestrogen-only HRT

Among the risk factors for breast cancer are:

- family history (plays a major role in this type of cancer)

Risk is doubled if a single first-degree relative is affected

Risk is increased fivefold if two first-degree relatives are diagnosed.

- genetic factors (BRCA1, BRCA2, ATM, p53, CHEK2, PTEN, CDH1, STK11, PALB2)

Pathogenic variants in BRCA1 and BRCA2 - responsible for disease in 45% of families with multiple cases of breast cancer only and in up to 90% of families with both breast and ovarian cancer.

- personal history of cancer (breast, endometrial, Hodgkin's lymphoma, chronic lymphocytic leukemia, melanoma, pulmonary adenocarcinoma)

- The absolute risk for patients with a strong family history of cancer = 2.0% (95% CI, 0.5-3.5%)
- RR for detection of breast cancer when there is a personal history = 1.42 (95% CI, 0.48-4.17) compared with family history.
- RR when both risk factors were present compared with having only a family history = 3.04 (95% CI, 1.05-8.86).

Sources:

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2. Collaborative Group on Hormonal Factors in Breast Cancer. Type and timing of menopausal hormone therapy and breast cancer risk: individual participant meta-analysis of the worldwide epidemiological evidence. *Lancet* 2019; 394: 1159–1168 .http://dx.doi.org/10.1016/ S0140-6736(19)31709-X

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Schacht DV, Yamaguchi K, Lai J, Kulkarni K, Sennett CA, Abe H. Importance of a personal history of breast cancer as a risk factor for the development of subsequent breast cancer: results from screening breast MRI. *AJR Am J Roentgenol*. 2014;202(2):289-292. doi:10.2214/AJR.13.11553

Genes associated with breast and/ or gynecologic cancer susceptibility

Cancer Susceptibility	Moderate-Penetrance Genes	High-Penetrance Genes
Breast cancer	ATM, CHECK2, RAD51D	BRCA1, BRCA2, CDH1, PALB2, PTEN, STK11, TP53
Ovarian cancer	EPCAM, MLH1, MSH2, MSH6, RAD51C, RAD51D	BRCA1, BRCA2
Endometrial cancer		EPCAM, MLH1, MSH2, MSH6, PMS2, PTEN

Sources:

1. Anothaisintawee T, Wiratkapun C, Lerdsitthichai P, Kasamesup V, Wongwaisawan S et al. Risk factors for breast cancer: a systematic review and meta-analysis. *Asia Pac J Public Health* 2013; 25 (5) : 368-387.

2. ***National Cancer Institute. *Breast Cancer Prevention PDQ*. (https://www.cancer.gov/types/breast/hp/breast-prevention-pdq)

3. Matei M, Azoicăi D. Epidemiologia cancerelor. În: Prisecari V: *Epidemiologie specială*. SA „Tipografia Reclama", Chişinău, 2015, 369-392. ISBN 978-9975-58-024-3.

Among the risk factors for ***breast cancer*** are:

- overweight and obesity;
- exposure to ionizing radiation;
- exposure to carcinogens in the environment (e.g. ethylene oxide);
- increased breast density; benign breast conditions (e.g. atypical hyperplasia);
- diet rich in fats;
- alcohol consumption;
- smoking;
- medical conditions and treatments (diabetes, autoimmune thyroiditis, increased bone mineral density; treatment with digoxin or diethylstilbestrol).

Sources:

1. Anothaisintawee T, Wiratkapun C, Lerdsitthichai P, Kasamesup V, Wongwaisawan S et al. Risk factors for breast cancer: a systematic review and meta-analysis. *Asia Pac J Public Health* 2013; 25 (5) : 368-387.

2. *** National Cancer Institute. *Breast Cancer Prevention PDQ*. (https://www.cancer.gov/types/breast/hp/breast-prevention-pdq)

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Risk factors for ovarian cancer

For **ovarian cancer**, the researchers identified the following risk factors:

- general factors (age, race/ethnicity, socio-economic status);
- reproductive factors (increased number of ovulations/ovulatory cycles, age at first menstruation under 12 years, age at menopause over 52 years, age at first birth over 35 years, nulliparity, infertility);
- exogenous hormones (hormone replacement therapy, fertilizer treatment);
- smoking
- occupational exposure to carcinogens
- ionizing radiation
- overweight and obesity

- perineal use of talcum powder
- medical conditions and treatments (endometriosis or diabetes)

Sources:

1. ***National Cancer Institute. *Colorectal Cancer Prevention PDQ*. (https://www.cancer.gov/types/colorectal/hp/colorectal-prevention-pdq)

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4. ***Cancer Research UK. *Bowel cancer risk factors* – aprilie 2015 (http://www.cancerresearchuk.org/cancer-info/cancerstats/types/bowel/riskfactors/bowel-cancer-risk-factors)

5. Johnson CM, Wei C, Ensor JE et al. Meta-analyses of colorectal cancer risk factors. *Cancer Causes Control* 2013; 24 (6) : 1207-1222.

For ovarian cancer, the researchers identified the following risk factors:

- family history (plays an important role in this type of neoplasm)

A large meta-analysis of 15 published studies estimated an odds ratio of 3.1 for the risk of ovarian cancer associated with at least one first degree relative with ovarian cancer.

Cancer site	Patients with 1 FDR				Patients with ≥ 2 FDRs				P-trend
	N	RR	95% CI	Power (%) RR = 1.2	N	RR	95% CI	Power (%) RR = 1.4	
Ovary	467	**2.42**	2.21-2.66	46.6	20	**11.36**	7.33 – 17.62	8.2	< 0.0001
Upper aerodigestive tract	239	1.08	0.95-1.23	52.7	4	1.46	0.55-3.90	9.1	0.1887
Oesophagus	70	0.94	0.74-1.19	22.8	0	-	-	-	0.6042
Stomach	349	1.08	0.97-1.2	56.4	3	0.57	0.18-1.76	10.5	0.2887
Small intestine	41	1.02	0.75-1.38	16.3	1	4.07	0.57-28.93	37.7	0.7269
Colorectum	1009	**1.06**	1.00-1.13	96.7	58	1.16	0.89-1.50	37.7	0.0377
Colon	659	1.04	0.96-1.13	88.3	28	1.33	0.92-1.92	22.0	0.141
Rectum	395	1.07	0.97-1.19	69.7	10	1.48	0.80-2.76	12.3	0.0974
Liver	277	***1.20***	1.06-1.35	48.9	4	1.64	0.62-4.38	8.6	0.0024
Pancreas	271	**1.14**	1.01-1.28	50.4	2	0.70	0.18-2.80	9.1	0.0595
Lung	655	1.05	0.97-1.14	89.4	27	1.04	0.71-1.52	26.3	0.2382
Breast	1243	***1.20***	1.14-1.28	99.2	88	***1.47***	1.20-1.82	43.8	<0.0001
Cervix	184	1.14	0.98-1.32	45.1	0	-	-	-	0.0862
Endometrium	317	***1.27***	1.14-1.42	54.4	4	1.40	0.53-3.73	9.0	<0.0001
Other female genitals	39	0.94	0.69-1.29	15.0	0	-	-	-	0.7144
Prostate	1320	1.02	0.96-1.08	99.6	121	1.14	0.95-1.36	56.2	0.2174
Testis	35	1.20	0.86-1.68	23.8	1	4.37	0.62-31.03	6.7	0.1904
Other male genitals	30	***1.74***	1.21-2.49	10.4	0	-	-	-	0.0056
Kidney	276	1.08	0.96-1.22	56.2	3	0.89	0.29-2.75	9.6	0.2458
Bladder	403	0.96	0.87-1.06	75.5	14	1.57	0.93-2.66	13.7	0.8195
Melanoma	339	**1.12**	1.00-1.25	78.4	8	1.02	0.51-2.04	16.1	0.0573
Skin	387	0.98	0.88-1.08	72.4	12	1.20	0.68-2.11	14.8	0.8309
Nervous system	279	1.08	0.96-1.22	68.4	2	0.49	0.12-1.97	11.3	0.2972
Thyroid gland	75	1.04	0.83-1.31	29.0	0	-	-	-	0.7147
Endocrine glands	148	0.97	0.83-1.14	45.5	1	0.72	0.10-5.10	8.2	0.6948
Connective tissue	61	1.06	0.83-1.37	21.9	0	-	-	-	0.6436
Non-Hodgkin lymphoma	296	1.08	0.96-1.21	63.6	5	1.27	0.53-3.05	10.5	0.1882
Hodgkin lymphoma	52	1.28	0.97-1.68	20.0	0	-	-	-	0.0887
Myeloma	141	1.07	0.90-1.26	33.6	0	-	-	-	0.4464
Leukaemia	250	0.99	0.87-1.12	60.7	2	0.50	0.13-2.01	10.4	0.6704
CUP	396	***1.25***	1.13-1.38	62.4	4	1.08	0.40-2.87	10.0	<0.0001
All cancers[a]	4553	***1.13***	1.09-1.18	100.0	2589	***1.29***	1.23-1.36	100.0	<0.0001
All cancers[b]	4340	***1.10***	1.06-1.15	100.0	2315	***1.20***	1.14-1.27	100.0	<0.001

For **ovarian cancer**, the researches identified the following risk factors:

- personal history of cancer (breast or colorectal)
- genetic factors (gene mutations: BRCA1, BRCA2, p-53, HNPCC, OVCA1, CYP1A1, HER-2/neu, CYP1A2, CHEK2, EMSY, p21, PTEN, SOD2, MPO, NQC1, B7-H4)

Risk factors for colorectal cancer

Factors that may increase the risk of ***colorectal cancer*** include both modifiable and non-modifiable factors.

- *age* (43% of cases are over 75 years old)
- *gender* (more commonly in men)
- *diet* (rich in red or processed meat; rich in animal fats; use of dietary sugars)
- *alcohol consumption*

Factors that may increase the risk of colorectal cancer include both modifiable and non-modisiable factors

- *smoking*
- *occupational exposure to carcinogenic substances* (exposure to asbestos)
- *ionizing radiation*; *history of infections* (with *H. Pylori*, *HPV*)
- *obesity and overweight*

Compared with women with a BMI of 18.5 to 22.9:

- RR = 1.37 (95% CI, 0.81-2.30) for overweight women (BMI, 25.0-29.9)
- RR = 1.93 (95% CI, 1.15-3.25) for obese women (BMI, 30.0).
- RR for each 5-unit increment in BMI = 1.20 (95% CI, 1.05-1.38; P = .01 for trend).
- Similar associations were observed among women without a family history of CRC and without lower endoscopy within the past 10 years.

- *alcohol consumption*;
- *Smoking;*
- *occupational exposure to carcinogenic substances* (exposure to asbestos);
- *various medical conditions* (adenomatous polyps; inflammatory bowel disease - Crohn's disease, ulcer-haemorrhagic rectocolitis; biliary lithiasis; diabetes; metabolic syndrome).
- *personal history of cancer* (colorectal, oesophageal, laryngeal, pulmonary, prostate, endometrial or breast, also in those with chronic lymphocytic leukaemia and melanoma)
- *family history*

• in persons with familial adenomatous polyposis, the risk of CRC by age 40 can be as high as 100%.

• persons with Lynch syndrome can have a lifetime risk of CRC of about 80%

- *genetic syndromes* (familial adenomatous polyposis - PAF; hereditary non-polyposis colorectal cancer).

Protective factors for cancers

In addition to the risk factors, there is a category of factors that can influence the risk of cancer occurrence, factors that address prevention strategies, both globally (for example: *"Global Action Plan for the prevention and control of NCDs 2013-2020"*), as well as at national level (example: "*Program for the prevention and control of non-communicable diseases"*).

Source: *** World Health Organization. *Global Action Plan for the prevention and control of noncommunicable diseases*. WHO Press, Geneva, Switzerland, 2013.

Diet. It has been studied as both RF and PF, being difficult to accurately evaluate only one of the effects because a person's diet also contains foods that increase the risk of cancer and foods that reduce this risk. Some studies support the hypothesis that diet rich in starch-free vegetables and different fruits can provide protection against oral, oesophageal and gastric cancers. Also, fruit consumption can protect against colorectal cancer.

Physical activity. Research shows that there is a strong relationship between physical activity and decreased risk for colorectal cancer. There is scientific evidence that sustain the protection of physical activity against postmenopausal breast cancer and endometrial cancer.

Chemoprevention consists of the administration of drugs that prevent the occurrence of cancers or recurrences: *tamoxifen* or *raloxifene* (treatment given for 5 years reduces the risk of breast cancer by 50%) or *finasteride* to reduce the likelihood of prostate cancer.

New substances are constantly being researched with the hope of identifying effective preparations for the prevention of different types of neoplasms. Thus, *COX-2 inhibitors* are studied for the prevention of colorectal and breast cancer (but increase the incidence of cardiovascular events) and *aspirin* for colorectal cancer.

Protective factors for breast cancer

Among the protective factors for **breast cancer** are:

- breastfeeding (the risk decreases by 4% for every 12 months of breastfeeding)
- physical activity
- celiac disease
- regular intake of aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs)
- diet (consumption of fruits and vegetables; fiber; carotenoids; soy; mushrooms; coffee) hysterectomy with ovariectomy (performed before menopause reduces the risk by 24-41%).

Protective factors for ovarian cancer

Regarding the **ovarian cancer**, risk-lowering factors include:

- multiparity; breastfeeding (decreases risk by 24%)
- the use of OC (reduces the risk by 25-28%)
- hysterectomy (decreases risk by 27-31%)
- ovariectomy; tubal ligation (30% risk reduction)
- the use of statins (decreases risk by 21%)
- the presence of erythematosus systemic lupus (decreases the risk by 34%)
- consumption of starch-free vegetables.

Protective factors for colorectal cancer

Protective factors for **colorectal cancer** include:

- physical activity; hormone replacement therapy (decreases risk by 16%)
- the use of OC (decreases risk by 14%)
- daily use of aspirin (a period of 5 years or more decreases the risk by **32-49**%)
- Parkinson's disease (decreases risk by 24%)
- diet (rich in fiber, garlic, milk, calcium) is a factor that is likely to decrease the risk for colorectal cancer.

Take home message

- There are many risk factors for cancers which are divided in modifiable risk factors and unchangeable risk factors
- For hereditary cancers the most important risk factors are family history and gene mutations which increase the susceptibility for the disease
- By identifying cancer risk factors we can address also the cancer risk for family members
- The Oncogenetics is the tool that can be used to manage cancers in patients, but also among their families.

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I.3.1. Essential clinical elements in hereditary colorectal cancer

Learning objectives

- know the definition and classification of hereditary colorectal cancer know the main genetic mutations related to each form of hereditary colorectal cancer;
- know the main genetic mutations related to each form of hereditary colorectal cancer;
- know the main clinical characteristics related to each form of hereditary colorectal cancer.

Introduction

- Colorectal cancer (CRC) — the 4th cause of mortality in the world
- Hereditary CRC (HCRC) - spectrum of conditions characterized by a specific mutation predisposing to CRC
- Phenotypically divided into nonpolyposis and polyposis syndromes
- 3-6% of CRC occurs in younger age
- Dramatic increases in CRC risk
- Correlate with other tumours, extracolonic manifestations

Selection criteria for predisposition to colon cancer

- Colon cancer diagnosed at <50 years
- Multiple colonic malignancies present, either synchronous or metachronous
- Multiple primary cancers diagnosed, either colonic or extracolonic
- Over a lifetime, ≥ 10 adenomas present or ≥ 2 histologically characteristic hamartomatous polyps
- Colon cancer in > 1 generation of the individual's family
- Clustering of extracolonic cancers in family members

Classification of HCRC

NON POLYPOSIS HCRC (HNPCC) (dominant autosomal transmission, 1.7-4.2% of CRC)
Lynch syndrome
Constitutional mismatch repair-deficiency syndrome
Familial colorectal cancer X syndrome
Lynch-like syndrome

POLYPOSIS HCRC (HPCC) (3-5% of CRC)	**Serrated polyposis syndrome**
Adenomatous polyposis syndromes • Familial adenomatous polyposis (FAP) • Attenuated familial adenomatous polyposis (AFAP) • MUTYH-associated polyposis (MAP) • Polymerase proofreading-associated polyposis • Adenomatous polyposis associated with germinal mutation in NTHL1	**Hamartomatous polyposis** • Peutz – Jeghers syndrome • Juvenile polyposis syndrome • PTEN – hamartoma syndrome (Cowden disease, Bannayan-Riley-Ruvalcaba variant, Lhermitte Duclos variant) Hereditary mixed polyposis syndrome

Lynch syndrome

- 1 -3% of all CRC
- Population prevalence: 1/279
- Transmission: autosomal dominant
- **Mutations in DNA mismatch repair (MMR) -** MLH1, MSH2, MSH6 or PMS2 **or epithelial cell adhesion molecules (EPCAM), leading to microsatellite instability (MSI)**

General characteristics:

- Average age of CRC onset: 44 years
- No pathognomonic symptoms
- Right-sided, mucin-rich, poorly differentiated tumours
- Multiple tumours (synchronous or metachronous) in 35% of cases - Multiple Lynch syndrome associated cancers
- It develops from adenomas with the following characteristics: low number, villous component, high-grade dysplasia, accelerated adenoma-carcinoma sequence (2-3 years)
- Good prognosis

Lifetime cancer risk related to Lynch genotypes

Site	MLH1		MSH2		MSH6		PMS2¶	
	Men	Women	Men	Women	Men	Women	Men	Women
Any Lynch cancer	**59%**	**80%**	**71%**	**75%**	**31%**	**71%**	**-**	**-**
Colorectal	34 to 47%	36 to 45%	37 to 47%	33 to 37%	14 to 22%	10 to 26%	19 to 20%	11 to 15%
Endometrial	NA	18 to 60%	NA	21 to 60%	NA	16 to 71%	NA	13 to 24%
Ovarian	NA	11 to 20%	NA	15 to 24%	NA	0 to 1%	NA	0%
Gastric	20%	8%	2%	9%	-		-	
Urinary tract	1.2%	3%	8%	10%	0.7%		-	
Brain tumours (gliomas)	1.7%*		2.5%*		-		-	
Biliary/ pancreatic	1.9%*		0.02%*		-		-	
Small bowel	0.4%*		1.1%*		Small bowel	0.4%*		1.1%*

* Not reported separately by sex.

Lifetime cancer risk related to Lynch genotypes EPCAM genes

Lifetime cancer risk	% (95% CI)
Endometrium	57 (22-82)
Stomach	11-19
Ovary	20 (1-66)
Hepatobiliary	2-7
Upper urinary tract	4-5
Pancreas	3-4
Small bowel	1-4
CNS (glioblastoma)	1-3

Lynch Syndrome - diagnosis

Amsterdam Criteria: "3-2-1 rule"	
• At least **three** relatives with cancers associated with Lynch syndrome: colorectal, endometrium, small intestine, ureter, renal-pelvis; at least one of them is first-degree relative to the other two	
• At least **two** successive generations affected	
• At least **one** relative with cancer associated with Lynch syndrome to be diagnosed before the age of 50 years	
• Exclusion of familial adenomatous polyposis	
• Histological confirmation	
Sensitivity	22%
Specificity	98%

Tumors should be tested for MSI in the following situations

Revised Bethesda Criteria	
• CRC diagnosed in a person < 50 years	
• Synchronous or metachronous CRC or other tumours of Lynch syndrome regardless of age	
• Presence of histological alterations suggestive of MSI (tumour infiltration with lymphocytes, Crohn-like lymphocytic reaction, mucinous /seal differentiation, medullary growth pattern) in a person under 60 years of age	
• CRC in a patient with one or more first-degree relatives with cancers associated with Lynch syndrome, with one of the cancers diagnosed under 50 years of age	
• CRC diagnosed in a patient with two or more Grade I or Grade II relatives with Lynch syndrome related tumours, regardless of age	
Sensitivity	82 %
Specificity	77%

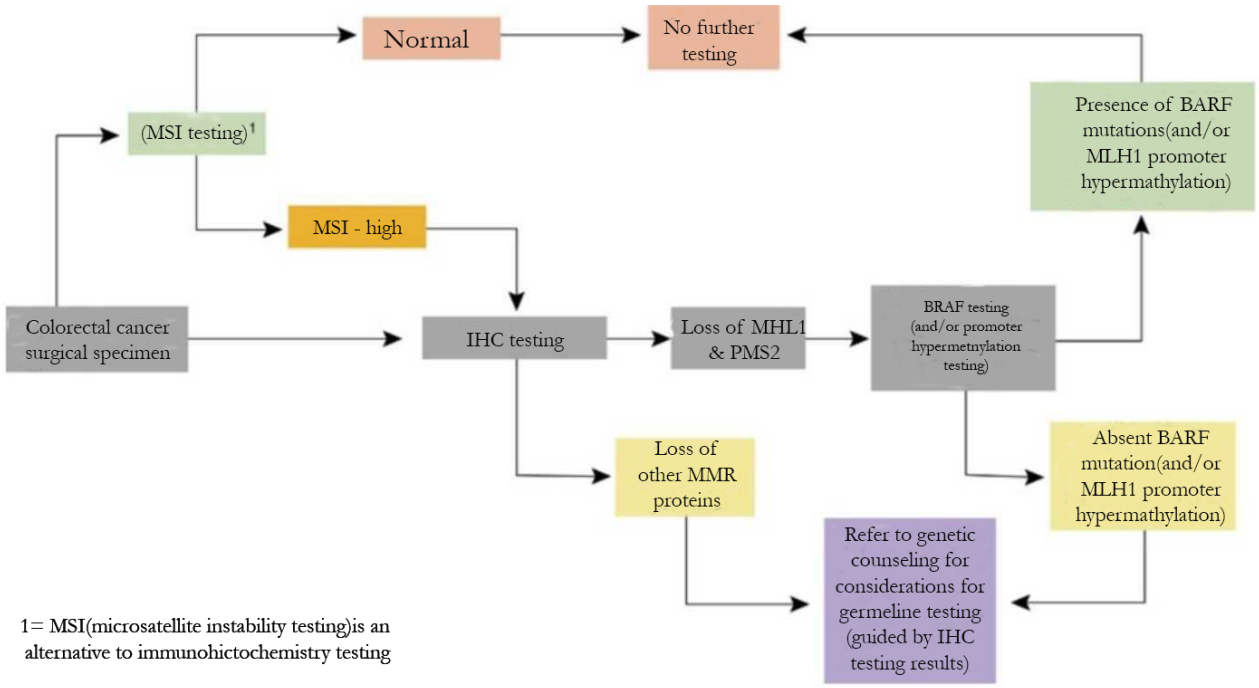
Lynch syndrome should be suspected:

- patients with synchronous or metachronous colorectal cancer (CRC)
- CRC prior to 50 years of age
- multiple Lynch syndrome associated cancers (e.g., CRC and endometrial, ovarian, stomach, small intestine, or renal pelvis/ureter)
- familial clustering of Lynch syndrome associated cancers.

Candidates for genetic evaluation:

- all newly diagnosed patients with CRC (alternatively, those diagnosed < 70 years);
- endometrial cancer < 60 years;
- first-degree relative of those with known MMR/EPCAM gene mutation;
- individuals with a CRC with > 5 percent chance of an MMR gene mutation by prediction models;
- family cancer history meeting Amsterdam criteria or revised Bethesda guidelines.

Lynch Syndrome - molecular diagnose



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Constitutional mismatch repair-deficiency syndrome (CMMRD)

• **biallelic mutations in one of the MMR genes:** MLH1, MSH2, MSH6, PMS2

Malignancies	Premalignancies and non-malignant tumours	Non-neoplastic features
• **Hematological malignancies (30%)** (non-Hodgkin's lymphomas, leukemias) • **Malignant brain and central nervous system tumours (50%)**(brain gliomas, supratentorial primitive neuroectodermal tumours, meduloblastoma) • **Lynch syndrome associated cancers (40%)**(CCR, small bowel, endometrium, bladder, ureter, renal pelvis, ovaries) • **Others**	• Adenomas/polyps: colon, rectum, duodenum, stomach • Hepatic adenomas • Neurofibromas • Optic gliomas • Pilomatricomas • Polyps of vocal cord	• Café au lait spots • Area of hyper/hypo skin pigmentation • Other signs reminiscent of neurofibromatosis 1 • Deffects in immunoglobuline class switch recombination (↓ Ig G2, ↓Ig G4, ↓ IgA, ↑IgM) • Agenesis of the corpus callosum with and without grey matter heterotopia • Cavernous brain haemangioma • Capillary haemangioma of skin • Combination of congenital malformations (asplenia, leftisomerism, ventricle septum defect) • Lupus erythematosus

CMMRD – Indications for testing in a cancer patient ≥ 3 points

Malignancies/premalignancies: one is mandatory; if more than one is present in the patient, add the points	
Carcinoma from the Lunch syndrome spectrum (colorectal, endometrial, small bowel, ureter, renal pelvis, biliary tract, stomach,bladder) at age <25 years	3
Multiple bowel adenomas at age <25 years and absence ofAPC/MUTYHmutation(s) or a single high-grade dysplasia adenoma atage <25 years	3
WHO grade III or IV glioma at age <25 years	2
non- Hodgkin's lymphomas of T-cell lineage or supratentorial primitive neuroectodermal tumours at age <18 years	2
Any malignancy at age <18 years	1

Additional features: optional; if more than one of the following is present, add the points	
Clinical sign of neurofibromatosis1 and/or ≥ 2 hyperpigmented and/orhypopigmented skin alterations Ø > 1 cm in the patient	2
Diagnosis of Lynch Syndrome in a first-degree or second-degree relative	2
Carcinoma from Lynch Syndrome before the age of 60 in first-degree, second-degree, and third-degree relative	1

A sibling with carcinoma from the Lynch Syndrome spectrum, high-grade glioma, supratentorial primitive neuroectodermal tumours non-Hodgkin's lymphomas	2
A sibling with any type of childhood malignancy	1
Multiple pilomatricomas in the patient	2
One pilomatricoma in the patient	1
Agenesis of the corpus callosum or non-therapy-induced cavernoma in the patient	1
Consanguineous parents	1
Deficiency/reduced levels of IgG2/4 and/or IgA	1

Diagnosis:

analysis of microsatellite instability (MSI) and/or immunohistochemical(IHC) staining of the four MMR proteins

Familial colorectal cancer X syndrome

- Meet the criteria of HNPCC (Amsterdam 1) but have **microsatellite stable** (no deleterious germline mutations in the MMR genes, no microsatellite instability, or no absence of immune histochemical staining of MMR protein);
- 2- 4% from HNPCC;
- Compared to Lynch syndrome:

- lower incidence of CRC;
- developing CRC at a later age;
- greater frequency in the distal colon;
- poor differentiation and more mucinous characteristics;
- distinctive morphological features, including tumor-infiltrating lymphocytes;
- fewer multiple tumors.

Lynch-like syndrome

- presence of MMR deficiency or MSI (excluding MLH1 hypermethylation) but lack a germline mutation;
- 60-70% of patients who fulfill Amsterdam criteria;
- Compared to Lynch syndrome:
  - most tend to have CRC in the right colon (93%) compared to those with Lynch syndrome (45%);
  - lower standardized incidence ratios for CRC (2.12 versus 6.04) and noncolorectal cancer (1.69 versus 2.81);
  - less likely to have synchronous or metachronous carcinoma.

Familial adenomatous polyposis (FAP)

- incidence of 0.6 to 3 per million inhabitants;
- 0.5-1% of CRC;
- Transmission: autosomal dominant; 20% to 30% of cases present as a result of a de novo mutation;
- The penetrance of FAP is 100%; incidence of CRC approaching 100% by the age of 50 years; 7% by age 21 and 95% by age 50;
- **germ-line mutation in APC gene;**

• **Genetic testing**:
- more than 10 cumulative adenomatous polyps are noted on a single colonoscopy;
- if an individual has 10 or more adenomas and a personal history of CRC;
- if an individual is found to have a total >20 adenomatous polyps in their lifetime.

Genotype-phenotype correlation in FAP

3 major phenotypes:

• profuse polyposis

- aggressive phenotype with early onset of polyposis, symptoms, and CRC-related death (at an average of 10 years earlier than typically described);
- deletions at codon 1309 and truncating mutations at codons 1250 and 1464.

• intermediate polyposis

- most mutations located between codon 157 and codon 1595.

• attenuated polyposis (AFAP)

- germline mutation in the APC gene is found in 10% of AFAP;
- reduced polyp burden (10—100 polyps); preferentially localised in the right colon;
- later age of onset; the age of onset of polyps between 20 to 30 years;
- lower risk of CRC; the diagnosis of CRC corresponds to 40 to 50 years;
- associations with gastric, duodenal, and thyroid cancer;
- some features of AFAP are similar to those of MUTYH-associated polyposis;

Familial adenomatous polyposis

• Over 100 colon polyps (usually between 1000 and 5,000);

• Adenomas may be sessile or pedunculated, with variable sizes: "carpet villous adenomas"(small, covering the entire surface of the large intestine) or moderate (polyps, less numerous, large and wide-ranging);

• Distributed throughout the colon, mainly distal;

• Average age of onset: 15,9 ± 5,4 years;

• Malignancy occurs 10-15 years after onset (83% by the age of 45,93% by the age of 50);

• Multiple cancers, usually synchronous, occur in 50% of cases;

Associated manifestations:

• Upper gastrointestinal tract polyps – gastric and duodenal;

• Duodenal cancers with typical periampullary location;

• Jejunal adenomas and carcinomas;

• Adenomas and carcinomas affecting the bile ducts, gallbladder and pancreas;

• **Congenital hypertrophy of the retinal pigment epithelium** (multiple bilateral lesions, intensely pigmented, round, oval or reniform; it is detected at the slit-lamp examination and it is a subclinical marker of the condition);

• **Desmoid tumours** of the abdominal cavity (benign tumours consisting of mature fibroblasts originated in the musculoskeletal structures; most occur 2-3 years after surgery);

• **Extra-intestinal malignancies**: papillary thyroid carcinoma, hepatoblastoma, biliary and pancreatic carcinomas, central nervous system malignant tumours, adrenal carcinomas.

MUTYH associated polyposis (MAP)

• germline mutations in alleles of MUTYH gene which is involved in base excision repair

•**Genetic testing** for MAP: in the case of clinically diagnosed polyposis without an identified APC mutation

• autosomal recessive (usually no family history of polyposis)
• 50 x increased lifetime risk of CRC with a mean age of diagnosis at 50 years
• CRC risk of 19% at 50 years and 43% at 60 years

•Phenotype:
• dominated by the presence of multiple colorectal adenomatous polyps/colorectal adenomatous polyposis (most often of the attenuated type);
•high frequency of degenerate forms at diagnosis in index cases (approximately 50% of cases);
•possibly involve the upper digestive tract (adenomatous polyps or duodenal polyposis);
•no extra-digestive manifestation (except for skin lesions: sebaceous hyperplasias, sebaceous adenomas, sebaceous carcinomas)

Polymerase proofreading-associated polyposis

• Two genes with autosomal dominant inheritance associated with multiple adenomas and early-onset CRC: POLE and POLD1;
• Mutations in these genes have been related to different phenotypes that range from a classic phenotype with gastroduodenal involvement to attenuated forms or characteristic tumours of LS;
• Oligopolyposis: 5- 100 adenomas;
• POLE: associated with adenomas, CRC;
• POLD1: Associated with adenomas, CRC, endometrial cancers, astrocytoma;

Adenomatous polyposis associated with germinal mutation in NTHL1
• an autosomal recessive inheritance;
• an increased risk of endometrial cancer in biallelic mutation carriers.

Lifetime cancer risk related to adenomatous polyposis syndromes

Syndrome	Genes	Lifetime cancer risk	% (95% CI)
FAP	APC	Colorectal Duodenum/periampullary Stomach Pancreas Thyroid Liver (hepatoblastoma) CNS (meduloblastoma)	100 4-12 < 1 2 1-2 1-2 < 1
AFAP	APC	Colorectal Duodenum/periampullary Thyroid	70 4-12 1-2
MAP	MUTYH	Colorectal Duodenum	80 4

Serrated polyposis syndrome

- Serrated polyps of the colon and rectum
- incidence of 1:100,00,0; prevalence 0.03-0.55%;
- **MSI in 50%; BRAF oncogene mutation** (differentiation from Lynch syndrome);
- **lifetime risk of CRC: 16-42%** (no other associated malignancies);

Diagnostic criteria (WHO, 2019)

- Criterion 1: >5 lesions/serrated polyps proximal to the rectum, all >5 mm, at least 2 >10 mm;
- Criterion 2: > 20 lesions/serrated polyps of any size at the level of the colon and rectum, out of which at least 5 are located proximal to the rectum.
- Histological types: hyperplastic polyp, serrated sessile polyp with or without dysplasia, classic serrate, adenoma, unclassified serrated adenoma;
- Most patients don't have family history.

Approach to genetic testing in adenomatous polyposis syndromes

APC testing

• Personal history of > 20 cumulative adenomas
• Known pathogenic APC family mutation
• Consider in:
- personal history of desmoid tumours
- hepatoblastoma
- papillary thyroid cancer
- multifocal bilateral CHRPE
- 10-20 cumulative adenomas

MUTYH testing

• cumulative number of adenomatous polyps (histologically proven) 15 regardless of age
• cumulative number of adenomatous polyps (histologically proven) between 10 and 14 before the age of 60 years
• cumulative number of adenomatous polyps (histologically proven) between 5 and 9 if at least one of the following additional criteria is validated and if somatic analyses are not in favour of Lynch syndrome: all these adenomatous polyps occurred before the age of 40; these adenomatous polyps are associated with cancer that occurred before the age of 60; at least 5 of these adenomatous polyps are of the "advanced" type (size 10 mm and/or of tubulovillous architecture or exclusive villous and/or associated with high-grade dysplastic lesions); association with one or more sebaceous adenomas or carcinomas or multiple and/or large sebaceous hyperplasia lesions before the age of 50; association with duodenal adenomas

Peutz - Jeghers Syndrome

• **STK11/ LKB1 gene pathogenic variant carriers** • incidence 0.9 - 1.2 per one million inhabitants;
• The lifetime risk of CRC is **39%**
• Hamartomatous gastrointestinal polyps
- location: small intestine, colon, stomach;
- sessile or pedunculated, polylobated, dimensions 0.1 - 3 cm;
- symptoms: rectal bleeding, intestinal occlusion, biliary obstruction.

Mucocutaneous pigmentation:

- multiple brown-black melanin spots, between 1 and 5 mm in size;
- lips, oral mucosa, genital, perianal, face, palms, plants, forearms, fingers;
- onset age is 1 to 2 years, becoming more and more numerous in time.

Malignant tumours

✓ intestinal: stomach (29%), small intestine (13%);
✓ extra-intestinal: breast (54%), ovary (21%), cervix (9-10%), testicle (1%), pancreas (11-36%), lung (15%) .

Juvenile polyposis syndrome

• SMAD4 and BMPR1A pathogenic variant carriers

• incidence: 1 per 1 million inhabitants;

• The lifetime risk of CRC is 39%;

• Stomach, pancreas, small bowel malignancies 21%;

• Hamartomatous polyps, occurring in children aged 4-14;

• Tens-hundreds, predominantly located at the level of the colon, ses sile or pedunculated, variable dimensions, red smooth surface, sometimes covered with whitish exudate;

• Symptoms: rectal bleeding, transrectal prolapse of the polyp, abdominal pain, diarrhea, delayed growth;

• Congenital malformations: intestinal malrotation, Meckel's diverticulum, hydrocephalus, heart malformations, polydactyly, palatoschisis.

PTEN — hamartoma syndrome

• mutation in the PTEN gene

• include Cowden syndrome and Bannayan—Riley—Ruvalcaba syndrome;

• CRC: 9-18%

Diagnostic criteria:

Clinical diagnosis (either of the following): 3 major criteria, one must be macrocephaly, Lhermitte-Duclos disease, or GI hamartomas OR 2 major and 3 minor criteria.

Clinical diagnosis in a family when one individual meets the revised PTEN hamartoma tumour syndrome criteria or has a PTEN mutation: any 2 major criteria with or without minor criteria; OR 1 major and 2 minor criteria; OR 3 minor criteria

Major criteria: breast cancer, follicular thyroid cancer, GI hamartomas (includes ganglioneuromas, but excludes hyperplastic polyps; > 3), Lhermitte-Duclos disease (adult), macrocephaly, macular pigmentation of the glans penis, multiple muco-cutaneous lesions (any of the following): multiple trichilemmomas (> 3, at least one of which is biopsy proven), acral keratosis (>3 palmoplantar keratotic pits an/or acral hyperkeratotic papules), muco-cutaneous neuromas (> 3), oral papilloma (tongue and gingiva), multiple (> 3) OR biopsy proven OR dermatologist diagnosed

Minor criteria: autism spectrum disorder, colon cancer, oesophageal glycogenic acanthosis (> 3), lipomas (> 3), intellectual disability, renal cell carcinoma, testicular lipomatosis, papillary or follicular variant of papillary thyroid cancer, structural lesions of the thyroid (e.g., adenoma and multinodular goiter), vascular anomalies (includes multiple intracranial developmental venous anomalies)

Selection criteria for in colorectal cancer genetic testing predisposition

I. Suspicion of Lynch syndrome (MLH1, MSH2, MSH6, PMS2, EPCAM)

II. Adenomatous polyps/polyposis (APC, MUTYH, POLE, POLD1)

III. Hamartomatous polyposis (STK11, BMPR1A, SMAD4,PTEN); clinical phenotype for Peutz-Jeghers syndrome, juvenile polyposis, Cowden disease.

Absence of multiple adenomatous polyps/polyposis

• Indications based on individual characteristics

- Any tumour of the Lynch spectrum, (including cutaneous) of MMR phenotype;
- If tumour phenotype not available: any Lynch spectrum tumour diagnosed at age < 41 years; 2 Lynch spectrum tumours, the 1 diagnosed at < 51 years; 3 Lynch spectrum tumours, regardless of age at diagnosis

Note: 1 advanced adenoma (> 1 cm and/or high-grade dysplasia) can replace one (and only one) tumour in the case of tumours multiple primitive phenotype

• Indications based on family history

Familial aggregation of cancers of the Lynch syndrome or POL3 spectrum validating the Amsterdam criteria or at least 2 of the 3 criteria Amsterdam

Multiple adenomatous polyps or adenomatous polyposis

• 15 colorectal adenomas regardless of age, characteristics of adenomas ("advanced" or not) and family history

• 5 - 14 colorectal adenomas and 2 of the following secondary criteria:
  - ≥ 2 advanced adenomas;
  - all adenomas occurred at age <51 years;
  - personal history of CRC diagnosed at age <61 years;
  - profuse glandulocystic gastric polyposis;
  - multiple sebaceous lesions;
  - consanguinity;

• Gastric adenomatous polyposis;

Take Home Message

• HCRC causes < 10% of new cases of CRC

• Family history (young age at onset, number and age of relatives) is very important;

• HRCC can be **non-polyposis** (e.g. Lynch syndrome with mutations of mismatch repair genes and microsatellite instability; Amsterdam, Bethesda criteria) and **polyposis** (familial adenomatous polyposis, MUTYH associated polyposis, serrated adenoma polyposis etc);

• Usually HCRC appears in young people, can be multiple, is located on the right colon, can associate other malignancy or extracolonic manifestations.

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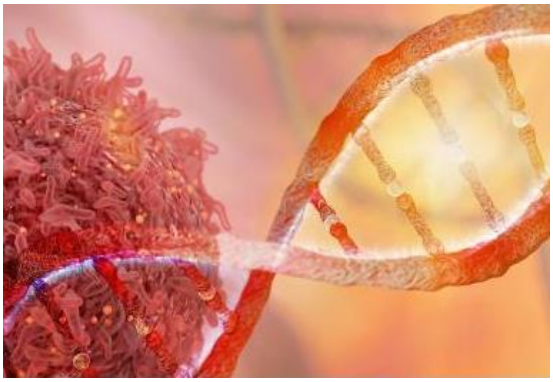
I.3.2. Essential clinical elements in hereditary breast and ovarian cancer

Learning objectives

- know the definition of hereditary breast and ovarian cancer
- know which are the tumour suppressor genes
- know which are the clinical differences between the BRCA1 and BRCA2 gene mutations
- know which are the breast cancer risk assessment guidelines
- know which are the Newer Risk Assessment Tools

Hereditary breast and ovarian cancer syndrome



The most common reasons for referral for genetic counselling and consideration of genetic testing. HBOC syndrome is characterized by an inherited predisposition (strong family history) of breast cancer (BC) and epithelial ovarian cancer (EOC).

HBOC syndrome prevalence

• HBOC syndrome can affect men as well as women and occurs in people from all ethnic and racial backgrounds

• The prevalence

- in the **general population** is estimated to be somewhere between 1 in 200 to 1 in 800;
- in **specific populations** is higher (in Ashkenazi Jewish people it is about 1 in 40 people).

Transmission

• HBOC syndrome involves a germline mutation (it occurs in the oocyte or the sperm cell) and can be transmitted from parents to children -» transmission can occur either maternally or paternally.

• *De novo* (sporadic) variations of the *BRCA1* or *BRCA2* gene have rarely been reported.

The transmission pattern is autosomal dominant

Genes involved in HBOC syndrome

Two separate and distinct tumour suppressor genes, **BRCA1** and **BRCA2,** account for the approximately 85% of all cases of hereditary breast and epithelial ovarian cancer (EOC).

These 2 genes account for a smaller percentage of isolated familial breast cancer cases in the absence of EOC.

Other genes (PTEN, CDH1, RAD51C, RAD51D, EPCAM, MLH1, MSH2, MSH6, PMS2, TP53, PAEB2 +/- ATM, CHEK2) account for a smaller number of hereditary breast and ovarian cancers.

• monoallelic mutations in **PALB2 gene** (partner and localizer of the BRCA2 gene) have also been shown to increase the risk of breast cancer in women as well as in men;

• PALB2 germline mutations seem to account for around 0.7-1.1% of all familial aggregation of BC.

Penetrance of tumour Suppressor Genes Mutations

The penetrance describes whether and to what degree a gene variation causes cancer in an individual/family.

- BRCA1 and BRCA2 genes have a high penetrance;
- RAD51C has a high penetrance in OC;
- ATM/CHEK2 (in BC) and BRIP1,RAD51D, MLH1, MSH2, and MSH6 (in OvC) have moderate penetrance.
- BRCA1 and BRCA2 mutation carriers have a cumulative BC risk at 70 years of age of around 57% and 49 % respectively;
- Cumulative OC risk is about 40% for BRCA1 and 18% for BRCA2 mutation carriers;
- There are other factors (probably additional genetic factors or environmental ones) that contribute to cancer development in people with mutations in their BRCA1 or BRCA2 genes.

Cancer types associated with BRCA2 mutations

- Breast
- Second primary breast
- Ovarian
- Male breast
- Prostate
- Pancreatic
- Melanoma (cutaneous & ocular)

BRCA1 mutation has not been associated with a significant increase in cancers other than breast and ovarian.

HBOC families' characteristics

- There are usually more cases of breast cancer than ovarian cancer
- By far an earlier age of onset than is seen in the general population;
- A higher likelihood of bilateral disease.

HBOC families

Markedly higher frequency of family members with breast cancer and EOC occurring in the same individual.

For some gene mutations, a strikingly higher risk for breast cancer in men.

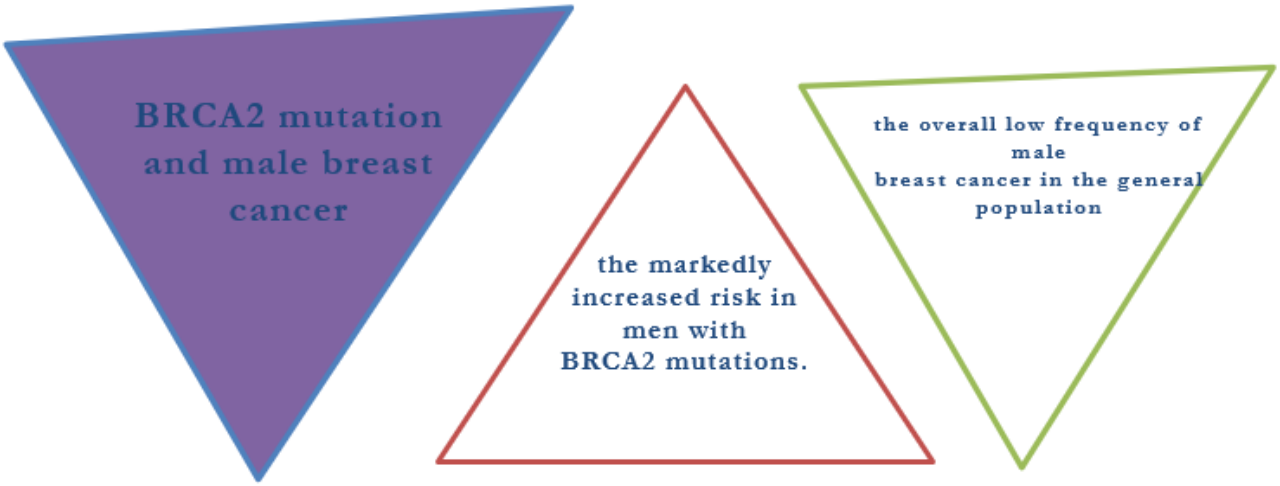
- Mutations in BRCA1/2 confer a markedly increased risk for developing breast cancer and EOC
- Both are associated with an approximately 85% - 90% risk for developing breast cancer by the age of 70.

• BRCA1/2 mutations are associated with a markedly elevated risk for developing EOC.

• BRCA1 mutations are associated with a higher risk for developing EOC than BRCA2

- BRCA2 mutations have been shown to increase the risk for male breast cancer compared with BRCA1.
- In many cases, affected females in the paternal lineage are either ignored or not considered on an equal basis with affected members from the maternal lineage because of a misperception that HBOC is a disease of women.

Any case of male breast cancer, regardless of age at diagnosis, should prompt the offering of genetic counselling and consideration of genetic testing for HBOC because of:



Clinical differences between the 2 BRCA 1/2 gene mutations

BRCA 2	BRCA 1
✓ An approximately 100-fold increased risk for male breast cancer among BRCA2 mutation. ✓ +/- an increased risk for early-onset prostate and pancreatic cancer.	✓ Only a potentially slight increased risk for male breast cancer.

Breast cancer risk assessment guidelines

USPSTF guidelines

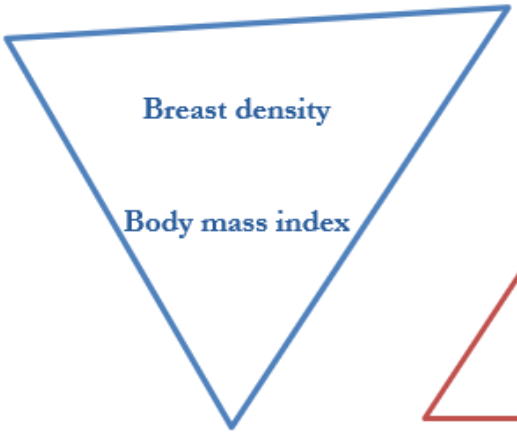
Gail Model

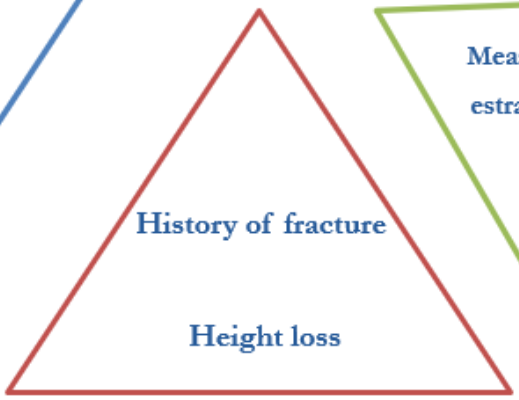
Breast Cancer Assessment Tool

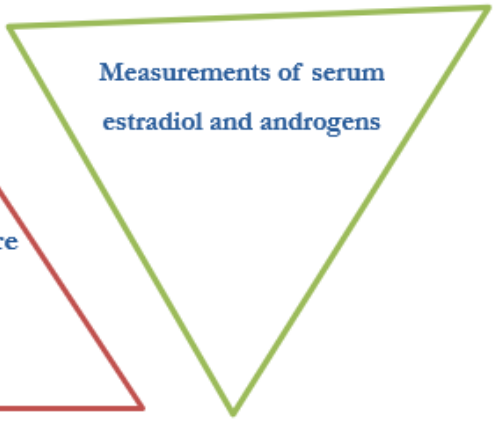
BRCA Pro

Newer Risk Assessment Tools

- Include factors other than personal and family history
- Provide a more accurate assessment of risk







American College of Obstetricians and Gynaecologists and the Society of Gynaecologic Oncologists guidelines

✓ Clarified the need for clinicians to incorporate risk assessment into their practices;

✓ Does not promote a more expansive role for genetic testing;

✓ Raises awareness of the marked increased risk of cancer development in individuals with tumour suppressor gene mutations and with family members with certain malignancies.

Clinical criteria for genetic testing

Based on clinical risk factors:

✓ age

✓ hormone receptor status

✓ ancestry with founder mutations

✓ personal and family history of cancer

Regardless of family history:

Women with synchronous or metachronous breast and ovarian cancer

Breast cancer ≤ 40 years

Bilateral breast cancer (the first diagnosed ≤ 50 years)

Triple-negative breast cancer ≤ 60 years

High-grade epithelial non-mucinous ovarian cancer (or fallopian tube or primary peritoneal cancer)

Ancestry with founder mutations

BRCA somatic mutation detected in any tumour type with an allele frequency > 30% (if it is known)

Metastatic HER2-negative breast cancer patients eligible to consider PARP inhibitor therapy

2 or more first degree relatives with any combination of the following high-risk features:

Bilateral breast cancer-I-another breast cancer < 60 years

Breast cancer < 50 years and prostate or pancreatic cancer < 60 years

Male breast cancer

Breast and ovarian cancer

Two cases of breast cancer diagnosed before age 50 years

3 or more direct relatives with breast cancer (at least one premenopausal) and/or ovarian cancer and/or pancreatic cancer or high Gleason (≥ 7) prostate cancer

Genes recommended for testing in HBOC syndrome

BRCA1 and BRCA2

PTEN

ATM/CHEK2 (in BC)

PMS2, EPCAM

RAD51C, RAD51D, MLH1, MSH2, and MSH6

CDH1, TP53, PALB2

Take Home Message

✓ HBOC is the most common reasons for referral for genetic counselling and consideration of genetic testing

✓ BRCA1 and BRCA2, account for the approximately 85% of all cases of hereditary breast and epithelial ovarian cancer

✓ There are many breast cancer risk assessment guidelines

✓ Equal attention must be paid to the assessment of paternal relatives of an individual being evaluated for a possible BRCA1/2 mutation

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1.3.3 Essential clinical elements of hereditary cancer/endocrine cancers

Learning objectives

1. Be able to classify the most important form of hereditary endocrine cancer, multiple endocrine neoplasia (MEN).
2. Be able to recognize the most important clinical signs of MEN 1 and MEN2 types.
3. Understand the relation between the gland/hormone involved and the clinical signs and/or symptoms.

Introduction

1. Hereditary endocrine tumours are an important group of diseases with great heterogeneity.
2. Tumour syndromes are described for all major endocrine glands, and like other cancers have been linked to activating mutations of oncogenes or inactivating mutations of tumour suppressor genes.
3. The best-described examples are activating mutations of RET proto-oncogene in neoplasia endocrina multipla 2 (MEN2) and inactivating mutations of MEN1 tumour suppressor gene in MEN1.

Multiple endocrine neoplasia classification

The tumours that make up MEN come from the neuroendocrin cells of the APUD system (amine precursor uptake and decarboxylation).

TYPES OF MEN	TUMOURS (estimated penetrance)
MEN1A	1. Hyperparathyroidism (90%): adenoma, carcinoma. 2. Enteropancreatic tumours (30-70%): Zollinger-Ellison syndrome, insulinomas, glucagonomas, VIPomas. 3. Pituitary tumours (30-40%): GH, ACTH, PRL producing or nonfunctional. Associated tumours: angiofibromas (88%), collagenomas (72%), adrenocortical tumours (35%), lipomas (33%), carcinoid and carcinoid syndrome tumours (10%), meningiomas (10%).
MEN2A	1. Medullary thyroid carcinoma (95%) 2. Pheochromocytoma (50%) 3. Hyperparathyroidism (20-30%) Variants: MEN2A+cutaneous amyloidosis, MEN2A+Hirschsprung's disease, familial medullary carcinoma
MEN2B	1. Medullary thyroid carcinoma (100%) 2. Pheochromocytoma (50%) Associated pathologies: mucosal neuromas, marfanoid habitus, megacolon.

MEN1 - general data

1. MEN1 is caused by an inactivating mutation of the MEN1 gene located on the long arm of chromosome 11 (11q13). The transmission is autosomal dominant but there may be sporadic cases.
2. The incidence is 0.25% (in post-mortem studies) and the estimated prevalence is between 0.02 and 0.2‰.
3. 95% of patients show clinical signs most commonly after the age of 50.
4. The clinical signs depend on the type of tumour (parathyroid, entero-pancreatic and pituitary).

5. The most frequent initial clinical manifestation is caused by hyperparathyroidism.
6. For a long time, these tumours can be asymptomatic, being discovered either during biological tests or due to related examinations (ophthalmological, radiological, etc.).

MEN1 - clinical signs caused by hyperparathyroidism

General characteristics

1. Hyperparathyroidism in MEN1 can have a long asymptomatic evolution and is usually detected incidentally by increased PTH dosing under hypercalcemia.
2. Clinical signs are a consequence of hypercalcemia, hypercalciuria and increased bone turnover due to PTH hypersecretion.

Clinical manifestations

1. Polyuria, polydipsia, constipation, generalized asthenia, depression, anorexia, nausea, epigastric pain, pyrosis.
2. Chronic or colicky lumbar pain due to nephrolithiasis. Bone pain, fragility fractures, bone tumours (fibrocystic osteitis).
3. The clinical manifestations depend on the size of the tumours and the type of hormone produced.

MENI - clinical signs caused by the enteropancreatic tumours (NETs)

General characteristics	Clinical manifestations
• Due to their extremely small size they do not show any symptoms for a long time ("cancer in slow motion"). • They usually metastasize before they becomes symptomatic. • The symptoms are nonspecific and may mimic other digestive disorders so that the condition remains undiagnosed.	• Flushes • Diarrhoea • Steatorrhea • Dyspeptic syndrome • Epigastric pain • Upper gastrointestinal bleeding • Mechanic jaundice • Hypoglycaemic episodes

TUMOR	INCIDENCE	CLINICAL MANIFESTATION	HORMONAL SECRETION
GASTRINOMA Zollinger-Ellison sd (ZES)	Up to 60% of patients with MEN1 have ZES	gastroesophageal reflux disease (GERD) and peptic ulcer disease (PUD), which are often refractory, with or without diarrhoea	GASTRIN
INSULINOMA	10–30% of NETs in MEN1	severe hypoglycaemia; occur primarily in patients younger than 40 years-old, with many of these tumours arising in patients younger than 20 years of age	INSULIN
GLUCAGONOMA	<3% of MEN1 patients	skin rash (necrolytic migratory erythema), weight loss, anaemia, and stomatitis	GLUCAGON
VIP-oma	Very rare	watery diarrhoea, hypokalaemia, and achlorhydria	VIP

MEN2 - general data

• MEN2 is caused by the activating mutation of a RET proto-oncogene, located in the centromeric region of chromosome 10 (10q11-2) and is autosomal dominantly transmitted.

• MEN2B is less common than MEN2A, representing only 5% of cases of MEN2;

• Thyroid medullary carcinoma is present in all forms of MEN2 and is usually the first sign of disease.

• MEN2A is classified in 4 variants:

1. classic form;
2. associated with cutaneous amyloidosis lichen (CAL);
3. associated with Hirschsprung's disease (HD);
4. Familial medullary thyroid cancer (FMTC).

• In 5%–9% of patients with MEN2A, and the large majority of patients with MEN2B, the RET mutation arises de novo and almost always from the paternal allele.

• There is no indication for evaluating the thyroid tumours of patients with sporadic MTC for the presence of somatic HRAS, KRAS, or NRAS mutations, or the RET codon M918T mutation

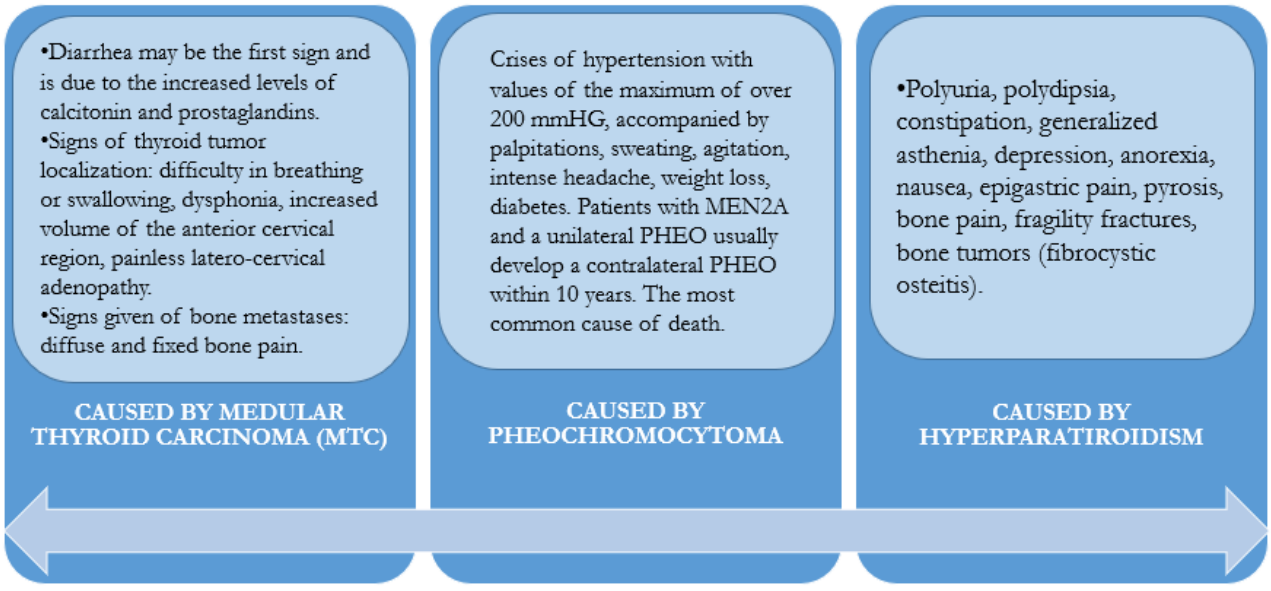
• Classical MEN2A is the most common MEN2A variant and in 95% of patients RET germline mutations occur in codons 609, 611, 618, or 620 of exon 10 or codon 634 of exon 11. Virtually all patients develop MTC and lower numbers develop PHEOs or HPTH, the frequency of each depending on the specific RET mutation.

• RET codon 634 mutations are associated with a high penetrance of PHEO, which according to some studies increased with age, being 25% by age 30 years, 52% by age 50 years, and 88% by age 77 years.

• There is a much lower penetrance of PHEO in patients with exon 10 RET codon mutations (609 [4%–26%], 611 [10%–25%], 618 [12%– 23%], and 620 [13%–24%]).

A RET codon 634 mutation is associated with a moderate penetrance of HPTH (up to 30%), and RET mutations in codons 609, 611, 618, and 620 are associated with a penetrance between 2% and 12%.

MEN 2a: clinical manifestations



Clinical characteristics and relationship between genotype and phenotype in patients with Sporadic MTC

• Usually occurs between the age of 40 and 60. Central and lateral compartment lymph node metastases are present in 14% and respectively 11% of patients with T1 tumours and in 86% and 93% of patients with T4 tumours.

The clinical behaviour is distant metastases may live for several years.

HPTH

• The HPTH in patients with classical MEN2A is usually mild and associated with few if any symptoms. From one to four parathyroid glands may be enlarged.

• A RET codon 634 mutation is associated with a moderate penetrance of HPTH (up to 30%), and RET mutations in codons 609, 611, 618, and 620 are associated with a penetrance between 2% and 12%.

• For practical reasons, screening for HPTH should be done concurrently with screening for PHEO.

• Is uncommon before puberty, occurring in90% of MEN1 individuals between 20 and 25 years of age, although mild-to-moderate hyperparathyroidism often emerges during adolescence with mild hypercalcemia. All individuals are affected by the age of 50 years. Progression is usually gradual but significant, hypercalcemia is occasionally evident in early adolescence.

• Generally, is a multiglandular disease and the parathyroid glands can become hyperplastic or develop adenomas.

• The growth of the glands is asynchronous and asymmetric, as each gland is considered a monoclonal lesion in which the germline mutation in the MEN1 gene confers on the parathyroid tissue a high susceptibility for the development of a tumour after the second somatic mutation.

• Morphologically, parathyroid glands in MEN1 may appear macroscopically normal, also because they can differ in terms of volume, weight, and size.

• Parathyroid adenomas in MEN1 can be ectopic, often located in the thymus, rarely within the thyroid gland, in the anterior mediastinum, in the pericardium, or surrounding the trachea, the oesophagus, and the carotid artery.

• Supernumerary glands are frequently found in up to 20% of MEN1 patients.

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MEN2A and CLA

• The CLA in MEN2A is characterized by dermatological lesions that are particularly evident in the scapular region of the back corresponding to dermatomes T2—T6.

• The classic symptom of CLA is intense pruritus that improves with sun exposure and worsens during periods of stress. Hyperpigmented lesions develop later, apparently secondary to scratching. The inciting lesion appears to be notalgia paresthetica, a sensory neuropathy involving the dorsal spinal nerves.

• The CLA may be present at a young age and prior to the onset of clinically evident MTC, thus serving as a precursor for the syndrome.

• The CLA in patients with MEN2A occurs almost exclusively in patients with the RET codon 634 mutation. PHEOs and HPTH occur in this variant with the same frequency as in classical MEN2A

MEN2A and HD

• RET germline mutations are present in 50% of patients with hereditary HD and in 15%—20% of patients with sporadic HD. Over 100 RET mutations have been described in HD, including microdeletions and insertions, nonsense or missense point mutations.

• The RET mutations in patients with MEN2A and HD are point mutations involving codons in exon 10: 609 (15%), 611 (5%), 618 (30%), and 620 (50%) (70,71).

• HD occurs in approximately 7% of patients with MEN2A.

• HD is almost exclude HD in always apparent shortly after birth; however, it is important to exclude HD in older patients.

MEN2B — clinical manifestations

1. **It appears at younger ages than MEN2A (infants and young children).**
2. **It has a higher mortality than MEN2A.**

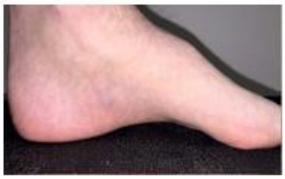
Approximately 75% of MEN2B cases are sporadic and affected patients have de novo RET mutations, while 25% of cases occur in families with previous or current manifestations of MEN2B.

Approximately 95% of patients with MEN2B have RET germline mutations in exon 16 (codon M918T) and fewer than 5% have RET germline mutations in exon 15 (codon A883F).

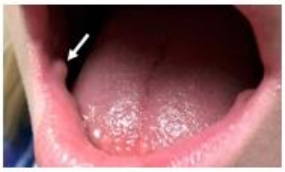
MEN2B

Unique physical appearance:	**Abdominal symptoms:**
➢ ophthalmologic abnormalities (inability to make tears in infancy, thickened and everted eyelids, mild ptosis and prominent corneal nerves) ➢ skeletal malformations (marfanoid body habitus, narrow long facies, pectus excavatum, high-arched palate, scoliosis, ganglioneuromatosis, *pes cavus*)	➢ bloating; ➢ intermittent constipation; ➢ diarrhoea; ➢ intestinal obstruction.

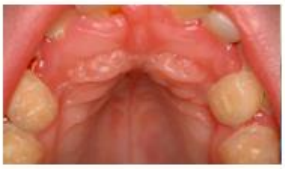
MEN2B - clinical manifestations



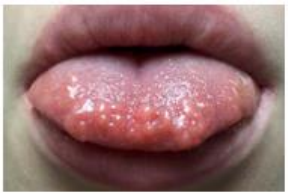
pes cavus

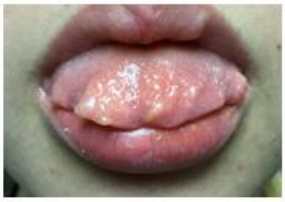


neuromas affecting the conjunctiva



high-arched palate





thickened lips and neuromas affecting the tongue

ptosis and everted upper eyelids

marfanoid body habitus

Endocrine-Related Cancer | F Castinetti *et al.* | Multiple endocrine neoplasia type 2B | 25:2 | T29–T39

HPTH in MEN2B

• The HPTH primarily occurs in patients with exon 11 RET codon mutations, most often in those with RET codon 634 mutations, and less frequently in patients with exon 10 RET codon mutations.

• In contrast to that occurring in families with MEN1, HPTH is mild and often asymptomatic.

• Enlarged parathyroid glands are occasionally found at the time of thyroidectomy for MTC in patients who are normocalcemic preoperatively.

Atypical MEN2B

• A rare group of patients have atypical MEN2B that develops around 20 to 30 years of age.

• The patients have double RET germline mutations appearing in tandem on the same allele and involving RET codon V804M and either RET codon Y806C, S904C, E805K, or Q781R.

• Evaluation of the tandem mutations by in vitro and *in silico* analysis provides information about their transforming ability (prediction scores). Using this methodology, each of the four reported double RET mutations had high transforming ability compared to the single mutations of the pairs, supporting the presence of a more aggressive MTC.

Relationship of Common RET Mutations to Risk of Aggressive MTC in MEN2A and MEN2B, and to the Incidence of PHEO, HPTH, CLA and HD in MEN2A

RET mutation	Exon	MTC risk level[a]	Incidence of PHEO[b]	Incidence of HPTH[b]	CLA[c]	HD[c]
G533C	8	MOD	+	+	N	N
C609F/G/R/S/Y	10	MOD	+/++	+	N	Y
C611F/G/S/Y/W	10	MOD	+/++	+	N	Y
C618F/R/S	10	MOD	+/++	+	N	Y
CF20F/R/S	10	MOD	+/++	+	N	Y
C630R/Y	11	MOD	+/++	+	N	Y
D631Y	11	MOD	+++	-	N	N
C634F/G/R/S/W/Y	11	H	+++	++	Y	N

K666E	11	MOD	+	-	N	N
E768D	13	MOD	+	-	N	N
L790F	13	MOD	+	-	N	N
V804L	14	MOD	+	+	N	N
V804M	14	MOF	+	+	Y	N
A883E	15	H	+++	-	N	N
S891A	15	MOD	+	+	N	N
R912P	16	MOD	-	-	N	N
M918T	16	HST	+++	-	N	N

Source: Wells SA Jr. (2018)

Take home message

1. Multiple endocrine neoplasias are extremely rare disorders.
2. Diseases like pituitary tumours, hyperparathyroidism or pheochromocytoma present in young patients must always raise the issue of a possible MEN.
3. Careful research of familial medical history is the first and the most important step in the establishment of MEN suspicion.

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I.4. Basis of medical genetics: Mendelian genetics, population genetics

Learning objectives

• Understand the structure of genetic material;
• Know and understand the mechanisms of variability;
• Understand the laws of heredity;
• Understand the basis of population genetics and the factors that modify the population equilibrium.

Summary

• DNA molecular basis of heredity
• Variability
• Genetic Testing
• Mendelian Genetics
• Genetics of populations
• Conclusions
• References

Introduction

• Genetics is the science of heredity and variability;
• The molecular substrate of genetics is the deoxy-ribonucleic acid;
• The variability is allowed by genetic re-combinations and mutations;
• The mutations could be identified using genomic or genic tests;
• The monogenic traits can be normal or abnormal and their genealogic transmission allows for the Mendel laws;
• In large populations, the gene's frequencies are in equilibrium (Hardy-Weinberg equilibrium);
• The factors that could modify the gene's equilibrium are genetic mutations, genetic drift, gene flow and natural selection.

Molecular basis of heredity:

deoxy-ribonucleic acid (DNA)

3 major functions.

- **Conserves** hereditary information
- **Express** hereditary information
- **Transmits** hereditary information

DNA conserves hereditary information

- Double chains macropolymer formed by **nucleotides**
- Information codified – unity of code:

CODON (3 contiguous nucleotides) ↔ **AMINO ACID**

- **GENOME** = totality of information from DNA.
- **GENE** = unity of hereditary information

"one gene → one phenotypic character"

- **MUTATION** (change of gene structure) → normal or abnormal gene variant.
- Mutations = **major causes** of disease or predisposition to disease
- DNA + proteines → **chromosomes (= fibers of chromatine)**
- **chromosomes** – morphologic substrate al of heredity;
  - somatic cells → ***46 chromosome*** (**2n** = diploid set);
  - sexual cells → ***23 chromosome*** (**n** = haploid set.
- chromosome = characteristic **succesion** of **genes**

Transcription + Translation

- Transcription - **copy** of gene information → molecule of **mRNA** (messenger):

- Translation – **decoding** genetic information of mRNA molecule → **peptide sequence**

DNA Replication + cell division

- DNA Replication - copy of genetic information of all DNA molecules → double the amount of DNA

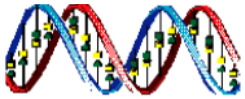
- Cell division – formation of two daughter cells
  - **Mitose** – somatic cells – transmission without changes;
  - **Meiosis** – germinal cells – transmission with genetic changes + **fertilization**

Mutations

changes in sequence
or
order of nucleotides from DNA

- accidental, permanent (±), hereditary
- Consequences:
  - neutral,
  - normale phenotypic variations,
  - disease
  - beneficial effects

DNA Mutations

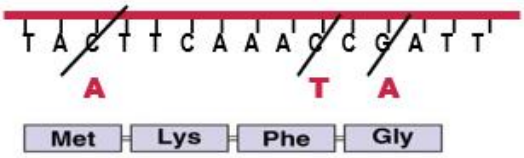


GENOMIC | CHROMOSOMIAL | GENE

CHROMOSOMAL ANOMALIES | PROTEIN ANOMALIES

CHROMOSOMAL DISEASES | MONOGENIC DISORDERS

HUMAN DISEASES 100% GENETIC

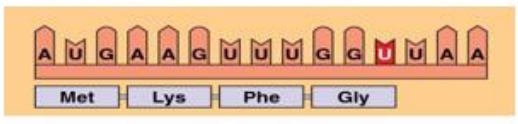


Gene

Protein

Silent mutation

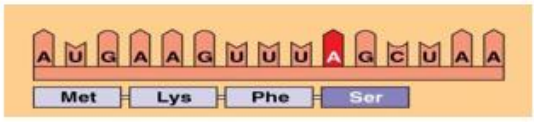
Without change in protein structure.



U instead C
other codon
→ same aa

Missesnse mutation

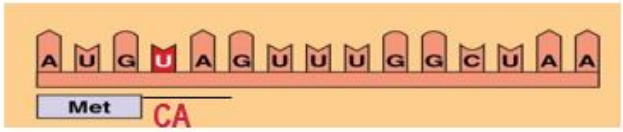
Change in protein structure.



A instead G
Other codon
→ other aa

Non-sens mutations

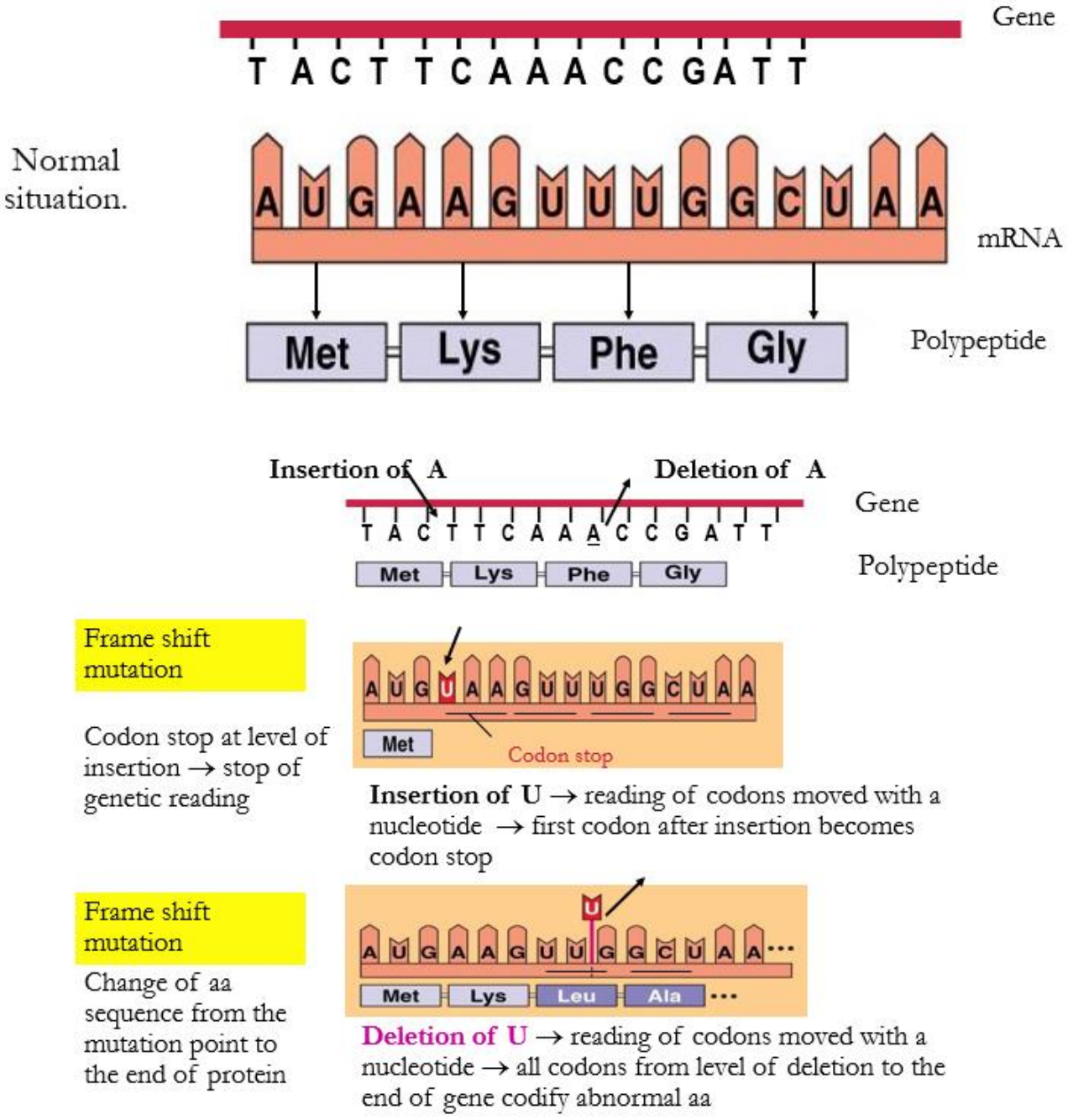
Shortening of amino acids sequence (prematurelly stop of genetic reading).



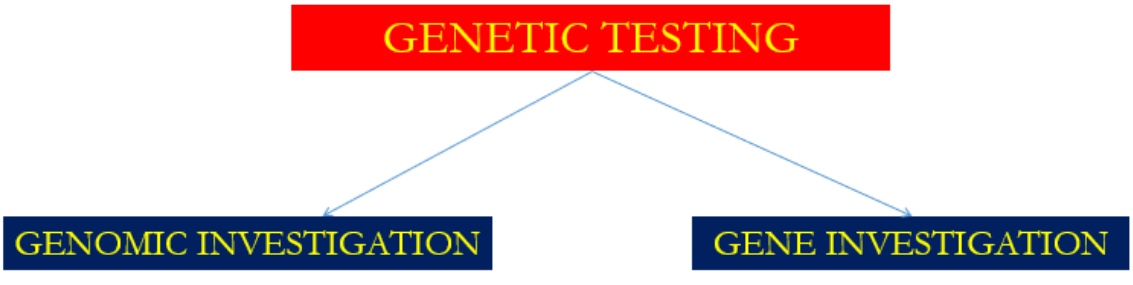
U instead A
codon stop
→ without
correspondence in aa

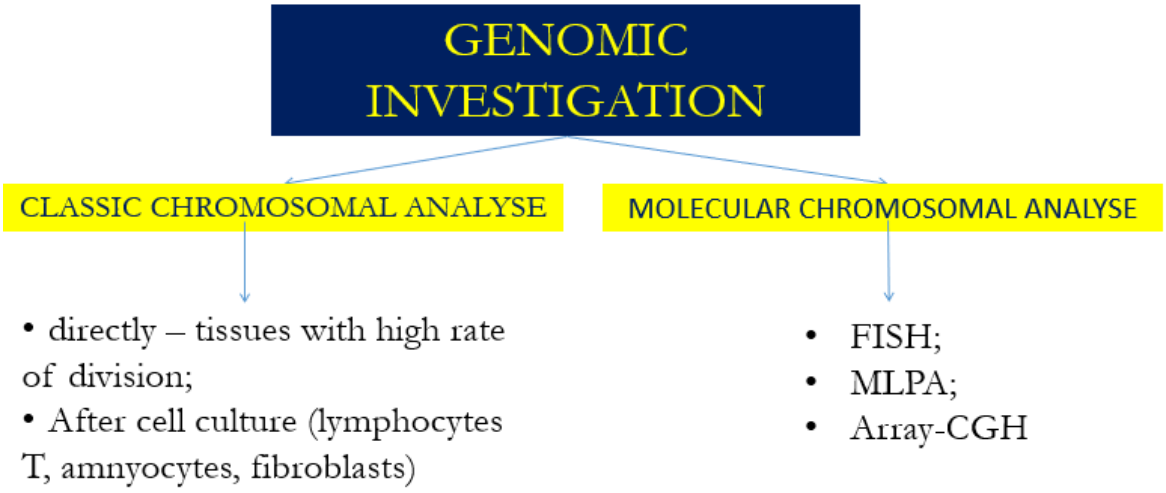
Insertions and deletions

1. Add or delete of one or more nucleotides in a gene.
2. Changes of mRNA and protein codified by gene.
3. Generate a **frame shift mutation** with different consequences in correlation with number of affected nucleotides: multiple of 3 or non/multiplu of 3



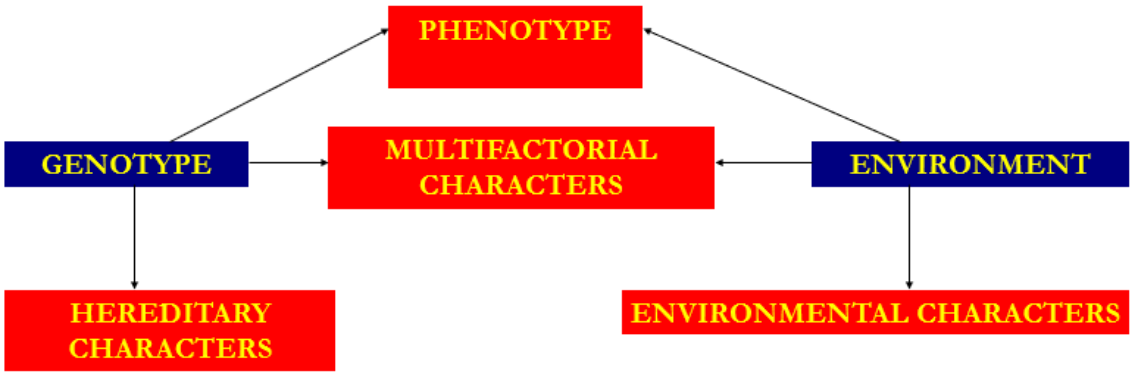
Campbell (3°éd.) — Figure 17.25 : 358





Etiology of phenotypic characters

Phenotypic characters:
hereditaries (genetic factors)
multifactorials (genetic factors + environment);
environment (environmental factors).



HEREDITARY CHARACTERS

Generated 100% by **genotype**;
• Could be:
- Species characters — only hereditaries;
- ex. **particular number of chromosomes** → reproductive barrier;
- normal hereditaries;
- abnormal hereditaries.

MONOGENIC DISEASES. MENDELIAN DISEASES

Monogenic diseases

Monogenic diseases are produced by **mutations** that interest **a single pair of alleles**.
Monogenic diseases presented **abnormal phenotypes**, generated by **hereditary factors**.
They represent o important part of genetic disorders and are numerous (> **9.500** disease).
Globally they are frequent - **1-2%** of new-borns have a monogenic disease
Hereditary transmission of these diseases is concordant with **Mendel laws**.
Identification of hereditary pattern of transmission in monogenic disorders request **familial history** and drawing of **pedigree**.

Autosomal dominant

A>n

AA, An

nn

X linked dominant

A>n

X^AX^A, X^AX^n X^AY

X^nX^n X^nY

MONOGENIC DISEASES

Autosomal recessive

N>a

aa

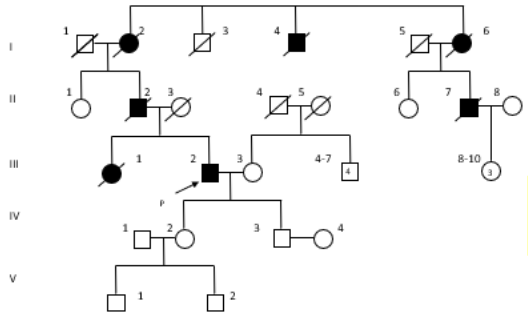
NN, Na

X linked recessive

N>a

X^aX^a X^aY

X^NX^N ,X^NX^a X^NY



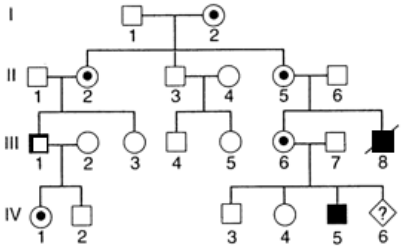
TYPE OF TRANSMISSION ?

AUTOSOMAL DOMINANT

A >n

DOMINANT
1. High frequency
2. Vertical transmission (parent to child)
3. Absent consanguinity
4. H + H → H

AUTOSOMAL
1. Transmission father – son
2. Affected father → affected daughters + healthy daughters
3. Healthy mother → healthy sons + affected sons



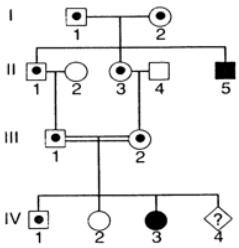
TYPE OF TRANSMISSION ?

X LINKED RECESSIVE

N >a

RECESSIVE
1. Small frequency
2. Horizontal transmission
3. Consanguinity often present
4. H + H → H + A

AUTOSOMAL
1. Healthy parents → affected children only boys
2. Healthy father → all daughters healthy



TYPE OF TRANSMISSION ?

AUTOSOMAL RECESSIVE

N >a

RECESSIVE
1. Small frequency
2. Horizontal transmission
3. Consanguinity often present
4. H + H → H + A

AUTOSOMAL
1. Healthy parents → abnormal daughters
2. Healthy father → affected daughters + healthy daughters

Monogenic diseases

Monogenic transmission = easy to recognise on pedigree if it is a regular transmission

In practice, many **difficulties of diagnosis and evaluation** of recurrence risk ← **variability** of phenotypic expression in gene activity (gene relationship and environmental influence):

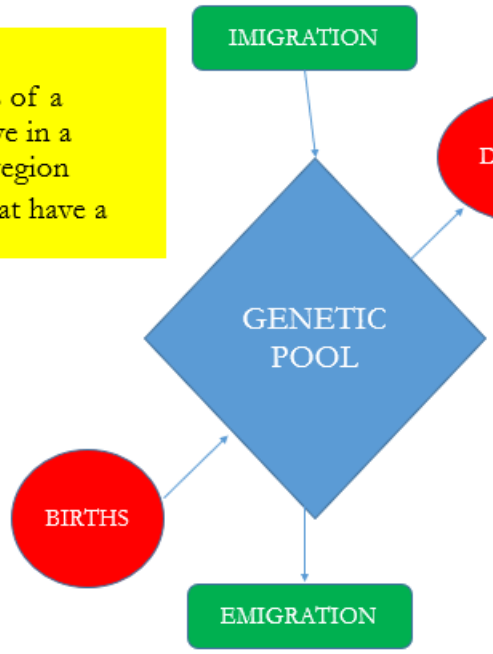
- **Incomplete penetrance,**
- **Variable expressivity ,**
- **pleiotropy,**
- **Genetic heterogeneity**
- **Organ specificity,**
- **Consanguinity.**

GENETICS OF POPULATION

Population - group of individuals who have a pool of genes, live in the same habitat and have a randomly reproduction (panmictic), without a selection of partners.

Biological population is a unit of **panmictic reproduction and an evolution unit**.

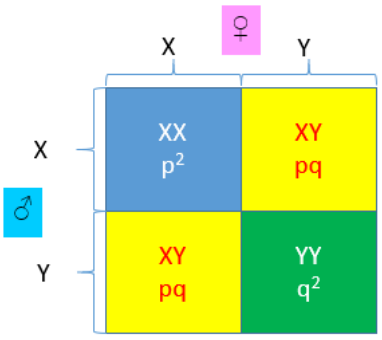
- Population
  - All individuals of a species that live in a geographical region
  - All peoples that have a *genetic pool*



- Genetic of population
  - Branch of genetics that studies processes of changes in gene frequency in a population
  - Analyses the genetic evolution of population

Genetic pool and Hardy Weinberg law

• Genetic pool — all alleles from a population

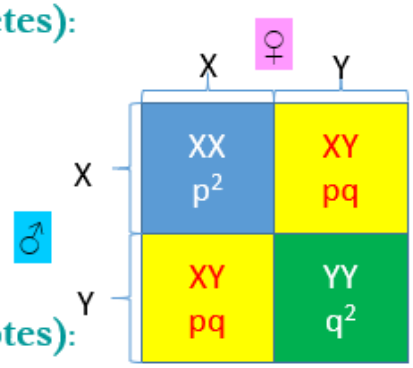


Hardy-Weinberg Law

• In a panmictic population in balance, without influences of migration, selection or mutation, for a locus that could be occupied by two alleles X and Y:

• Frequencies of alleles X and Y, noted p and q, are constant in succession of generations.

Frequencies of alleles (in gametes):



Frequency (X) = p

Frequency (Y) = q

⇒ $p + q = 1$

Frequencies of genotypes (in zygotes):

XX XY YY

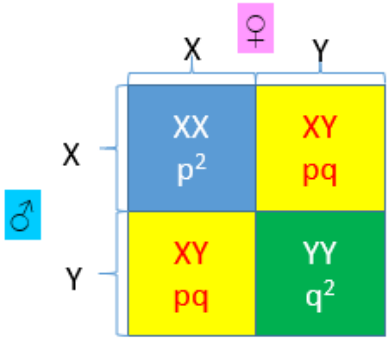
Frequency (XX) = $p_X \times p_X = p_X^2$

Frequency (XY) = $(p_X \times q_Y) + (q_X \times p_Y) = 2p_Xq_Y$

Frequency (YY) = $q_Y \times q_Y = q_Y^2$

⇒ $p^2 + 2pq + q^2 = 1$

Estimation of allelic frequency:



Genotypes: XX XY YY

Frequency : p^2 2pq q^2

⇒ Frequency of allele X:

$p = p^2 + ½ (2pq) = p^2 + pq$

⇒ Frequency of allele Y:

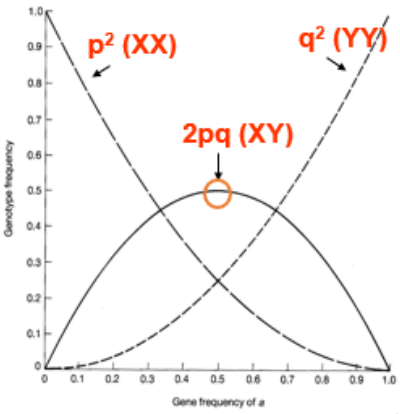
$q = q^2 + ½ (2pq) = q^2 + pq$

THE NEW MUTATIONS LEAD TO A NEW EQUILIBRIUM AFTER SEVERAL GENERATIONS

ALLELIC FREQUENCIES ARE IDENTICAL IN SUCCESSIVE GENERATIONS

FREQUENCY OF HETEROZYGOTE IS MAXIM 0,5

consequences of Hardy – Weinberg equilibrum



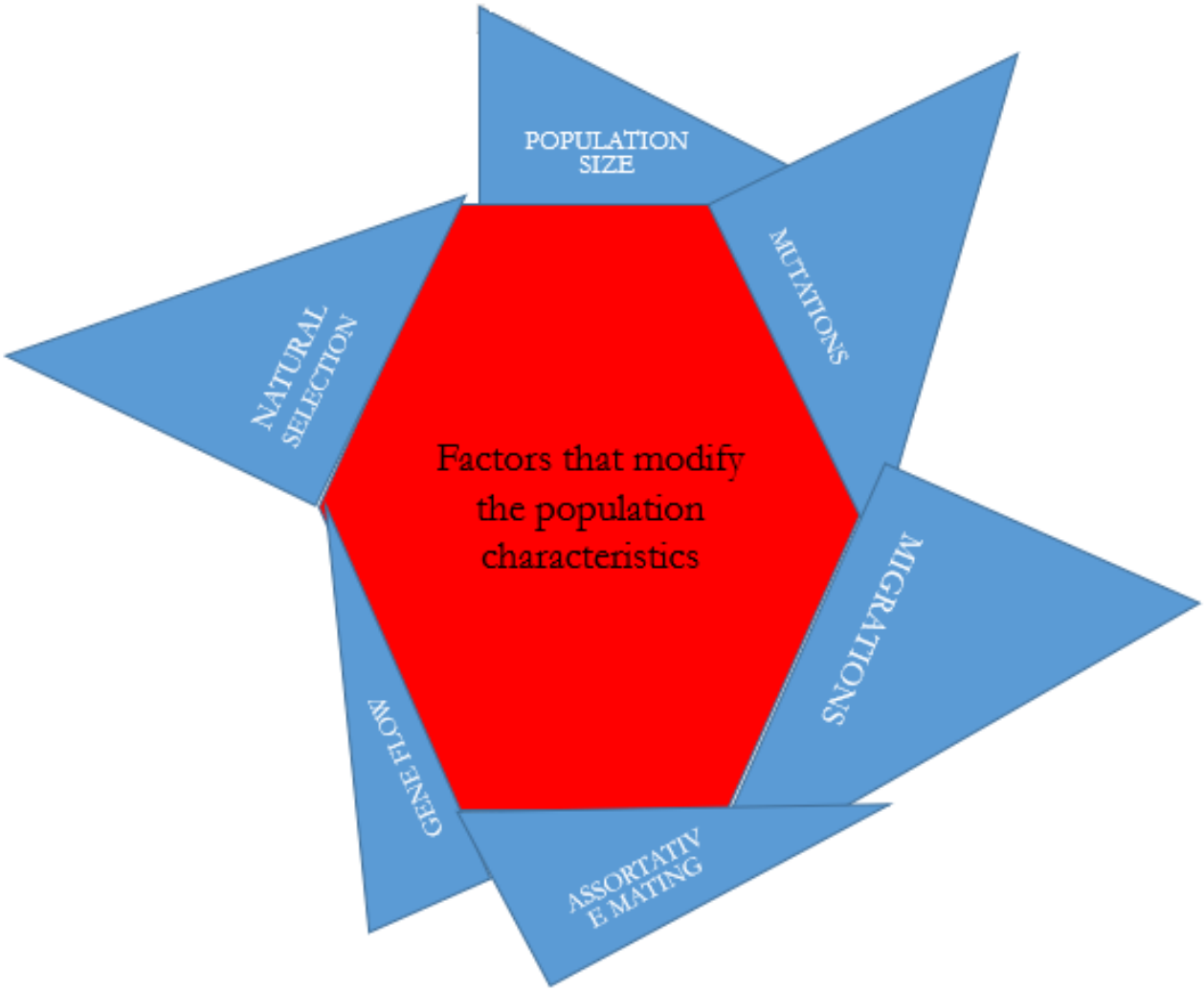
genetic variation
induced by random mutations

over time gene frequency is changed by genetic drift, gene flow and natural selection

REAL POPULATIONS

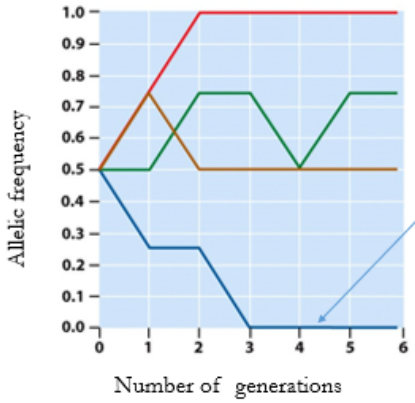
new variants have small phenotype effects and produced gradual changes

diversification improves the individuals' characteristics

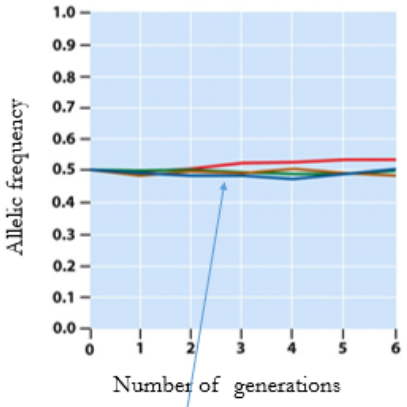


POPULATION SIZE

SMALL POPULATION

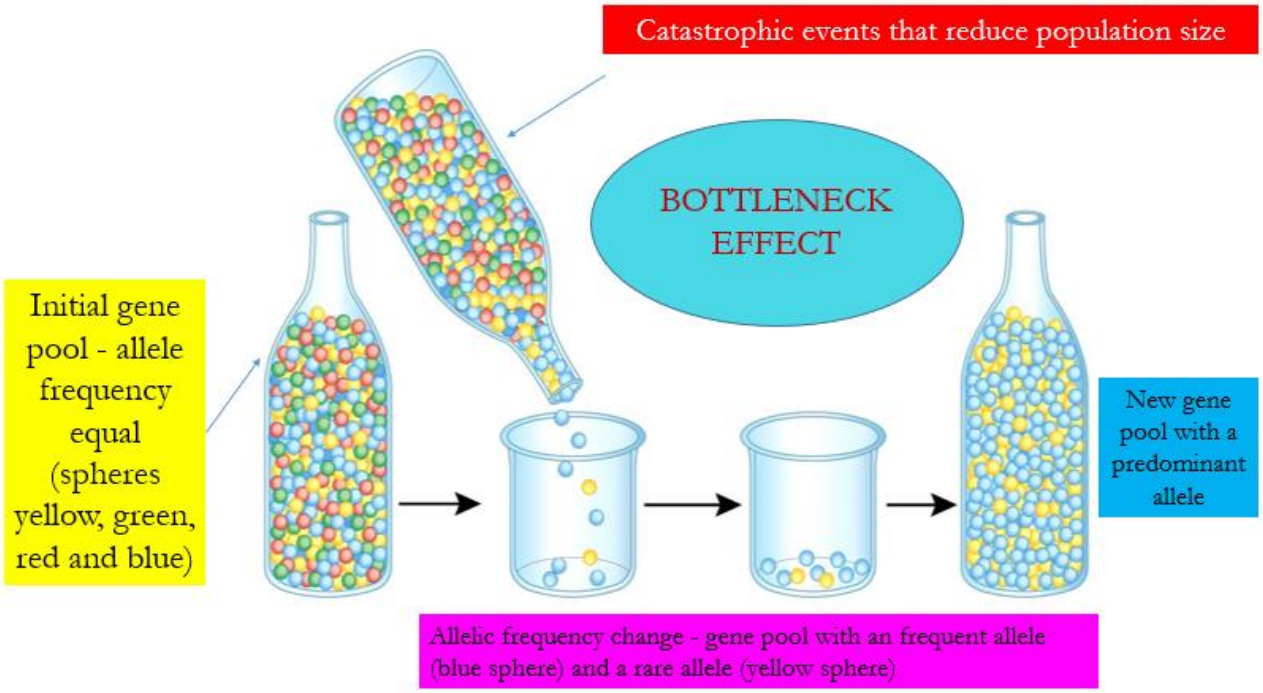


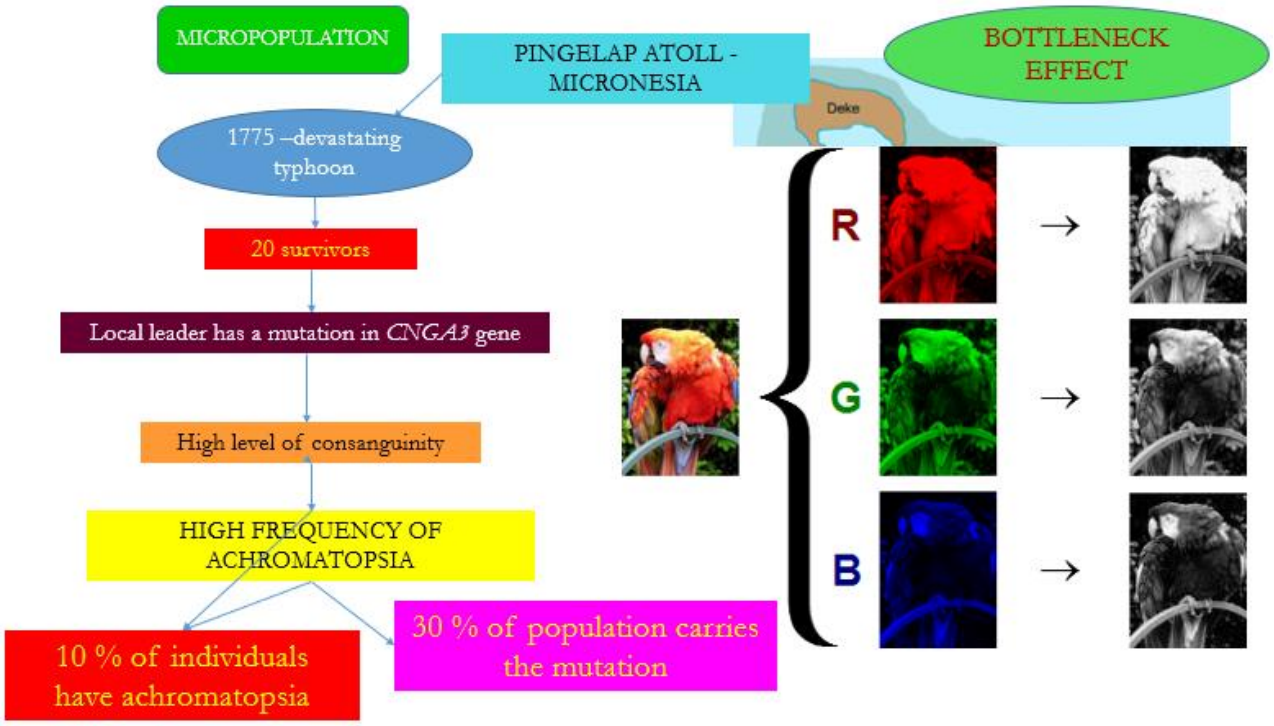
Certain allele extinction



Maintaining relatively constant the allele frequency

BIG POPULATION


Catastrophic events that reduce population size
BOTTLENECK EFFECT
Initial gene pool - allele frequency equal (spheres yellow, green, red and blue)
New gene pool with a predominant allele
Allelic frequency change - gene pool with an frequent allele (blue sphere) and a rare allele (yellow sphere)


MICROPOPULATION
PINGELAP ATOLL - MICRONESIA
BOTTLENECK EFFECT
Deke
1775 –devastating typhoon
20 survivors
Local leader has a mutation in CNGA3 gene
High level of consanguinity
HIGH FREQUENCY OF ACHROMATOPSIA
10 % of individuals have achromatopsia
30 % of population carries the mutation
R
G
B

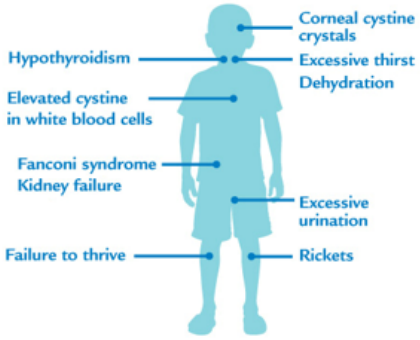
MICROPOPULATION

NEPHROPATIC CYSTINOSIS

FOUNDER EFFECT

AUTOSOMAL RECESSIVE LYSOSOMAL STORAGE DISEASE

GENERAL POPULATION
INCIDENCE – 1/100,000
FREQUENCY OF HETEROZYGOTES Na – 1/150



AMISH POPULATION from ONTARIO REGION
INCIDENCE – 1/144
FREQUENCY OF HETEROZYGOTES Na – 1/6

religious isolated community

common origin– small number of common ancestors

FOUNDER EFFECT

CANCER

THE MUTATION IS CONSERVED IN POPULATION BECAUSE THE ONSET OF DISEASE IS AFTER REACHING REPRODUCTIVE AGE

BREAST CANCER– MUTATIONS BRCA1/2

MELANOMA – MUTATIONS CDKN2A

LYNCH SYNDROME – MUTATIONS MMR

50% OF CASES OF LYNCH SYNDROME FROM FINLAND – ONLY TWO MUTATIONS IN MLH1 GENE

> 50 MUTATIONS WITH FOUNDER EFFECT IN DIFFERENT POPULATIONS

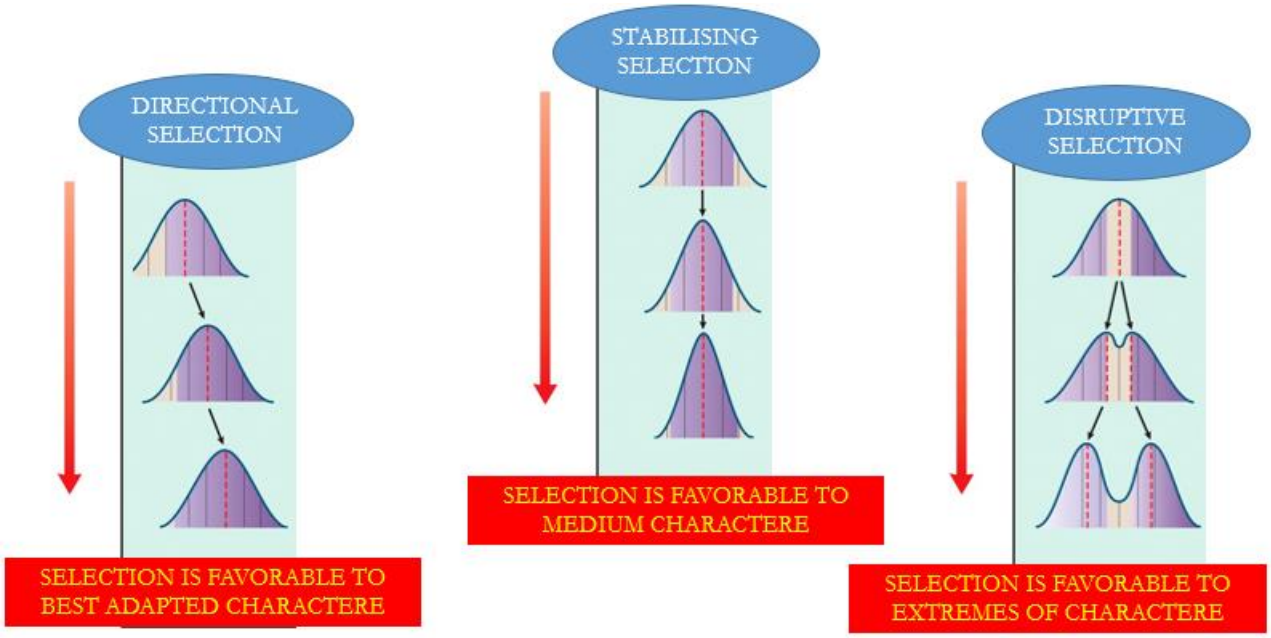
NATURAL SELECTION

FAVOURABLE

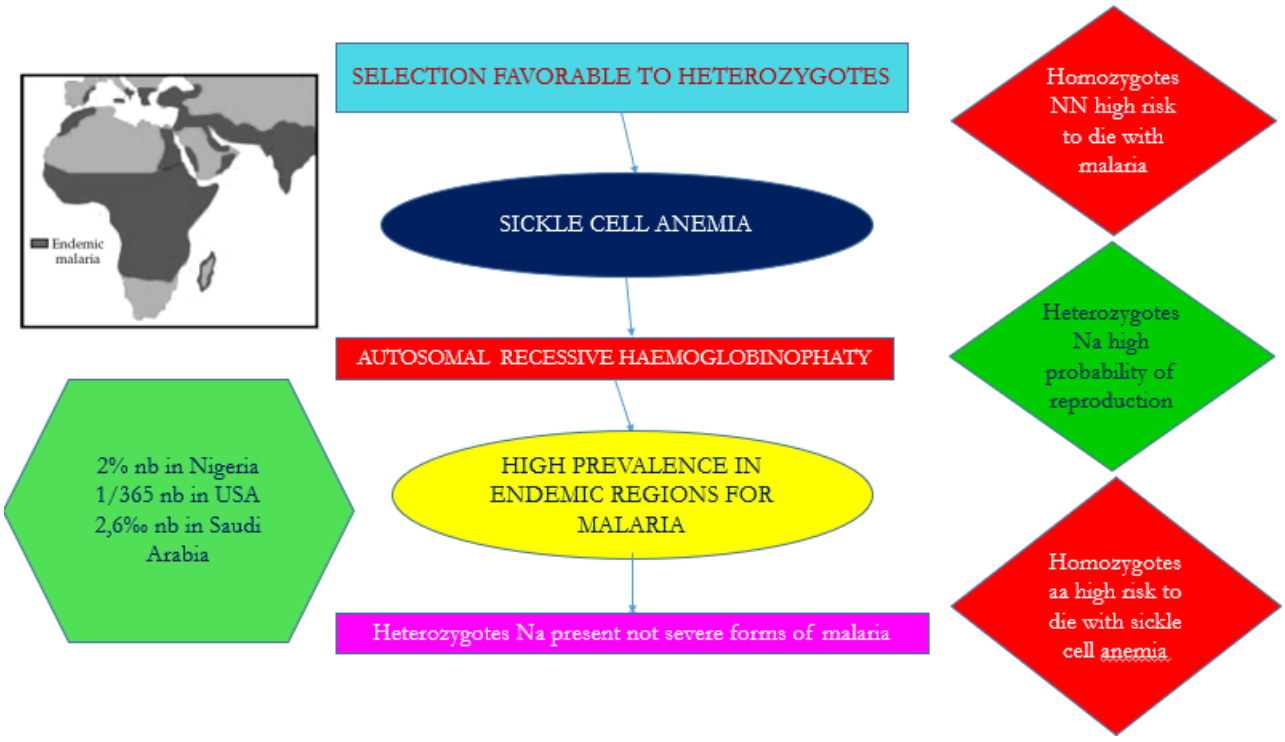
CARRIERS OF MUTATION HAVE BIG CHANCES TO SURVIVE AND REPRODUCTION

NONFAVORABLE

CARRIERS OF MUTATION HAVE SMALL CHANCES TO SURVIVE AND REPRODUCTION



TYPES OF SELECTION



Change of mutation effect by modification of environmental conditions

85% from patients with **hereditary hemochromatosis** (HE) in Europa have the same mutation (in codon 282 from gene *HFE*) linked with allele *HLA A3*

Mutation relative new (< 10.000 years)

Selective advantage for heterozygotes Na – low risk for anemia

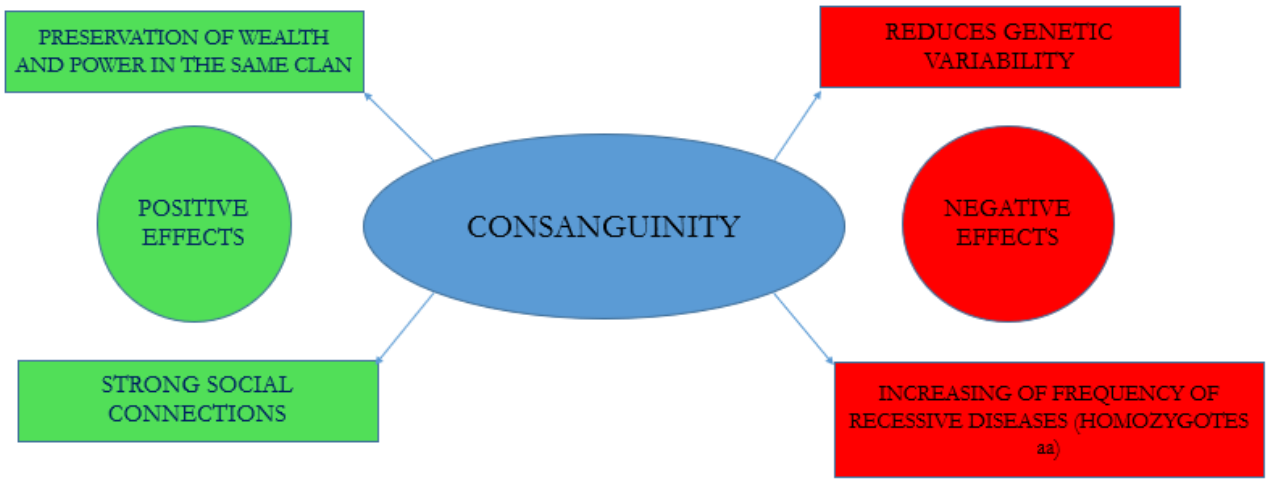
Limited effect of disease in geographic regions with iron deficit in alimentation

Severe cases in homozygotes aa after iron supplementation of flour

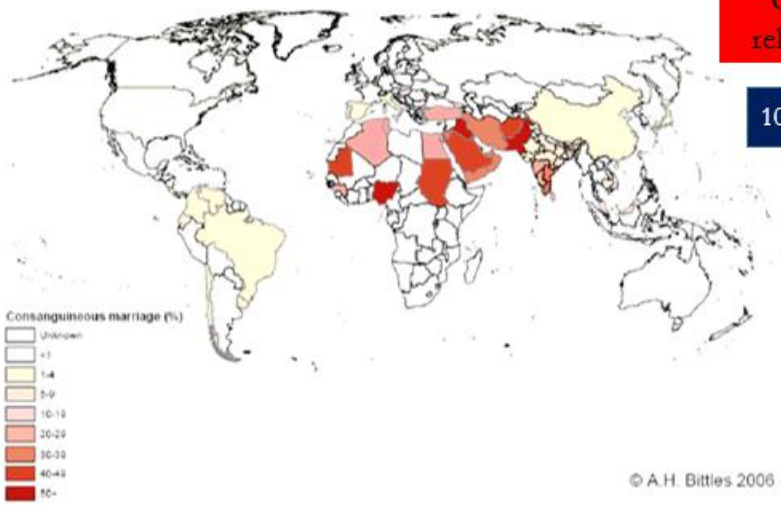
Hereditary hemochromatosis

- Hereditary disease (AR) frequent in Western Europe, 1:400
- High intestinal absorbtion of iron produces accumulation of iron in liver (cirrosis), heart (heart insufficiency), pancreas (diabetes), skin (bronze pigmentation), endocrine glands.
- Gene *HFE is located on* chromosome 6 (6p) linked with gene HLA A

Assortative mating



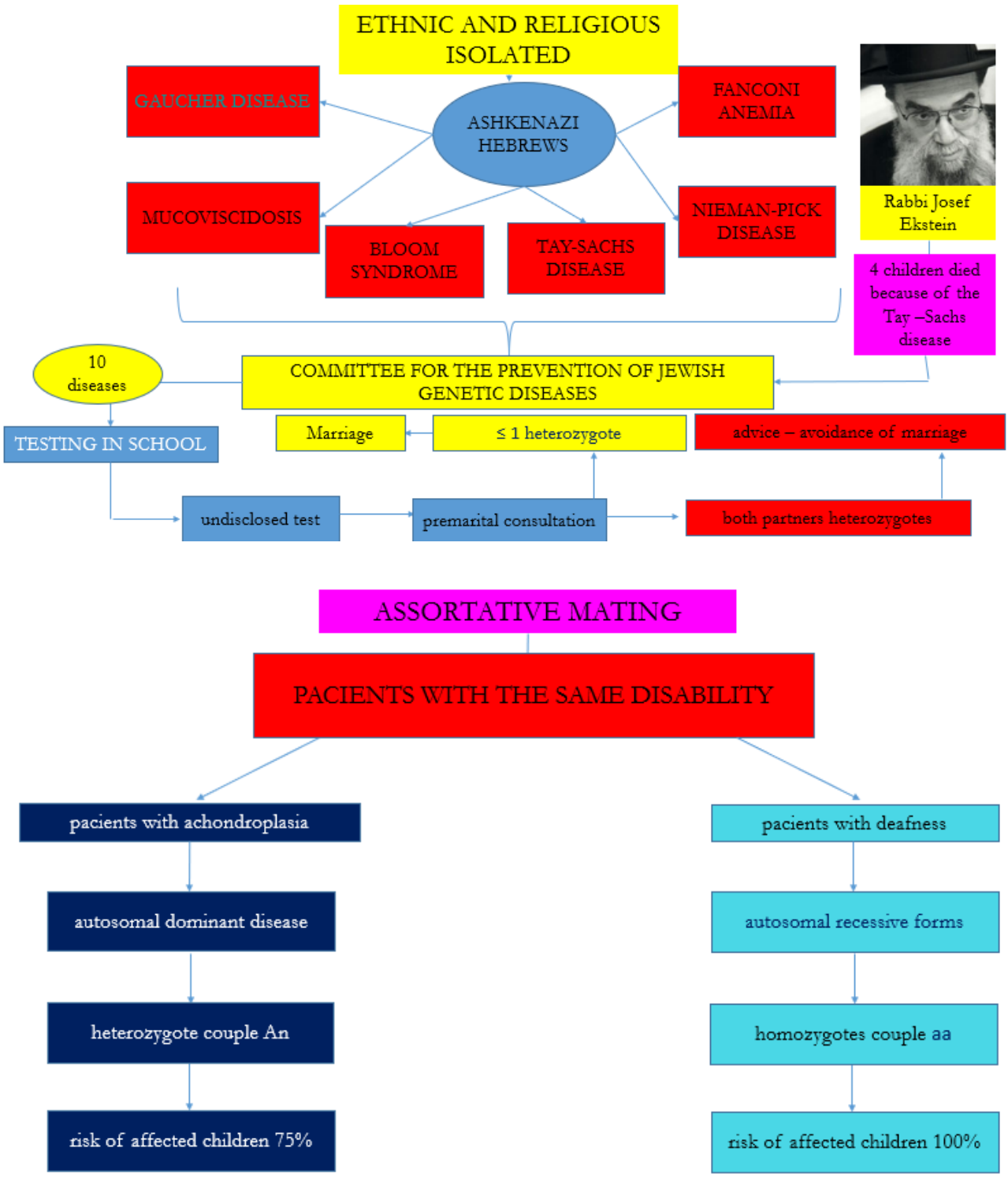
Consanguinity in world



Consanguinity – marriage between relatives (close to 2nd degree cousins)

10,4% of couples are consanguines in world

Reduction of consanguine marriages by globalisation



Conclusions

• ADN is a molecular substrate of heredity;

• ADN has 3 function: stock, express and transmit hereditary information;

• Genetic information is conserved under codified form, unity of cod is codon or triplet (three adjacent nucleotides);

• Expression of genetic information is made by two successive processes: transcription and translation; • Transmission of hereditary information requests DNA replication, cell division and fertilisation;

• Variability is ensemble of events that generate differences between individuals, between population and between species;

• Variability is allowed by genetic recombinations, genetic mutations and populational migrations • Mutations are divided in: gene mutations, chromosomal mutations and genome mutations;

• The most frequent mutations are gene mutations;

• Gene mutations are divided in: nucleotide substitutions and frame shift mutations (deletions and insertions);

• Testing of genetic diseases requests genomic and gene testing methods;

• Genomic testing is allowed by classic chromosomal analyse and molecular chromosomal analyse (FISH, MLP A, array-CGH);

• Gene testing is allowed by PCR, Sanger sequencing and NGS;

• Phenotypic characters are divided in: hereditaries, multifactorials and environmentals;

• Hereditary phenotypic characters (normal or abnormal) are transmitted monogenic in concordance with Mendel laws;

• Mendel formulated two universal laws: segregation law and independent assortment law.

• Abnormal monogenic hereditary characters are represented by monogenic diseases: dominant or recessive, autosomal or X-linked.

• Population is a panmictic reproduction unity that have a gene pool • In ideal conditions, the population's gene pool is in equilibrium.

• Hardy-Weinberg law — when a locus could be occupied by two alleles, gene frequencies and genotypes frequencies are constant if population size is big, the marriages are randomly, gene flow is absent, natural selection is also absent and are not produced new mutations;

• In reality, for the small populations Hardy-Weinberg equilibrium is modified by the bottleneck effect and by the founder effect;

• Natural selection could favourable or nonfavourable for a certain genotype and it presents changes during the evolution of species;

• Assortative mating are represented by consanguineous marriages, marriages between individuals that belong of the same social group, marriages between individuals belonging to the same population group (in geographic and religious isolated communities) and by marriages between individuals with the same disability;

• Consanguineous marriages increase the likehood of children to be homozygotes for a recessive mutation and have a high incidence in isolated communities;

• Assortative mating between individuals with same disability is associated with a high risk of abnormal children that have the same diseases like theirs parents.

Take home message

• Genetics is the science of heredity and variability;
• Molecular substrate of genetics is DNA;
• Changes in the DNA structure allow variability;
• In large populations, the alleles' frequency for a certain locus are in equilibrium.

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I.5 The biological and molecular basis of the hereditary monogenic and multifactorial risk of cancer

Learning objectives

✓ Monogenic inheritance pattern
✓ Multifactorial inheritance pattern.

Introduction

✓Genetic susceptibility to cancer is divided into **monogenic** and **polygenic predisposition, completed by environmental factors.**

✓**Monogenic diseases** are generated by constitutional mutations of single genes, with a Mendelian inheritance, which leads to an increased susceptibility to various cancer types

✓In **polygenic diseases**, is present a familial aggregation of affected individuals without a specific type of inheritance; in these cases are presented mutations in genes with "moderate risk" or some DNA polymorphisms that interact with environmental factors and increase the risk of cancer development in people with these alterations.

Monogenic and multifactorial inheritance patterns in cancer predisposition

Monogenic inheritance pattern

- classic Mendelian inheritance patterns;

Multifactorial inheritance pattern

- multiple genes, often together with environmental factors.

Monogenic inheritance pattern

Most monogenic diseases - caused by mutations that reduce the functionality/stability of a single protein by altering its three-dimensional structure, like:

- **point mutations** (e.g., alterations in single nucleotides that change the amino acid sequence)
- **insertions/deletions** in the DNA sequence that encodes the protein
- **changes in the non-coding DNA** that interfere with gene splicing

Multifactorial inheritance pattern

Complex features from **multifactorial pattern:**

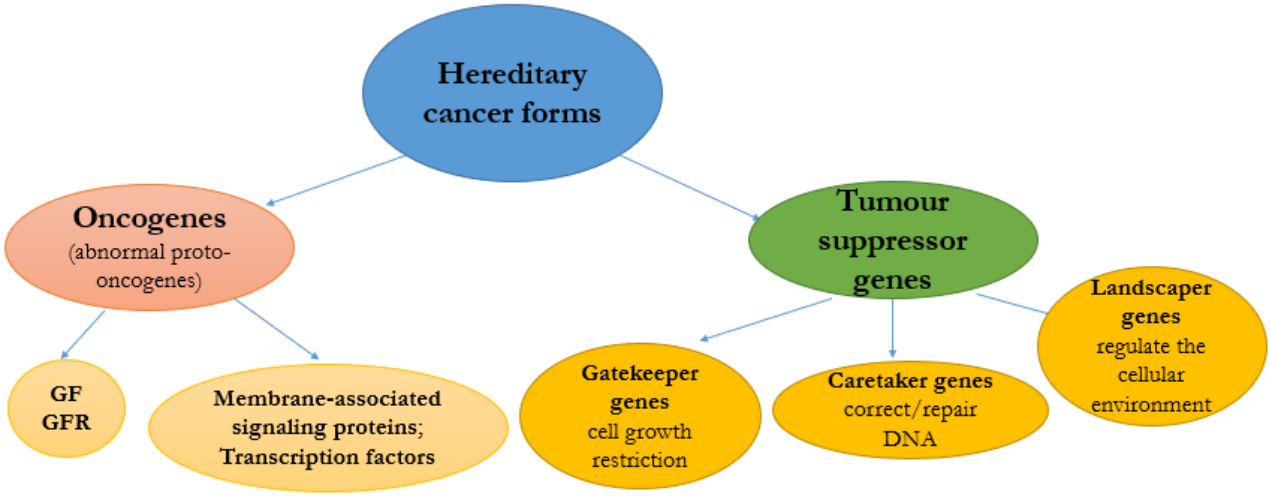
- variation of multiple genes and their interaction with behavioural and environmental factors;
- do not readily follow predictable patterns of inheritance.

Distinction between **monogenic** and **complex traits:**

- monogenic features can be influenced by variation in multiple genes - "modifier genes"
- complex traits can be predominantly influenced by variation in a single gene

Monogenic predisposition to cancer

✓ diseases caused by **constitutional mutations of single gene**



Knudson theory of hereditary carcinogenesis

• hereditary tumours - a combination of inherited germ-line mutation with an acquired somatic-cell mutation

• Somatic mutations — the key mechanism — "target" genes — critical sites of mutations in carcinogenesis:

- oncogenes — *ras, myc*

- tumour suppressor genes/antioncogenes — recessive genes (retinoblastoma, familial polyposis coli and colon cancer, familial breast cancer) — the most frequently mutated genes in human cancers — strong predisposition for heterozygosity for germ-line mutations

• "**two-hit hypothesis**" - loss of two alleles of a tumor-suppressive gene/antioncogene

• The somatic mutational hypothesis for the origin of cancer implies:

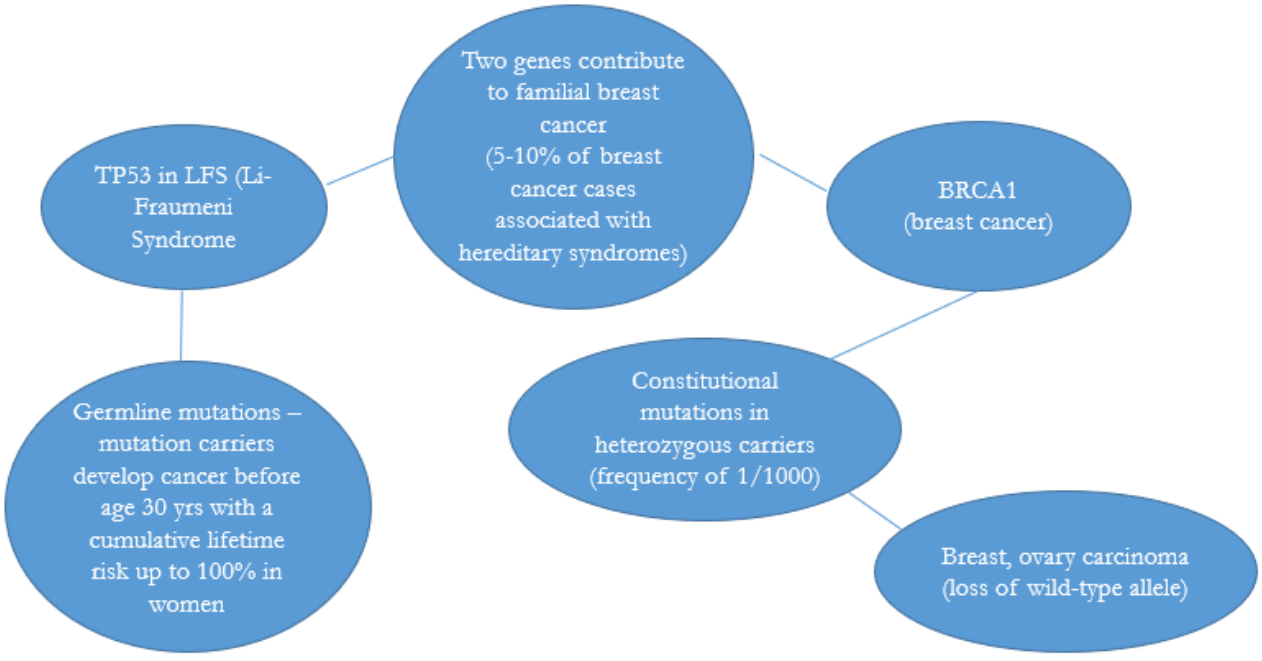
(1) spontaneous mutations produce an irreducible cancer "milieu";

(2) this background can be increased by mutagen agents that can modify the host genome by addition or deletion of genetic material;

(3) spontaneous or induced mutations should establish the elevated risk of cancer;

(4) inherited initiating mutation should be considered susceptible for cancer.

Familial breast cancer



Monogenic predisposition to cancer

✓ Pedigrees of monogenic diseases with **autosomal dominant inheritance** — occurrence of the disorder in all generations (**vertical transmission**), among men and women, among almost 50% of the relatives, excepting:

1. **"de novo" germline mutations** — disease absent among ancestors and siblings of the proband (individuals with genetic counselling); subsequent generations can be affected
2. **mosaic mutation -** present only in some of the tissues, arise in the fetus "de novo" during pregnancy; single individuals in the family are affected; mutation can be inherited only if present in sex cells;
3. **"low penetrance" mutations** (penetrance represents the proportion of mutation carriers who develop cancer) - single individuals only are affected
4. **mutations predisposing to disease occurring among one gender only** - e.g. only women with BRCA1 gene mutation will develop ovarian cancer. Men can transmit the mutation to their offspring.
5. **small families few relatives**.

Evaluation of the pedigree and clinical data of families with aggregations of cancers should exclude phenocopies (accidental malignancy not related to mutation responsible for the aggregation of malignant tumour).

Polygenic predisposition to cancer

• **polygenic inheritance** — usually single individuals are affected in the family;

• **panels of DNA mutations/polymorphisms**

- identified in breast cancers, colorectal cancers, malignant melanomas, ovarian and prostate cancers;

- associations of "moderate risk" gene mutations, polymorphisms and influence of environmental factors - significantly increase the risk of cancer development in individuals with these alterations.

Proposed classification of genetic diseases combining "gene-centric" model with "pathway-centric" model

The mechanisms and the proposed designation of genetic diseases

Mechanism	Proposed designation
a single gene mutation associated with a single alterated pathway phenotype	monogenic-monogryphic disease
a single gene mutation with a pathogenetic cascade of altering pathways	monogenic-polygryphic disease
chromosomal abnormalities altering several pathways	chromosomal-oligogryphic diseases
rearrangement of a chromosomal locus containing several genes, two of which alter two pathways	chromosomal-digryphic disease
mutations in different genes involved in a single pathway	monogenic-homeogryphic diseases
alterations to shared pathways due to the complex interaction between genetic and environmental factors	polygenic/multifactorial-homeogryphic diseases
alteration to a specific pathway, because of a variety of single-gene mutations, chromosome abnormalities and genetic-environmental interactions	polygenic/multifactorial-monogryphic disease

gríphos (Greek) = pathway (in the context of molecular biology)

Take home message

- Most cancers have a multifactorial etiology, due to a combination of different genetic and environmental factors.
- Very few common cancers have a strong inherited susceptibility.
- There is a minority of monogenic cancer with mendelian inheritance which confer an a wide range of cancers.
- Mutations in tumour suppressor genes/antioncogenes represent the most influential genetic change that lead to cancer
- There is a susceptibility of heterozygous individuals for DNA repair deficiencies.

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Learning objectives

At the end of this presentation, learners will be able to:

• define the scope, benefits and limits of molecular oncogenetic diagnostic • describe the most frequently used laboratory methods, their advantages and disadvantages;

• evaluate individual risk criteria and identify the main population target groups;

• understand the complexity and cost/effectiveness of molecular oncogenetic diagnostic;

• evaluate the consequences of the results for the patients and for their families;

MOLECULAR ONCOGENETIC DIAGNOSTIC - COMPLEXITY AND RESPONSIBILITY

- **Why is it so expensive ?**
- **Why does it last so much time ?**
- **What is the risk of errors ?**
- **Why human responsibility is so important ?**

Introduction - Cancer in general population

• Breast cancer — most frequent cancer in women and 2nd cause of mortality (due to cancer)

• Ovarian cancer — 4th cause of mortality (due to cancer) in women

• Colorectal cancer — third most frequent cancer worldwide

• Hereditary cancer (breast/ovarian, colorectal)

— 5 - 10% of all cancer cases;

— 25% of cases diagnosed before age 30;

• Small proportion but important target group: early intervention can save lives !

•Accent on:

✓ Early detection

✓ Prevention

✓ Prophilaxy

Introduction - Cancer risk factors

POLYMORPHISMS

Sporadic multifactorials : 60 - 85%
Lifestyle: 66%
Age
Tobacco
Reproductive life and endocrine hormones
Alimentation: 30-60%
Sedentarism: 10-30% ?
Obesity
Environment: 2% ?
Stress
Ionisant radiations
Chemical toxics (air, water, food)
Infections: 16-25 % (2/3 virus, 1/3 bacteria, few parasites)
Immune deficiencies

MUTATIONS

5-10 % hereditary monogenic multifactorial predispositions

10-30 % hereditary polygenic multifactorial susceptibilities

FAMILIALS

Although all cancer are GENETIC (caused by genetic mutations), only a small proportion are HEREDITARY.

Oncogenetics

Hereditary monogenic susceptibility to cancer
Inherited gene mutation = Important lifetime cancer risk

The most common syndromes presenting a hereditary risk:

- **HBOC (Hereditary Breast and Ovarian Cancer):**
  Principal genes involved: *BRCA1, BRCA2*
  Other possible genes: *CHEK2 PALB2, ATM, PTEN, RAD51C, CDH1, STK1,* etc...
- **HNPCC (Hereditary Non-Polyposis Colorectal Cancer):**

  Principal genes involved: MMR (DNA mismatch repair genes)
  ***(MSH2-* 35%, *MLH1-* 25%, *MSH6-* 15%, *PMS2, EPCAM*)**

- **FAP (Familial adenomatous polyposis):**

Principal genes involved: *APC*

Hereditary breast cancer (5-10%)

~ 1/3 of cases caused by 2 genes: BRCA1 (17q21) and BRCA2 (13q12.3)

HEREDITARY PREDISPOSITION = GERM-LINE *BRCA* MUTATIONS

BRCA genes are responsible for up to 90% of hereditary breast and ovarian cancer (HBOC)

Thousands of deleterious mutations already reported in reference database:
- NCBI ClinVar
- Breast Cancer Information Core (BIC) mutation database
- University of Maryland (UMD) database
- Leiden Open Variation (LOVD) database

Oncogenetics

ONCOGENETICS - medical and diagnostic follow-up op patients and of their families, which present a hereditary monogenic risk of cancer.

Hereditary risk factor: positive and negative predictive value.

Oncogenetics demonstrated its efficiency in the western world more than 20 years ago, in terms of incidence and prognosis of breast/ovarian/colorectal cancer.

Prevention — major economic benefit for health systems.

Oncogenetics can save lives !

Keywords:
- Oncogenetics Department
- Personalized Follow-up Oncogenetic Program (Personalized Medicine)
- Interdisciplinary Consultancy Team
- Prevention
- Early detection
- Prophylaxis

Who needs to attend an oncogenetic consultation ?

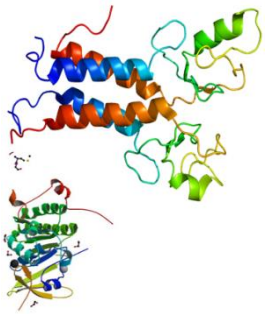
COMMON SITUATIONS — autosomal dominant risk

- 3 or more cancer cases within the same family line;
- 2 or more cancer cases within a small family line or for a rare cancer;
- 1 or 2 cancer cases in young persons (early-onset);
- Several cancers at the same patient;

INDICATIONS FOR CONSULTATIONS — simple situations

- Ovarian cancer at a woman < 70 years;
- Colorectal cancer at a patient < 40 years;
- Breast cancer in a woman < 35 years;
- Breast cancer in men;
- Medullary or Basal-like/Triple negative breast cancer;
- Digestive polyposis;
- Multiple cancers;
- Cancer in a monozygous twin;

The BRCA genes: HBOC



BRCA1 **(17q21): 5592 coding bp, >100 kb genomic DNA, 1863 amino acids (220 kDa) Tumour Suppressor,**

Transcriptional activator, DSB repair on DNA, Cell cycle regulation, Genome integrity maintain (*Caretaker)*

BRCA2(13q123):10257 **coding bp, >70 kb genomic DNA, 3418 amino acids (380 kDa) Functional similarities with BRCA1, DSB repair on DNA, Cell cycle regulation, Genome integrity maintain (*Caretaker*)**

MMR genes: HNPCC

MSH2 (2p21): 3145 coding bp, >80 kb genomic DNA, 934 amino acids (105 kDa)
Mismatch lesion repair on DNA, within a complex Mismatch repair (MMR) proteins, MutS Complex with MSH3 and MSH6, MutL Complex with MLH1;

MLH1 (3p22.2): 2524 coding bp, ~60 kb genomic DNA, 756 amino acids (85 kDa);
Mismatch lesion repair on DNA, within a complex Mismatch repair (MMR) proteins. MutL Complex with MSH1;

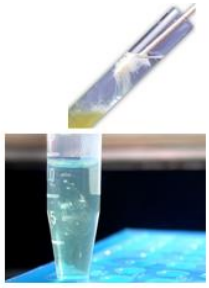
MSH6 (2p16.3): 4330 coding bp, ~25 kb genomic DNA, 1360 amino acids (160 kDa);
Mismatch lesion repair on DNA, within a complex Mismatch repair proteins. MutS Complex with MSH2 and MSH3;

PMS2 (7p22.1): 5093 coding bp, ~45 kb genomic DNA, 862 amino acids (96 kDa), partner of MLH1;

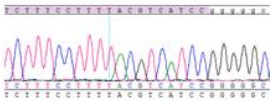
EPCAM (2p21): 1547 coding bp, ~17 kb genomic DNA 314 aminoacids (35 kDa) — located upstream of MSH2; novel mutational mechanism causing Lynch syndrome by epigenetic inactivation of

... For **FAP**, there is the **APC gene** (5q21-q22), 8538 coding bp, >108 kb genomic DNA, 2844 amino acids (310 kDa), Tumour Suppressor, Transcriptional activator, Microtubules stabilisation;

Molecular oncogenetic diagnostics

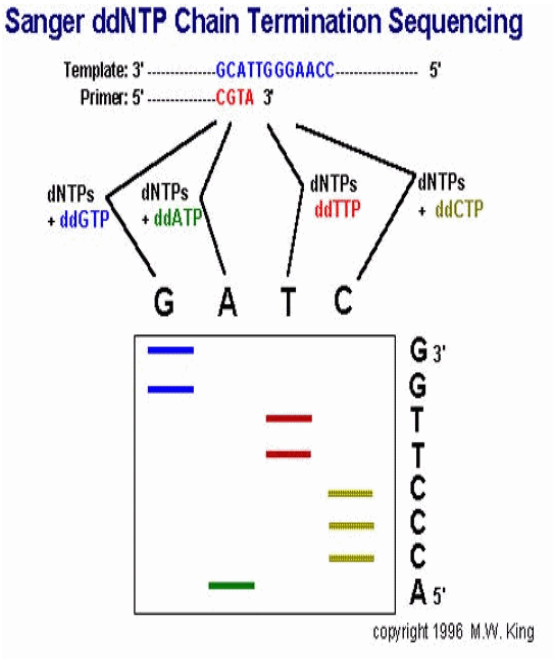


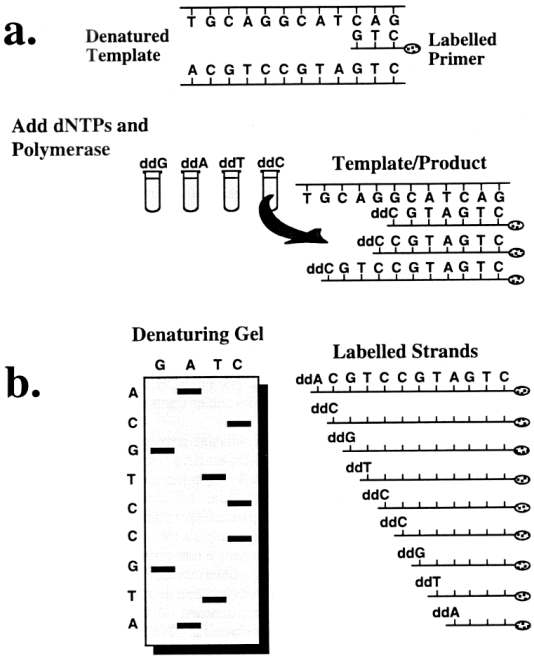
1. **DNA EXTRACTION**
2. **MUTATIONS PRE-SCREENING**
3. **PCR AMPLIFICATION (REGIONS OF INTEREST)**
4. **PURIFICATION OF PCR PRODUCTS**
5. **SEQUENCING OF AMPLICONS**
6. **PURIFICATION OF SEQUENCING PRODUCTS**
7. **CAPILLARY ELECTROPHORESIS**
8. **INTERPRETATION OF THE RESULTS (most important !!!)**

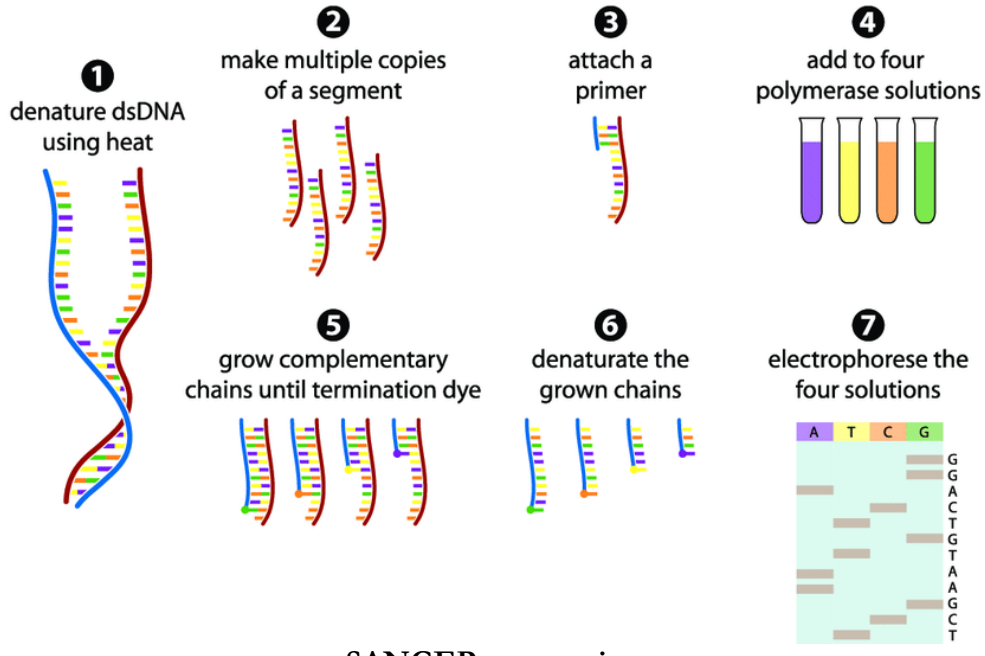


Oncogenetics - how to detect a mutation?

COMPLETE GENE SEQUENCING is the correct and exact method for diagnosis.

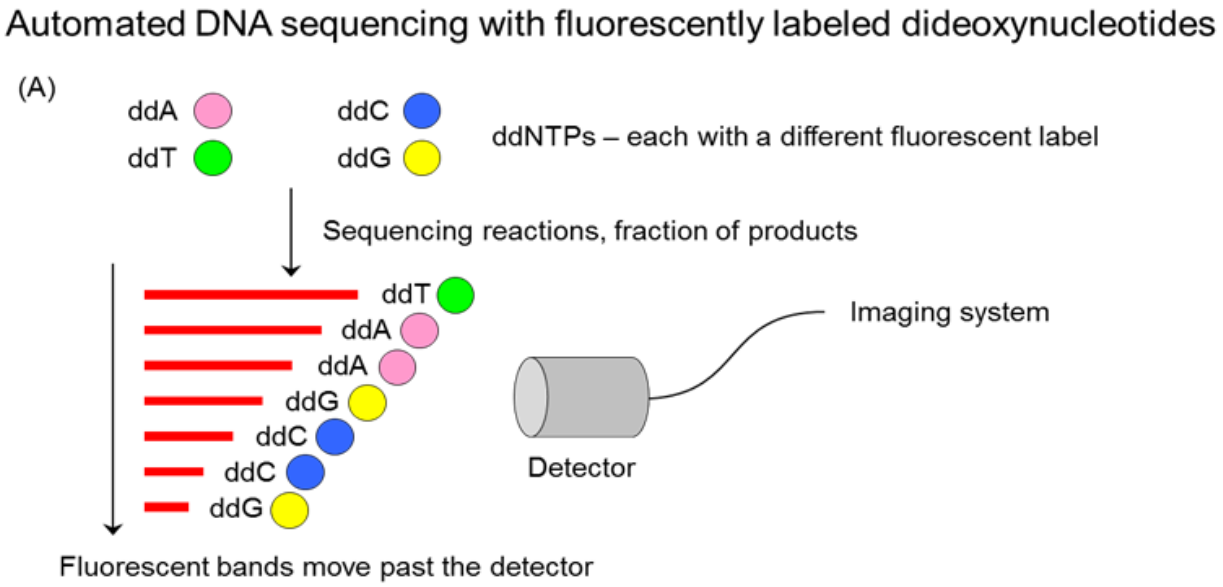




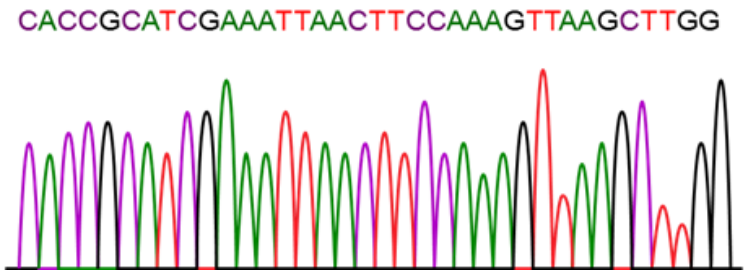


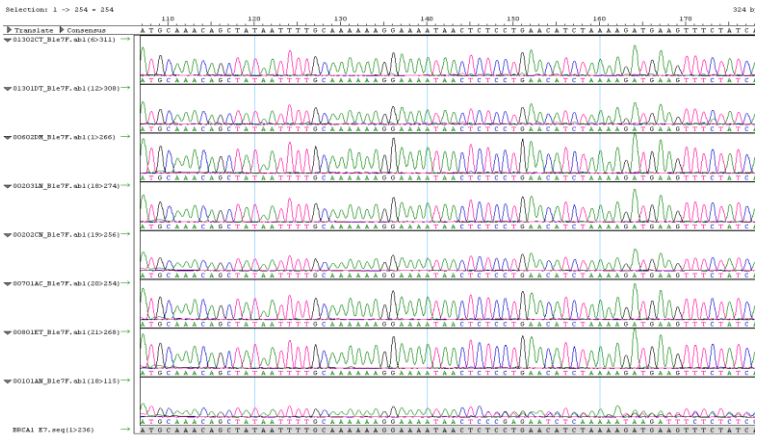
SANGER sequencing

SANGER sequencing in the 2000s



(B)





NGS sequencing in the 2020s

Next-generation sequencing (NGS), also known as high-throughput sequencing, is the catch-all term used to describe a number of different modern sequencing technologies. These technologies allow for sequencing of DNA and RNA much more quickly and cheaply than the previously used Sanger sequencing, and as such revolutionised the study of genomics and molecular biology. Such technologies include:

Illumina (Solexa) sequencing

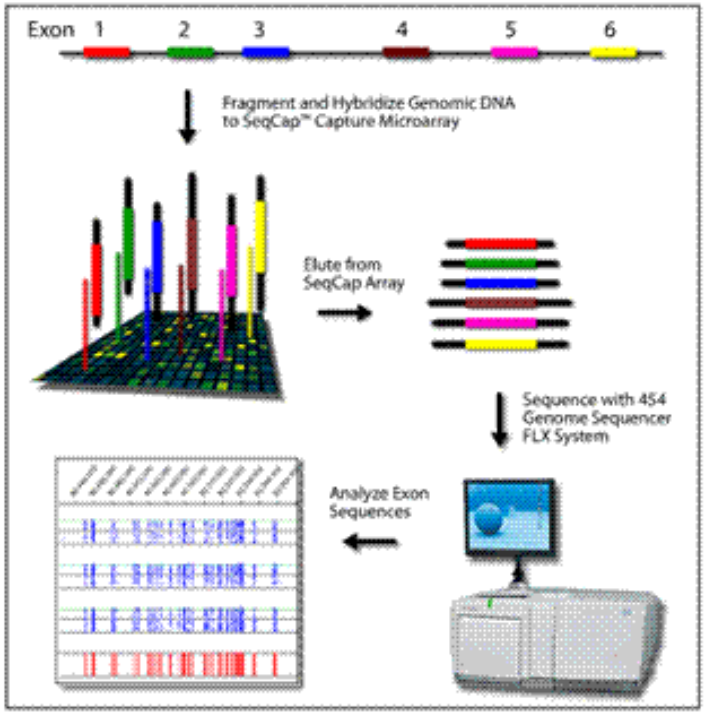
Illumina sequencing works by simultaneously identifying DNA bases, as each base emits a unique fluorescent signal, and adding them to a nucleic acid chain.

Roche 454 sequencing

This method is based on pyrosequencing, a technique which detects pyrophosphate release, again using fluorescence, after nucleotides are incorporated by polymerase to a new strand of DNA.

Ion Torrent: Proton/PGM sequencing

Ion Torrent sequencing measures the direct release of H+ (protons) from the incorporation of individual bases by DNA polymerase and therefore differs from the previous two methods as it does not measure light.



NGS is already the reference worldwide for Oncogenetic Molecular Diagnostic.

NGS implementation is more and more important in all UE and will be very soon the gold standard

WGS vs. WES

Whole genome sequencing (WGS) attempts to sequence the entirety of the genome. Due to the difficulty in sequencing technically challenging regions of the genome with current sequencing platforms (high GC content, large repeat regions, centromeres, telomeres, etc), in reality, WGS only covers 95% to 98% of the genome. Exome sequencing, sometimes called 'whole exome sequencing' (WES), instead focuses on just the protein coding sequences.

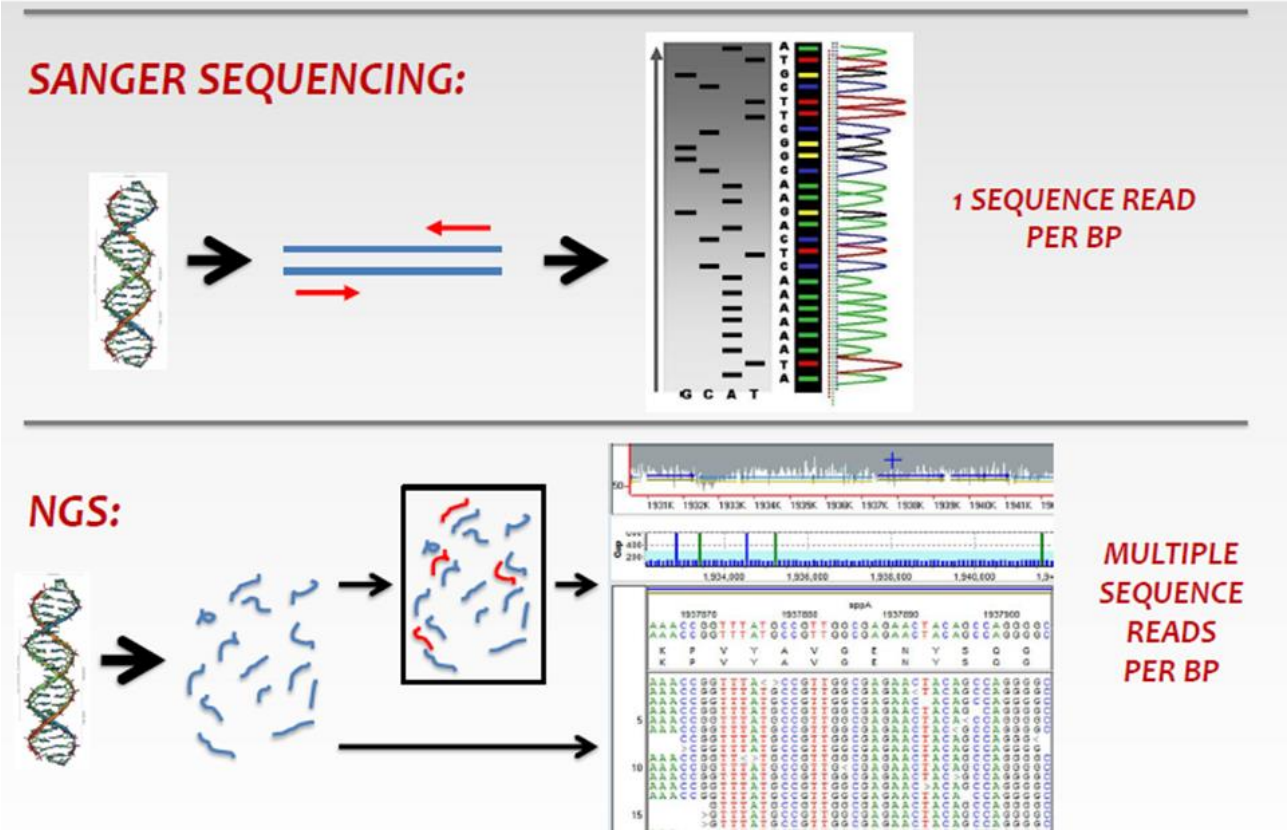
WES Advantages - **save money and time**. Even though WES samples are typically sequenced to a higher depth (100X vs 30X), the reads are focused on only ~2% of the genome, so less overall sequence is needed (= lower costs). This is achieved through an enrichment/pulldown process where DNA or RNA baits are used to hybridize with the protein-coding portion of the genome, isolating it from the non-coding portion. The amount of sequence needed for a 100X

exome sample is ~5-6Gb, substantially less than the ~90Gb needed for WGS ^ **lower data storage costs + quicker, cheaper and easier data analysis**. Since the coding region of the genome has been characterized to a substantially higher degree, advocates of WES feel there's a better chance of interpreting variants in a meaningful way.

WGS Advantages - The enrichment steps involved in WES lead to non-uniform coverage, generating both 'hot spots' with too much coverage (a waste of sequencing power) and regions with too little coverage (leading to missed variant calls). For example, a region dense with SNPs can interfere with the capture process, as the enrichment baits may not hybridize as efficiently. Because WGS doesn't require an up-front enrichment step, it generates much **more uniform coverage of the genome +** take advantage of **longer reads**. Most human exons are <200b, anything longer than 2x100 paired end reads for WES will essentially be wasted. The longer reads available for whole genome sequencing allows for better determination of copy number variations, rearrangements and other structural variations (very important in cancer studies.

Illumina has added new life to the debate with the launch of their HiSeq X Ten sequencing platform and the substantial reduction in the cost to generate whole genome sequencing data that it brings. With real world prices of whole genome sequencing ranging between $1500 and $2000 on AllSeq's marketplace, WES doesn't have as strong of a price advantage anymore. This is leading to a rise in the **popularity of WGS**. Once Illumina lifts the restrictions on what samples and applications can be run on the HiSeq X Ten (no timetable yet provided), perhaps public opinion will swing back in favour of WES.

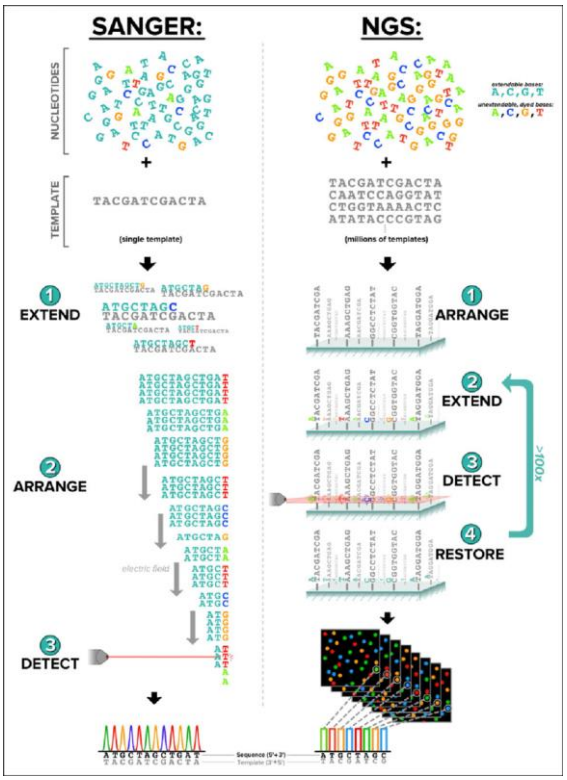
Sanger (traditional) versus NGS (future)



NGS (future)

Massive parallel sequencing (allows for sequencing multiple fragments at one time) NGS comprised of multiple different types of sequencing technologies:

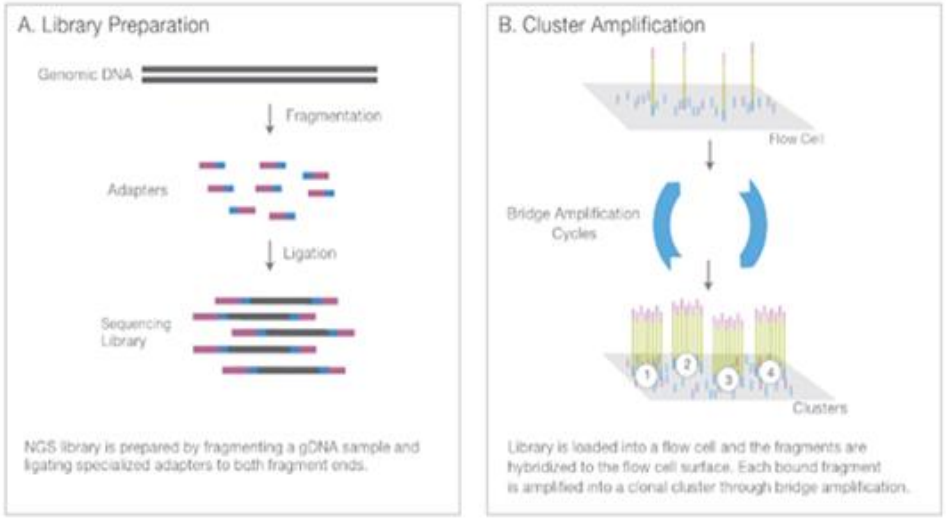
- Ion torrent
- SOLiD sequencing
- Illumina
- ChIP-Seq
- RNA Seq

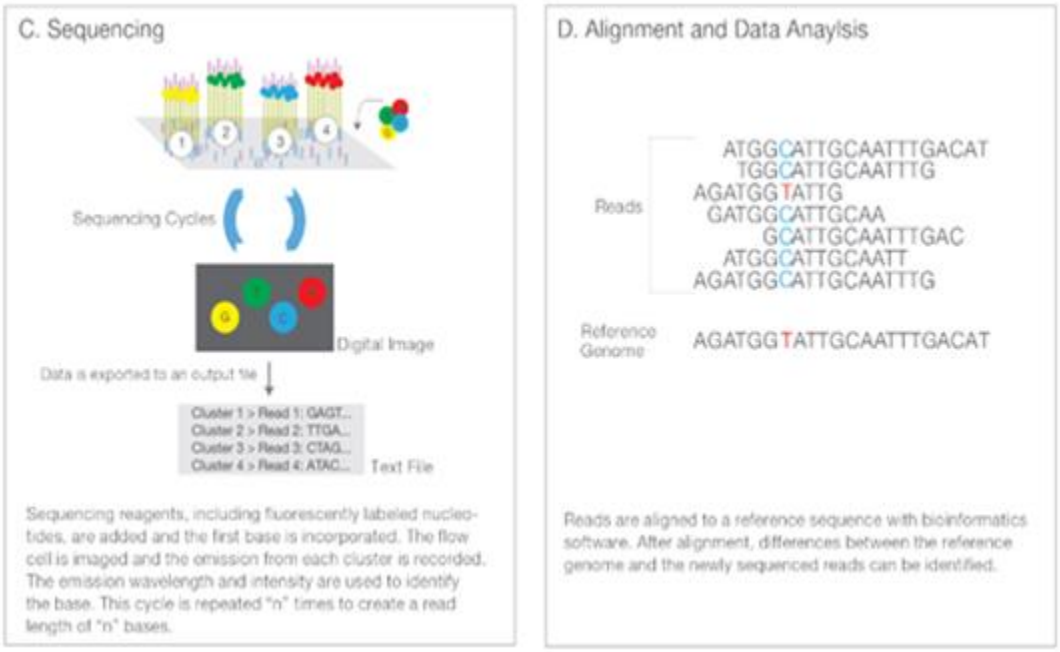


Features	Sanger	NGS
Generation	1st	2nd_jrd
Year	Late 1990s-early 2000s	2006-Current
Sequencing Samples	Cloning, PCR	DNA Libraries
Preparation Steps	Simple	Complex
Data Collection	96-384 well plates	1-16 slides
Data	1 Read/Sample	103-106 Reads/Sample
Whole genome effort/cost	Hundreds of Scientists $3 billion/10 years Large machines	1-2 Scientists $1000/Hours Counter-top machine

NGS – the workflow

1. Library Preparation: Technology determines type of sequencing
Non-targeted: Whole Genome Sequencing (30x-60x)
Targeted: Exome and Gene Panel sequencing (>100x)
***Multiplexing:** barcoded adapters to sequence more than one sample in a single run
2. Cluster Amplification
3. Sequencing:
Single-End: only provides forward sequence
***Paired-End:** provides forward and reverse sequence
Primary Analysis: FASTQ file
4. Alignment and Data Analysis: can be performed by different bioinformatic platforms
Secondary Analysis: BAM — VCF files
Data clean up, variant calling, some variant interpretation ***Improving scalability**





NGS - the systems

Sequencing System[a]	Estimated system cost	Consumable cost per single-end run (paired-end run)	Read Length per single- end run (paired-end)	Gigabases sequenced per single- end run (paired-end)	Run time per single-end run (paired-end)	Raw accuracy
454 Genome Sequencer FLX	$500,000[b]	n/a[c]	250-300 bp (2 X 110 bp)[d]	0.1 Gb[e] (0.1 Gb)	7.5 hours (7.5 hours)	99.5%
Illumina Genome Analyzer	- $400,000	$3000 (n/a)[f]	36 bp[9] (2 X 36 bp)	1.5 Gb (3.0 Gb)	2.5 days (5 days)	>98.5%
ABI SOLID™ System	$525,000	$3390[n] ($4390)	35 bp (2 X 25 bp)'	3 Gb[1] (4 Gb)	5-7 days[k] (10 days)	99.94%
Helicos Heliscope	n/a	n/a	25-35 bp[1]	7.5-10 Gb	3-7 days	>99%

+ "Third"-Generation Sequencing Technologies
Pacific Biosciences
Oxford Nanopore
Ion Torrent
Others…

Multi-Gene (NGS) Panels

Sanger: one gene; NGS: 2 to > 500 genes

- Genetic tests to look at dozens of genes related to cancer;
- Similar cost and turnaround time as gene specific testing;
- Higher risk of uncertain results.

Example: Breast NGS: Walsh et. al. 2013 (ASHG Platform Presentation)

- 800 families with negative BRCA1/2 testing
- 206 tested positive with NGS BROCA panel (26%)
- Of the 26% with a new positive results
  - 39% (80/206) had BRCA1/2 mutations
  - 37% carried mutations in CHEK2, PALB2, or TP53
  - 20% carried mutations in 10 less characterized genes

Table 1 Genes Included in Next-Generation Sequencing Multigene Cancer Panels

Cancer type	No. of genes	Gene list*
Breast cancer	17	*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, MRE11A, MUTYH, NBN, NF1, PTEN, RAD50, RAD51C, RAD51D, TP53, PALB2*
Colorectal cancer	17	*APC, BMPR1A, CDH1, CHEK2, EPCAM, GREM1, MLH1, MSH2, MSH6, MUTYH, PMS2, POLD1, POLE, PTEN, SMAD4, STK11, TP53*
Paragangliomas/Pheochromocytomas	12	*FH, MAX, MEN1, NF1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, VHL*
Renal cancer	19	*MLH1, MSH2, MSH6, PMS2, PTEN, TP53, VHL, EPCAM, FLCN, TSC2, TSC1, SDHB, MET, MITF, SDHC, SDHD, SDHA, FH, BAP1*
Pancreatic cancer	13	*APC, ATM, BRCA1, BRCA2, CDKN2A, EPCAM, MLH1, MSH2, MSH6, PMS2, STK11, TP53, PALB2*
Ovarian cancer/uterine cancer	24	*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, PMS2, PTEN, RAD50, RAD51C, RAD51D, STK11, TP53, PALB2, SMARCA4*

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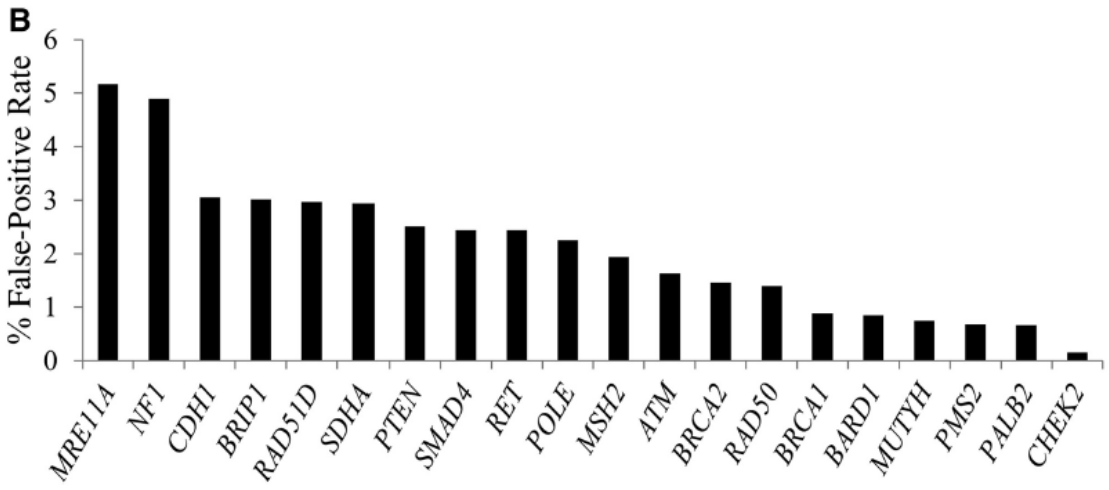
Analytica Chimica Acta

Volume 1046, 10 January 2019, Pages 154-162

A multi-gene panel beyond *BRCA1/BRCA2* to identify new breast cancer-predisposing mutations by a picodroplet PCR followed by a next-generation sequencing strategy: a pilot study

Marcella Nunziato [a, b, 1], Maria Valeria Esposito [a, b, 1], Flavio Starnone [a, b], Maria Angela Diroma [a], Alessandra Calabrese [a, c], Valentina Del Monaco [a], Pasqualina Buono [a, d], Giuseppe Frasci [c], Gerardo Botti [c], Massimiliano D'Aiuto [c], Francesco Salvatore [a, b] ✉, Valeria D'Argenio [a, b] ✉



The Journal of Molecular Diagnostics, Vol. 18, No. 6, November 2016

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jmd.amjpathol.org

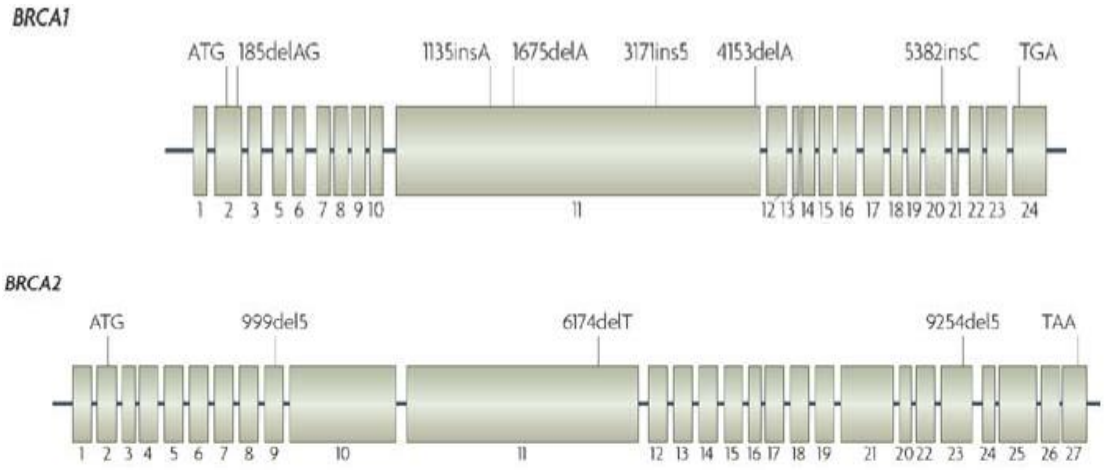
Sanger Confirmation Is Required to Achieve Optimal Sensitivity and Specificity in Next-Generation Sequencing Panel Testing

Wenbo Mu, Hsiao-Mei Lu, Jefferey Chen, Shuwei Li, and Aaron M. Elliott

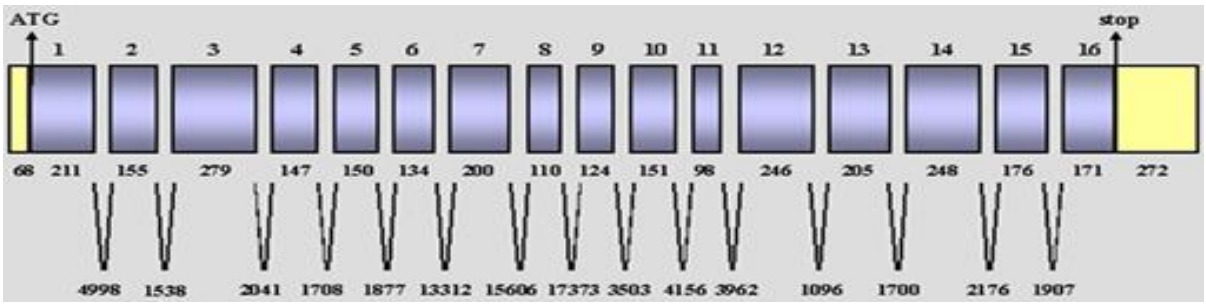
From Ambry Genetics, Aliso Viejo, California

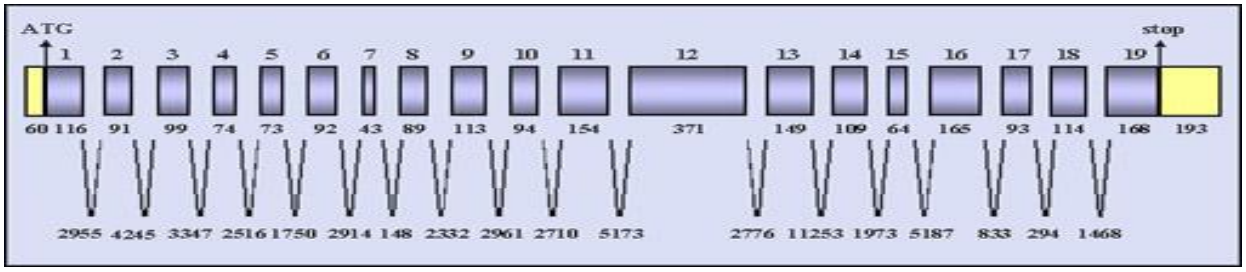
COMPLETE SANGER GENE SEQUENCING The genetic material that needs to be decrypted

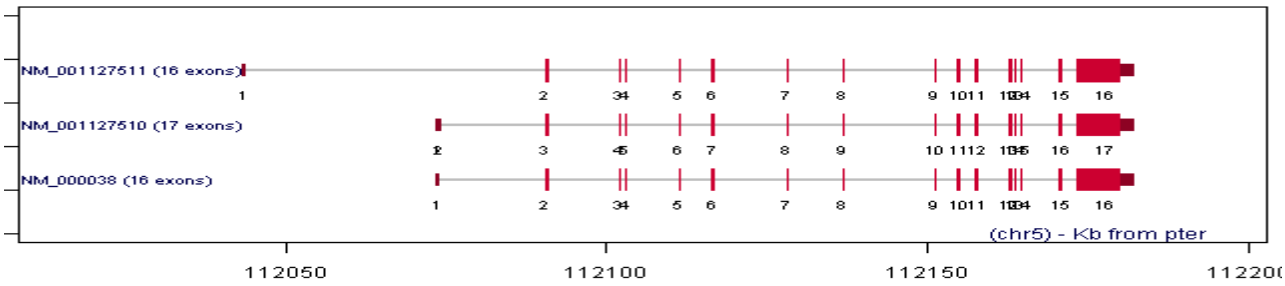
HBOC:
BRCA1 >2000 germ-line mutations identified.
Several recurrent/founder mutations.
Distribution among all **22 exons. 32 amplicons**
BRCA2 >2000 germ-line mutations identified.
Few recurrent/founder mutations.
Distribution among all **26 exons**. **44 amplicons**



HNPCC:
MSH2: >300 germ-line mutations identified. No recurrent/founder mutations. Distribution among all **16 exons. 16 amplicons**
MLH1: >300 germ-line mutations identified. Few recurrent/founder mutations. Distribution among all **19 exons. 19 amplicons**
MSH6: few germ-line mutations identified. No recurrent/founder mutations. Distribution among all **10 exons. 15 amplicons**







FAP:
APC: >300 germ-line mutations identified.
No recurrent/founder mutations.
Distribution among all **16 exons. 36 amplicons**

Complexity and problems

PROBLEM:

- Forward + Reverse sequencing of coding regions + exon/intron boundaries
- Long Exons are sub-divided in small amplicons (e.g. 15 fragments for B2- e11)
- The genes are very very long ! (kb)
- Any identified variation must be verified on a second independent sample

IN FACT: The genetic material to be decrypted:

HBOC: 15849 nucleotides = 76 amplicons = 48 exons
HNPCC: 9999 nucleotides = 50 amplicons = 45 exons
FAP: 8538 nucleotides = 36 amplicons = 16 exons

Forward + Reverse sequencing = double work ! (assume 100% success/(real) efficiency = 60-70%)

COMPLETE SANGER GENE SEQUENCING The complexity and the problems

PROBLEM:
• Forward + Reverse sequencing of coding regions + exon/intron boundaries
• Long Exons are sub-divided in small amplicons (e.g. 15 fragments for B2- e11)
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IN FACT: The genetic material to be decrypted:

HBOC: 15849 nucleotides = 76 amplicons = 48 exons
HNPCC: 9999 nucleotides = 50 amplicons = 45 exons
FAP: 8538 nucleotides = 36 amplicons = 16 exons

Forward + Reverse sequencing, all work doubles !!!!!
(supposing 100% success/efficiency - in reality 60-70%)

1. Very long genes (thousands of nucleotides), numerous exons;
2. Thousands of different mutations already identified;
3. About 100.000 nucleotides to "read" for a "simple" BRCA test;
4. Numerous benign common polymorphisms present in those genes;
5. About 50% of identifies sequence variants are not pathogenically clear (unclassified variants);
6. The continuous danger of false-positives/false-negatives.

• Responsible of the diagnostics — highly specialized + responsibility;
• Large cost;
• Long time to interpret;
• Coherent organization of the workflow.

Molecular Oncogenetics Diagnostic Interpretation of the results: In-silico analysis

1. Simulation of the sequence variant effects over the protein/metabolism
• Deleterious certain effect: STOP codons or frameshift = truncated or aberrant protein
- Deleterious probable effect: deletions/insertions
- Deleterious possible effect: alteration of essential amino acids, alteration of splicing sites
- Effect difficult to predict: MONONUCLEOTIDIC SUBSTITUTIONS
- Neutral effect: silent mutations & SNP

Classification of sequence variants according to biological significance

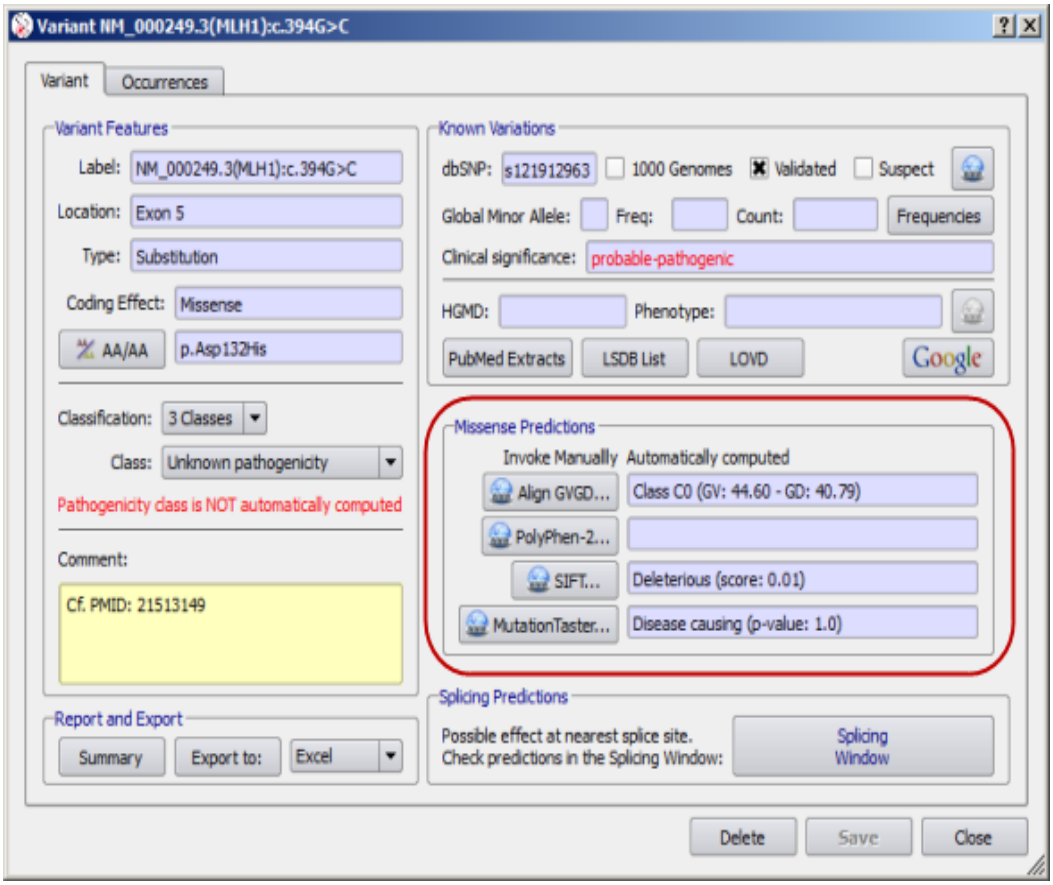
Class	Significance	Pathogenicity probability
5	Definitely Pathogenic	> 0,99
4	Likely pathogenic	0,95 - 0,99
3	Uncertain	0,05 - 0,949
2	Likely Not Pathogenic or of Little Clinical Significance	0,001 - 0,0049
1	Not Pathogenic or of No Clinical Significance	< 0,001

Different types of sequence variants can be identified

1. **Deleterious mutations** — clear pathogenic effect (premature termination of protein synthesis, frameshift, alteration of essential amino acids, alteration of splicing sites, etc...
2. **Unclassified variants (UVs)** — *a priori* unknown effect (premature terminations at terminal sites, alteration of non-essential amino acids, alteration of splicing sites, delins, substitutions, etc .....**~40% of identified BRCA sequence variants are UVs !**
3. **Common polymorphisms (SNPs)** (substitutions) - can they be risk modifiers ?

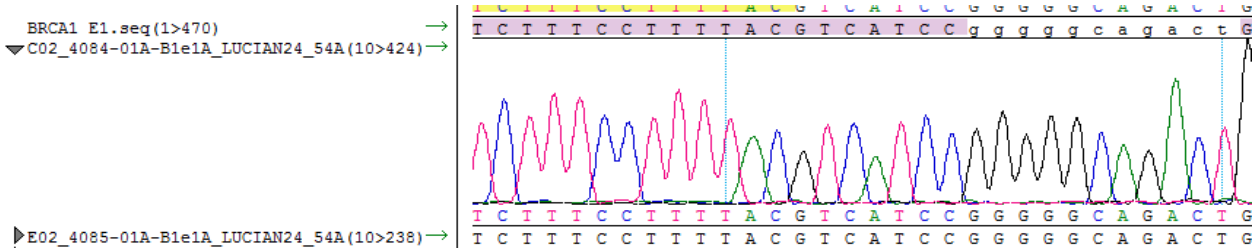
Informatized analysis of sequence variants - ALAMUT, Grantham, GVGD, PolyPhen, SIFT, ESE finder, MAXENTSCAN, NNSPLICE, GENESPLICER, etc....

But... Pathogenicity class is NOT automatically computed !!!

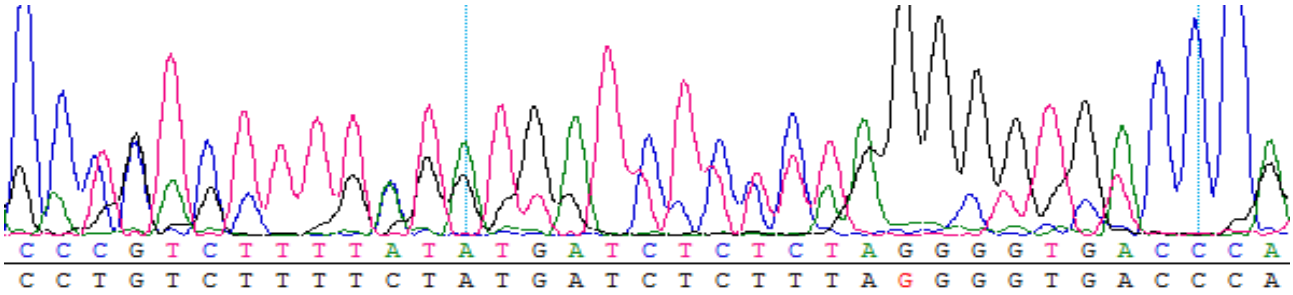


The real "face" of mutations. What do we expect to see ?

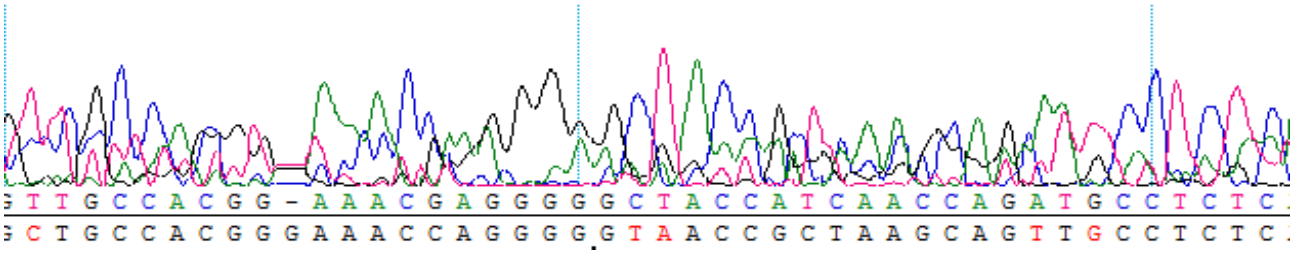
Normal, "clean" sequence



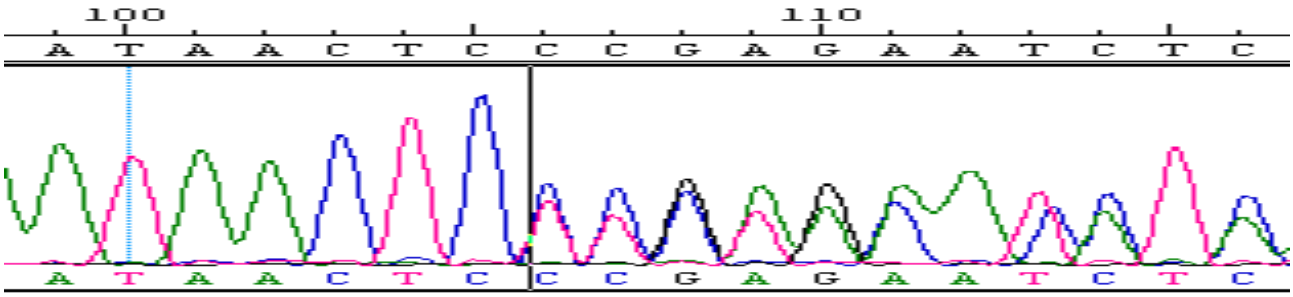
Contaminated, "dirty" sequence



Or a … "very dirty" one



Doubled sequence (case of deletions/insertions/frameshift)

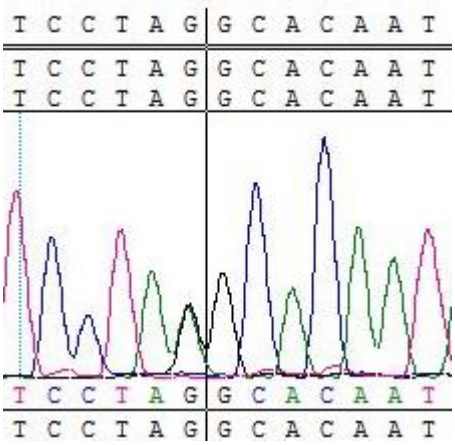


Ref : TTTTGCAAAAAAGGAAAATAACTCTCCTGAACATCTAAAAGATGAA

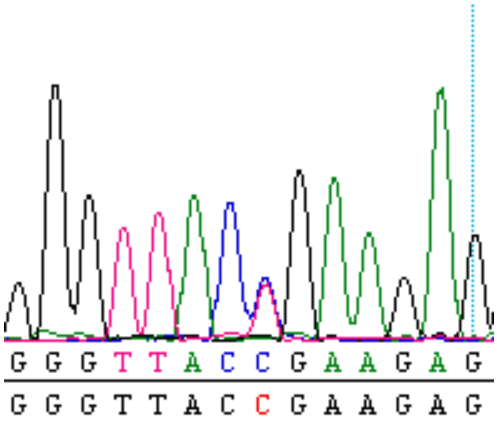
Alela 2 : TTTTGCAAAAAAGGAAAATAACTC--CTGAACATCTAAAAGATGAA

BRCA1 c.342_343delTC (p.pro115Stop)

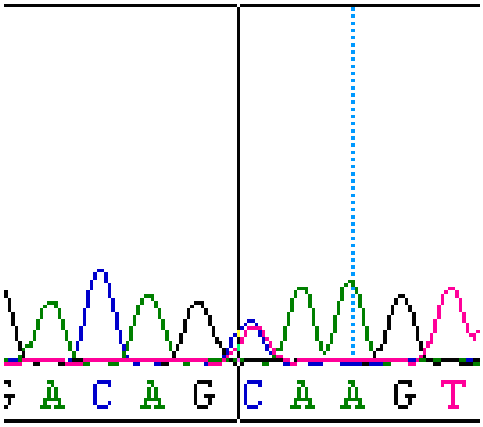
But, unfortunately, most often …… the mutations are SNPs



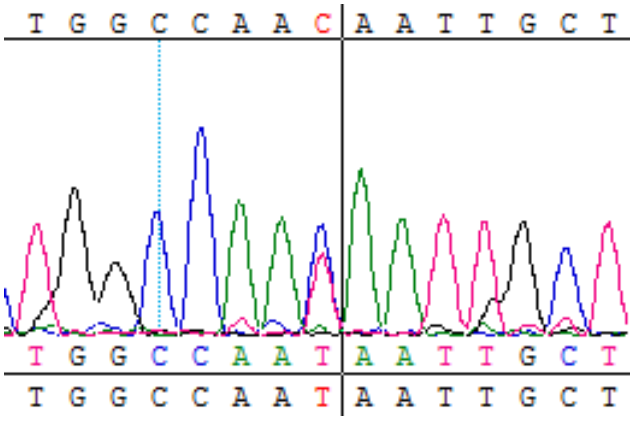
BRCA2 c.6938-1G>A



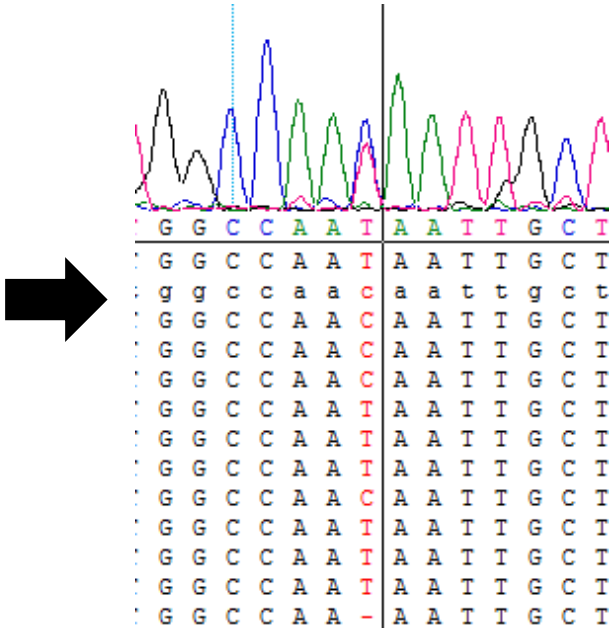
BRCA1 c.3607C>T (R1203X)



BRCA2 c.8680C>T (E2894X)



BRCA1 IVS7-34C/T



POLYMORPHISM !

Aligning the sequences from several Patients can be useful for identifying benign SNPs, but *in-silico* analysis is **mandatory** !!!

Molecular Oncogenetics Diagnostic Errors can occur

1. **Due to manipulations (less frequent)** - contamination
— mixing/inverting samples — coding errors
2. **False positives (very rare)**
— identifying a mutation which doesn't exist — "over-evaluating" an UV
3. **False negatives (quite often !)**
— not identifying a mutation which exist — "sub-evaluating" an UV

Where can this happen ?
- Gathering of samples (blood, tissue, buccal swab...) - Transport of samples;
- DNA extraction (manual vs automated);
- Dilution, sampling and coding DNA samples; - Introducing data in DNA database;
- Writing of work planning/protocols;
- Preparation and distribution of reaction mixtures; - Verifying PCR products by gel electrophoresis;
- Purification of PCR products;
- Purification of sequencing products;
- Transfer of reactions from plate to plate(manual vs automated); - Programming and preparing the sequencer;
- Pre-analysis of data;
- Export/import of data in specialized software.

Angelina Jolie

May 2013: double mastectomy
March 2015: bilateral annexectomy

"I went through what I imagine thousands of other women have felt. I told myself to stay calm, to be strong, and that I had no reason to think I wouldn't live to see my children grow up and to meet my grandchildren..."

Conclusions

- Molecular oncogenetic diagnostic is addressing targeted population from high-risk families;
- Germ-line mutations are identified by DNA sequencing of predisposition genes;
- The main steps in molecular diagnostics are DNA extraction, PCR, amplicon sequencing and capillary; electrophoresis, but the most important is data interpreting !
- Each identified sequence variant should be interpreted at a database, biochemistry, molecular biology and bioinformatics level;
- The most important error causes to avoid are false positives and false negatives.

Take home message

- Laboratory activity in molecular oncogenetics laboratory is logical and complex, routine but interesting;
- The molecular oncogenetic diagnostic is based on DNA manipulation part (extraction, PCR, sequencing) and interpretation part (much more difficult !!!);
- The in-silico analysis is helping the responsible of diagnostic activity, but the final decision is human based !!!;

• All steps have error possibilities — the diagnostic is needing high attention, expertise and responsibility;

• The molecular oncogenetic diagnostic results always open a way to research, but research results should be also implemented in further diagnostic.

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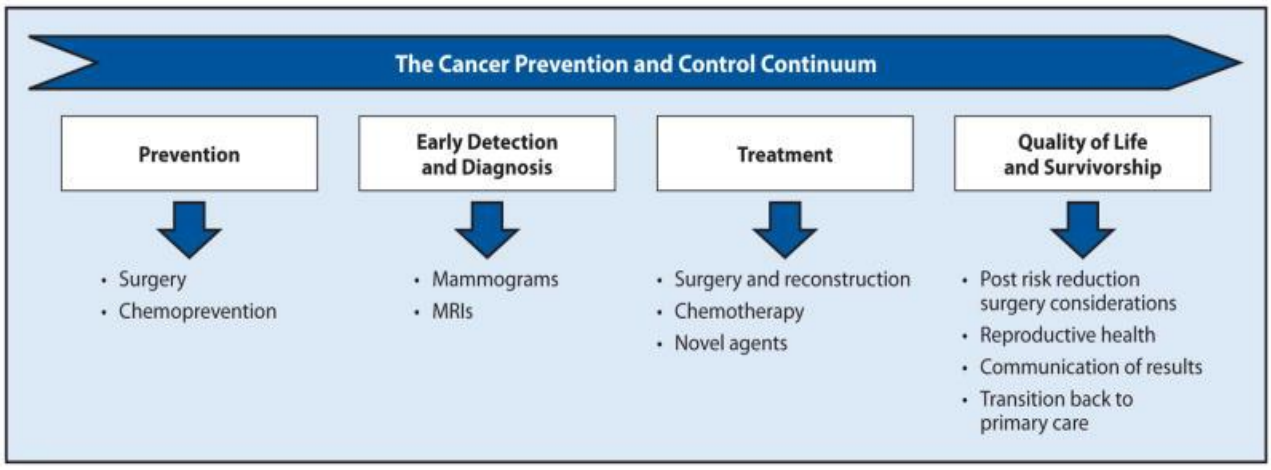
1.7. The monitoring of individuals with hereditary risk of cancer

Learning objectives

- Concept of inheritance of susceptibility;
- Gene variants;
- Monitoring of individuals with breast/ovarian cancer syndrome;
- Monitoring of individuals with colorectal cancer syndrome.

Introduction

- Cancer is caused by DNA damage.
- Every individual has a risk of developing cancer by chance (exposure to risk factors).
    - 90—95% have their roots in the environment and lifestyle (Preetha Anand - Cancer is a Preventable Disease that Requires Major Lifestyle Changes)
- Some genes may generate a high risk of developing certain types of cancer (genetically predisposed).
    - Up to 10% of cancers occur through the inherited mutation of a group of genes called cancer predisposition genes (Qing Wang - Cancer predisposition genes: molecular mechanisms and clinical impact on personalized cancer care: examples of Lynch and HBOC syndromes)
- Getting tested is a personal choice, but:
    - Genetic screening has the ability to diagnose people before cancer occurs.
    - It can provide vital data to help predict the odds of a dissease as well as provide time to plan for care.



Model of cancer prevention in individuals with genetic risk

Tuya Pal - Genetic Risk Assessments in Individuals at High Risk for Inherited Breast Cancer in the Breast Oncology Care Setting

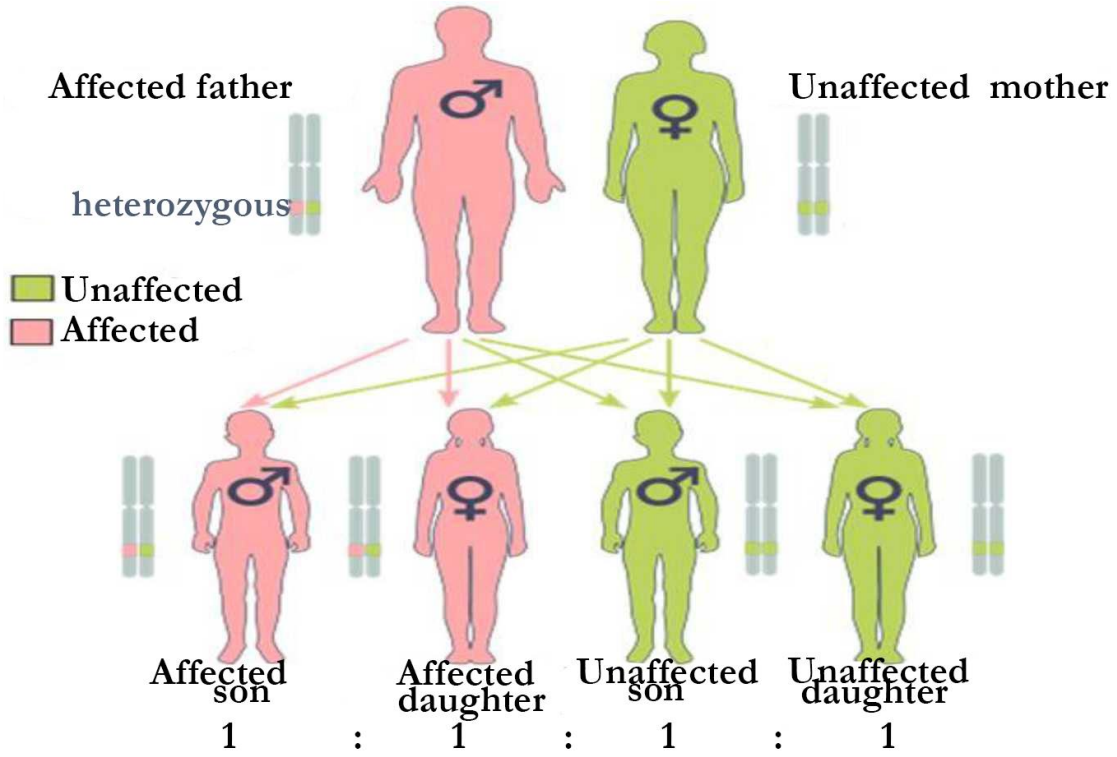
Do those who inherit their susceptibility to cancer always have cancer?

- The penetration of variants;
- Variation of expressivity;
- Environmental risk factors.

NO

Example: BRCA 1 and 2 mutations

- 50% risk to inherit the mutation if one parent has the mutation;
- BRCA 1 mutation > 50-85% breast cancer (up to 70 years) and 40-60% ovarian cancer (up to 85 years); 25-30% risk for second cancer;
- BRCA 2 mutation > 40-57% breast cancer up to age 70, and 13-23% ovarian cancer up to 70 years.



Autosomal dominant model

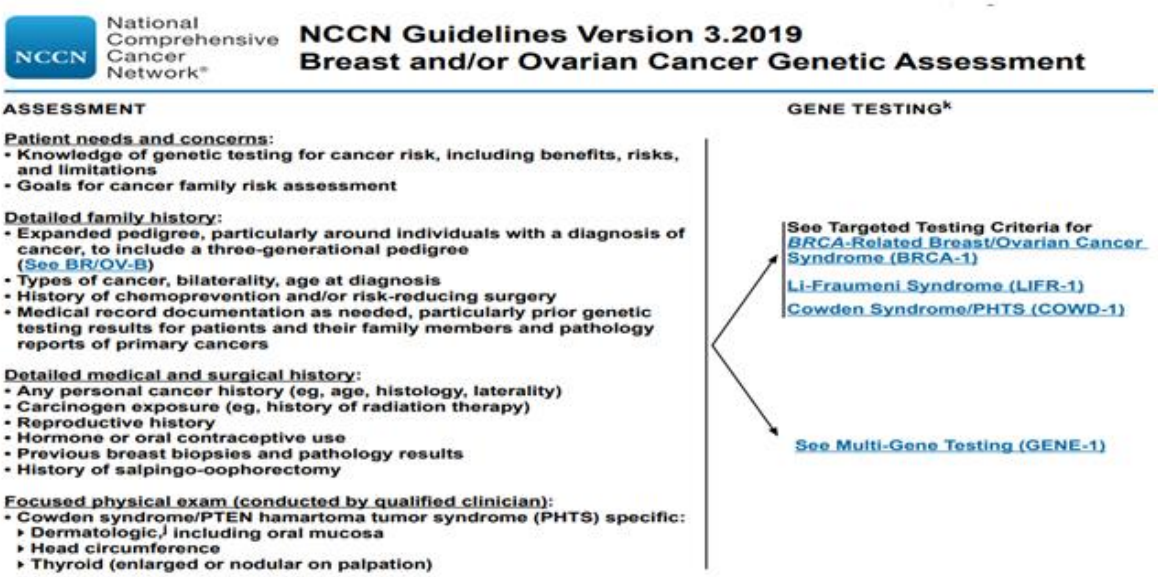
Gene variants

- Genetic tests are not 100% sensitive. There is no test that can identify all BRCA mutations.

Variants

- **Pathogenic mutations:** Changes have a significant risk of cancer.
- **Mutations with suspected pathogenicity:** Although not shown, variants are suspected of being harmful.
- **Variants of unknown significance (VUS):** It is unclear whether the change has clinical significance.
- **Variant in favour of polymorphism:** Although it is not clear, it is considered that the variant is not pathogenic.
- **Benign polymorphism:** Modification is classified as harmful.
- Breast and ovarian cancers can occur without a predisposition.

What is important to evaluate?



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NCCN Guidelines Version 3.2019
Breast and/or Ovarian Cancer Genetic Assessment

ASSESSMENT

Patient needs and concerns:
- Knowledge of genetic testing for cancer risk, including benefits, risks, and limitations
- Goals for cancer family risk assessment

Detailed family history:
- Expanded pedigree, particularly around individuals with a diagnosis of cancer, to include a three-generational pedigree (See BR/OV-B)
- Types of cancer, bilaterality, age at diagnosis
- History of chemoprevention and/or risk-reducing surgery
- Medical record documentation as needed, particularly prior genetic testing results for patients and their family members and pathology reports of primary cancers

Detailed medical and surgical history:
- Any personal cancer history (eg, age, histology, laterality)
- Carcinogen exposure (eg, history of radiation therapy)
- Reproductive history
- Hormone or oral contraceptive use
- Previous breast biopsies and pathology results
- History of salpingo-oophorectomy

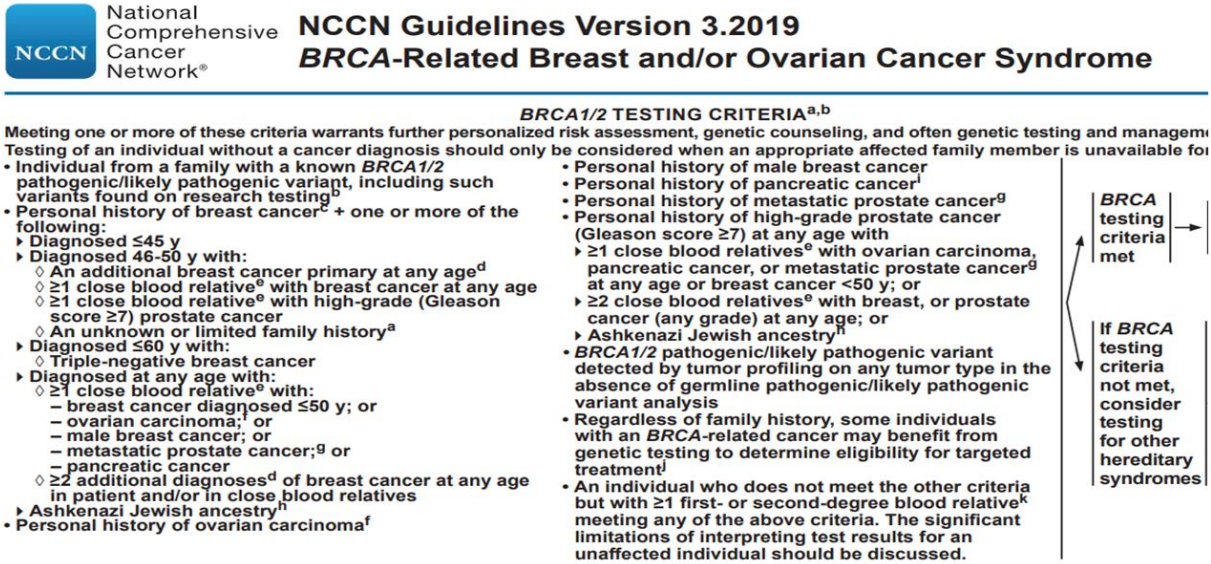
Focused physical exam (conducted by qualified clinician):
- Cowden syndrome/PTEN hamartoma tumor syndrome (PHTS) specific:
  - Dermatologic,[j] including oral mucosa
  - Head circumference
  - Thyroid (enlarged or nodular on palpation)

GENE TESTING[k]

See Targeted Testing Criteria for *BRCA*-Related Breast/Ovarian Cancer Syndrome (BRCA-1)
Li-Fraumeni Syndrome (LIFR-1)
Cowden Syndrome/PHTS (COWD-1)

See Multi-Gene Testing (GENE-1)

Who should be tested?



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NCCN Guidelines Version 3.2019
***BRCA*-Related Breast and/or Ovarian Cancer Syndrome**

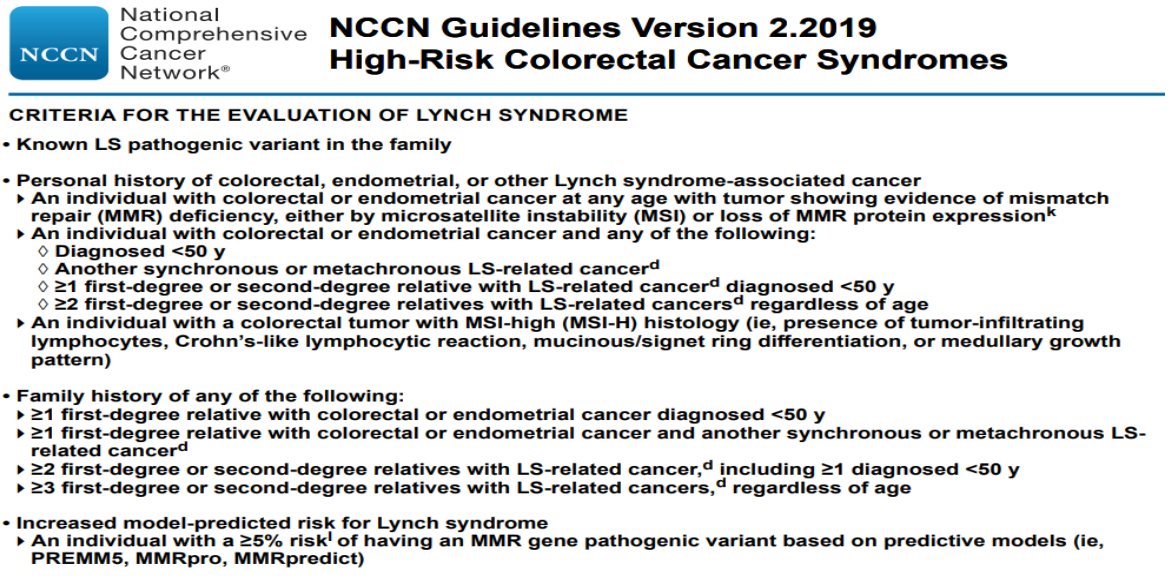
***BRCA1/2* TESTING CRITERIA[a,b]**

Meeting one or more of these criteria warrants further personalized risk assessment, genetic counseling, and often genetic testing and managem
Testing of an individual without a cancer diagnosis should only be considered when an appropriate affected family member is unavailable fo

- Individual from a family with a known *BRCA1/2* pathogenic/likely pathogenic variant, including such variants found on research testing[b]
- Personal history of breast cancer[c] + one or more of the following:
  - Diagnosed ≤45 y
  - Diagnosed 46-50 y with:
    - ◊ An additional breast cancer primary at any age[d]
    - ◊ ≥1 close blood relative[e] with breast cancer at any age
    - ◊ ≥1 close blood relative[e] with high-grade (Gleason score ≥7) prostate cancer
    - ◊ An unknown or limited family history[a]
  - Diagnosed ≤60 y with:
    - ◊ Triple-negative breast cancer
  - Diagnosed at any age with:
    - ◊ ≥1 close blood relative[e] with:
      - breast cancer diagnosed ≤50 y; or
      - ovarian carcinoma;[f] or
      - male breast cancer; or
      - metastatic prostate cancer;[g] or
      - pancreatic cancer
    - ◊ ≥2 additional diagnoses[d] of breast cancer at any age in patient and/or in close blood relatives
  - Ashkenazi Jewish ancestry[h]
- Personal history of ovarian carcinoma[f]
- Personal history of male breast cancer
- Personal history of pancreatic cancer[i]
- Personal history of metastatic prostate cancer[g]
- Personal history of high-grade prostate cancer (Gleason score ≥7) at any age with
  - ≥1 close blood relatives[e] with ovarian carcinoma, pancreatic cancer, or metastatic prostate cancer[g] at any age or breast cancer <50 y; or
  - ≥2 close blood relatives[e] with breast, or prostate cancer (any grade) at any age; or
  - Ashkenazi Jewish ancestry[h]
- *BRCA1/2* pathogenic/likely pathogenic variant detected by tumor profiling on any tumor type in the absence of germline pathogenic/likely pathogenic variant analysis
- Regardless of family history, some individuals with an *BRCA*-related cancer may benefit from genetic testing to determine eligibility for targeted treatment[j]
- An individual who does not meet the other criteria but with ≥1 first- or second-degree blood relative[k] meeting any of the above criteria. The significant limitations of interpreting test results for an unaffected individual should be discussed.

BRCA testing criteria met →

If *BRCA* testing criteria not met, consider testing for other hereditary syndromes



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NCCN Guidelines Version 2.2019
High-Risk Colorectal Cancer Syndromes

CRITERIA FOR THE EVALUATION OF LYNCH SYNDROME

- Known LS pathogenic variant in the family
- Personal history of colorectal, endometrial, or other Lynch syndrome-associated cancer
  - An individual with colorectal or endometrial cancer at any age with tumor showing evidence of mismatch repair (MMR) deficiency, either by microsatellite instability (MSI) or loss of MMR protein expression[k]
  - An individual with colorectal or endometrial cancer and any of the following:
    - ◊ Diagnosed <50 y
    - ◊ Another synchronous or metachronous LS-related cancer[d]
    - ◊ ≥1 first-degree or second-degree relative with LS-related cancer[d] diagnosed <50 y
    - ◊ ≥2 first-degree or second-degree relatives with LS-related cancers[d] regardless of age
  - An individual with a colorectal tumor with MSI-high (MSI-H) histology (ie, presence of tumor-infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet ring differentiation, or medullary growth pattern)
- Family history of any of the following:
  - ≥1 first-degree relative with colorectal or endometrial cancer diagnosed <50 y
  - ≥1 first-degree relative with colorectal or endometrial cancer and another synchronous or metachronous LS-related cancer[d]
  - ≥2 first-degree or second-degree relatives with LS-related cancer,[d] including ≥1 diagnosed <50 y
  - ≥3 first-degree or second-degree relatives with LS-related cancers,[d] regardless of age
- Increased model-predicted risk for Lynch syndrome
  - An individual with a ≥5% risk[l] of having an MMR gene pathogenic variant based on predictive models (ie, PREMM5, MMRpro, MMRpredict)

Expert recommendations for screening BRCA mutation carriers for breast cancer. CBE: clinical breast examination

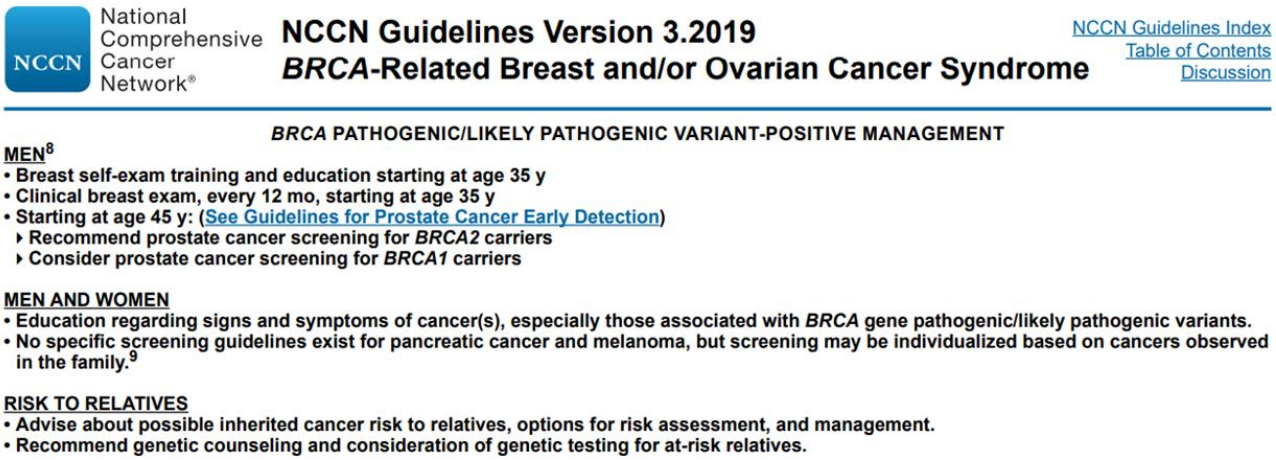
Organization	Annual MRI	Annual Mammography	Screening Ultrasound	Other
NCCN [18] 2018 (U.S.)	Aged 25–75	(**with consideration of tomosynthesis**) Aged 30–75 Aged 25-75 if MRI not possible	Not recommended	Breast awareness aged 18+ Semi-annual CBE aged 25+
NICE [17] 2017 (U.K.)	Aged 30–49 Aged 50–69 only if mammo-graphically dense breasts	Aged 40–69	Aged 30–49 if MRI not possible	Breast awareness
ESMO [19] 2016 (Europe)	Aged 25+	Aged 30+	Aged 25+ if MRI not possible	Breast awareness Semi-annual CBE aged 25+
CCO [20] 2018 (Canada)	Aged 30–69	Aged 30+	Aged 30–69 if MRI not possible	Breast Awareness

Source: Ellen Warner - Screening BRCA1 and BRCA2 Mutation Carriers for Breast Cancer

BRCA pathogenic/likely pathogenic

- Screening
  - 25 y — clinical breast exam every 6-12 months • 25-29y — annual breast MRI
  - 30-75y — annual mammogram/MRI
- Prevention
  - Risk-reduction mastectomy
  - Risk-reduction salpingo-oophorectomy
  - Risk reduction agents (chemoprevention)

Monitoring and therapeutic interventions



National Comprehensive Cancer Network®

NCCN Guidelines Version 3.2019
***BRCA*-Related Breast and/or Ovarian Cancer Syndrome**

NCCN Guidelines Index
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Discussion

***BRCA* PATHOGENIC/LIKELY PATHOGENIC VARIANT-POSITIVE MANAGEMENT**

MEN[8]
- Breast self-exam training and education starting at age 35 y
- Clinical breast exam, every 12 mo, starting at age 35 y
- Starting at age 45 y: (See Guidelines for Prostate Cancer Early Detection)
  - Recommend prostate cancer screening for *BRCA2* carriers
  - Consider prostate cancer screening for *BRCA1* carriers

MEN AND WOMEN
- Education regarding signs and symptoms of cancer(s), especially those associated with *BRCA* gene pathogenic/likely pathogenic variants.
- No specific screening guidelines exist for pancreatic cancer and melanoma, but screening may be individualized based on cancers observed in the family.[9]

RISK TO RELATIVES
- Advise about possible inherited cancer risk to relatives, options for risk assessment, and management.
- Recommend genetic counseling and consideration of genetic testing for at-risk relatives.

Lynch syndrome. Screening

Colonoscopy

- annually, beginning at age 20-25, or ten years younger than the earliest age of diagnosis in the family, whichever comes first.
- 2 to 5 years prior to the earliest age of diagnosis in the family, if under the age of 25 and repeat every 1-2 years.

Endometrial sampling

- beginning between ages 30-35
- no current scientific evidence - annual endometrial samplings may be useful in select patients

Transvaginal ultrasound

- beginning ages 30-35/at the clinician's discretion/Sensitivity/specificity issues

CA 125

- data does not support routine ovarian screening for LS

Urinalysis

- Annually, beginning at age 25-35 + Selected individuals (MSH2)

Gastroscopy

- No clear data. Selected individuals (family history of gastric/small bowel cancer)
- EGD with extended duodenoscopy (to distal duodenum or into the jejunum) and polypectomy every 3-4 years beginning at the age of 40.

Others

- Dermatologic exam
- Capsule endoscopy
- Neurologic exam

Lynch syndrome. Prevention

- Total abdominal colectomy with ileorectal anastomosis in the event of adenomas not amenable to endoscopic rescection
- Subtotal colectomy with preferences of patient actively elicited.
- Hysterectomy
    - Mortality reduction?
    - Reduction in cancer incidence
- Risk-reduction salpingo-oophorectomy
- Chemoprevention
    - Consider aspirin (no clear data)
    - Oral contraceptives
- Lifestyle modifications

Important problems

- When does cancer occur?
- Effects on fertility and pregnancy
- Timing
- Available methods
- Patient preferences
- Lifestyle considerations

Take home message

- Getting tested is a personal choice. Every decision must be respected.
- Genetic tests are not 100% sensitive.
- Gene variants must be understood and the results must be explained by a geneticist.
- Monitoring and therapeutic interventions can considerably reduce the risk of death.
- Definition of risk
- Identify the consequences
- Interpretation of results
- Adaptation of the strategy
- Extending testing to family members
- Adaptation of treatment when cancer occurs

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NCCN Guidelines at https://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf

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1.8. Psychological aspects of hereditary cancer – general notions

Learning objectives

• understanding the role of psycho-oncology in the bio-psycho-social approach to malignancy
• understanding the need for psycho-social assistance in cancer prevention and treatment
• knowledge of the psycho-social implications of hereditary cancer

Introduction

Although early detection methods and new cancer treatments lead to an increased survival rate, the prevalence and impact of symptoms associated with the disease are increasing, which can greatly affect quality of life and may even limit the use of procedures with prophylactic or curative potential.

Oncological patients and their family members experience significant practical, psycho-social and spiritual concerns with an impact on their quality of life.

In the scientific literature there are numerous studies on the relationship between the social environment and the psychological adaptation to the disease.

Psycho-oncology - discipline necessary in the holistic approach to oncological disease

• the need to educate people in the community in order to early detect the first symptoms of the disease

• the need to adopt preventive behaviours that can contribute to reducing the risk for disease or recurrence

• the need to improve the quality of life of the oncological patient, to reduce the emotional reactivity and to mobilize the individual resources in the fight with the disease

• the need for collaboration between biomedical and psycho-social sciences specialists

Psycho-social oncology = specialized in dedicated cancer care
understanding and management of the following aspects related to oncological disease:
- psychological
- emotional social
- spiritual
- quality of life and functional aspects

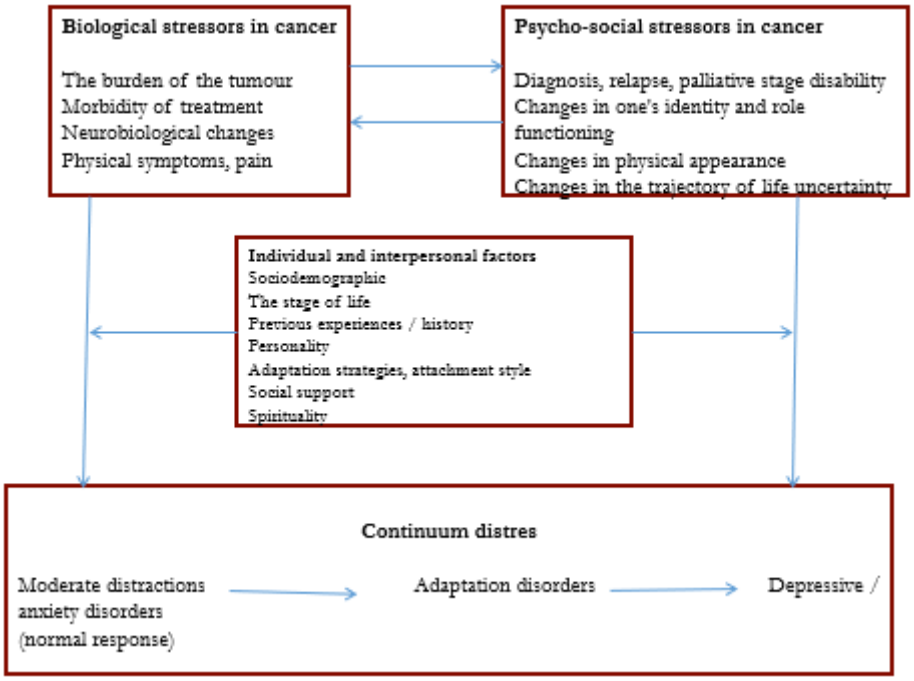
having an approach to the person as a whole (a holistic approach) and covering a range of human needs (Sackett et al., 1996).

One of **the goals of psycho-oncology** is to identify the psycho - neuroimmunological mechanisms for regulating the decision- making process and for adopting sanogenic behaviors, at the level of motivation, beliefs, attitudes with impact on cancer control.

The need for psycho-social assistance in the prevention and treatment of cancer

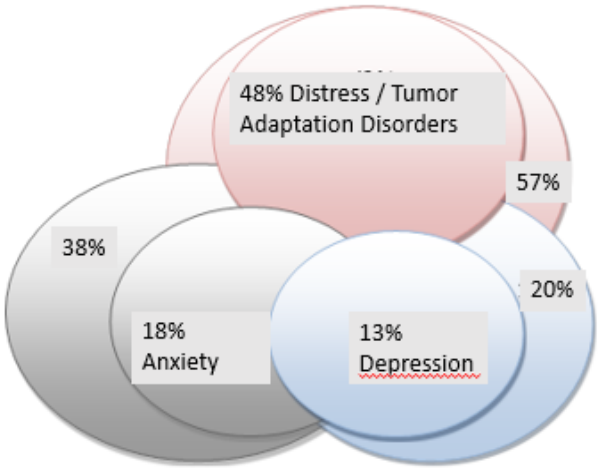
• Psycho-social assistance in oncology focuses in particular on emotional stress related to cancer which is internationally recognized as the 6th Vital Sign on cancer care, as well as managing complex disease problems.

Obvious evidence suggests that changes caused by stress at the neuroendocrine, neuroimmune and neurotransmitter levels may contribute to the part of a group of behavioural changes called "disease-related behaviour"



Tumoral distress model after Zimmermann et al. (2007); Lo et al. (2008)

- It is estimated that there is in fact a continuum of stress from normal fear and sadness, worry, leading to more severe symptoms, identified among the diagnostic criteria of more serious psychiatric disorders such as depression or anxiety.
- According to the standards of psycho-social oncology published by *The Canadian Association of Psychosocial Oncology,* psycho-social assistance in oncology provides support to people with cancer who experience difficulties in adapting to illness, emotional stress, changes in family and social relationships, difficulties in planning the next stage of life.
- psycho-social health services in oncology address people at risk, diagnosed patients, cancer survivors, patients who need palliative care, families;
- studies and reports highlight the impact and emotional or psycho-social burden of the patient and their families;
- More than 35% of patients with cancer experience stress and require psycho-social interventions particular to oncology or supportive interventions with a view to managing the burden of the disease or improving the quality of life.



Rates of stress, anxiety and depression by interview (inner circle) and self-reporting (outer circle) based on meta-analysis research, according to Krebber et al. (2014)

✓ Depression and anxiety represent for the oncological patients not only a psychological burden, which can also have negative effects on health and behavioural implications, such as reduced adherence to treatment/medical recommendations, quality of life, high rates of suicidal ideation or suicide, mortality.

✓ Oncological patients and their family members experience significant practical, psycho-social and spiritual concerns with an impact on their quality of life;

✓ In the scientific literature there are numerous studies on the relationship between the social environment and the psychological adaptation to the disease;

✓ The social environment can have both beneficial and harmful effects

✓ People at higher risk of developing cancer than the general population and the general population itself have the right to psychosocial and supportive care needed to manage concerns and fears about the risk of having cancer or concerns related to screening;

✓ They are also entitled to receive information which can assist them in pursuing primary, secondary or tertiary prevention programmes, in changing behaviour and lifestyle towards sanogenesis and any perceived challenges related to cancer, prevention or screening.

✓ According to the standards of psycho-social assistance in oncology, people with hereditary risk have the right to receive genetic counselling and genetic testing that fully integrates psycho-social and supportive care to facilitate informed decision-making about risk reduction options (prophylactic surgery, preventive chemotherapy e.g.).

Psycho-social implications of hereditary cancer

✓ Individuals do not work in isolation, they share health issues and beliefs with family members and the social environment they belong to, when their health is threatened.

✓ In addition to beliefs, family interactions also influence the mechanisms of psychological adjustment and adaptation to illness or to the threat to health.

✓ There are studies that highlight the impact of chronic diseases on the family throughout the life cycle and on the family dynamics in relation to the behaviour towards the disease, the adherence and the course of the disease.

✓ Daly (2015), in the study *A Family - Centered Model for Sharing Genetic Risk,* draws a parallel between using the family-centered model in the oncological context and in the context of the genetic risk of cancer, considering that, in order to understand the problems that families face in the context of the information on genetic risk, a systemic approach is required;

✓ This approach should include the nature of family relationships, the temporal dimension, the life cycle stage, the communication models, the cultural beliefs and the social network.

✓ Previous cancer experiences in the family affect the psychological adaptation for genetic testing

✓ Experiences with affected or deceased relatives can directly affect adaptation, but can also indirectly influence the representations of the disease and how to cope, adapt to the disease or threat to health

✓ Zakowskiet et al. (1997), Erblich et al. (2000) argue that women with a family history of breast cancer have reported a higher level of breast cancer distress if they lost their mother or parent due to cancer.

✓ There may be a direct relationship between psychological distress and the number of cancer experiences with close relatives, parents or losses suffered during childhood or adolescence due to the disease; in this context, the family system model - genetic disease (FSGI), being a longitudinal model, can help to better manage and predict diseases with genetic risk

✓ According to the study by Kaphingst et al. (2009) on a representative sample of the general population (N = 5813), people who consider having a family history of cancer or genetics as a factor that could reduce the risk of cancer, seek significantly more information about cancer. About 80% of respondents also believe that their family history of cancer

➢ In two prospective studies performed on subjects undergoing genetic testing for BRCA1and

BRCA2 or gene mutations responsible for HNPCC, predictors for the emotional distress generated by hereditary cancer were identified.

➢ When subjects were evaluated before genetic testing and retested for up to 6 months after reporting test results (van Oostrom et al., 2007b), emotional distress prior to testing, lack of hope, number of first-degree relative affected by cancer and strong emotional representations of the disease are predictive factors for the emotional suffering generated by hereditary cancer.

➢ The study of 7172 adults by Kowalkowski et al. (2012), highlights the fact that there is significant association between cancer history and cancer perceptions; people with a family history of cancer are more concerned that they will develop the disease in the future compared to people without a cancer history.

➢ Moreover, a family history that cancer disease, most behaviour or lifestyle of cancer often, is causes individuals to not caused by the considered persons.

➢ Regarding the family history of cancer genetic mutations, Bradbury et al. (2009), in the qualitative, retrospective study, on 22 adult descendants with parents carrying BRCA1 and BRCA2 mutations, concludes that

➢ the majority of the descendants (77%) believe that the disclosure of the genetic mutation history did not have a significant impact on the emotional state, for some generating even a behavioural change for health.

Take home message

✓ Psycho-social oncology is a specialty in cancer care dedicated to understanding and managing the following aspects related to oncological disease: psychological, emotional, social, spiritual, quality of life and functional aspects, using a holistic approach and covering a series of needs.

✓ Of the oncological patients, over 35% experience stress and require psycho-social interventions specific to oncology or supportive interventions in order to manage the burden of the disease as best as possible and to improve the quality of life.

✓ Hereditary cancer, being a family problem, can directly or indirectly affect all its members in terms of individual representations about the disease, strategies of adaptation and psychological adjustment to the disease or to the threat to their own health.

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Learning objectives

After completing the course students should be able to:

• identify the ethical issues that stems from a particular clinical situation (eg: genetic testing)

• critically integrate the ethical insights into a coherent arguments in the field of oncogenetics

• to decide when it's morally justifiable to respect the confidentiality and when it's ethically permissible to disclose the information about a patient to a third party

• present the advantages as well as the negative consequences of genetic testing

Introduction

1. Ethical theories and principles
2. Informed consent and confidentiality in genetic testing
3. Ethical issues regarding genetic testing
4. Conclusions

Ethical theories

• Have you recently made any difficult choices

• When going to the doctor, do you make decisions by yourself or do you discuss it with other family members?

• What are the limits of a personal choice? (if any)

Ethics

• refers to principles that define the **right, good and appropriate** behaviour

• these principles do not always produce a single moral resolution, but but provide a means of evaluating and deciding among competing options.

Bioethics

• Takes into account life-related issues, including life creation and the process of death, through the lens of ethical principles

The scope of medical ethics includes:

• recognition of ethical dilemmas
• promotion of ethical practice
• development of ethical codes and guidelines
• resolution of ethical conflicts

Ethical theories

1. Principlism
2. Deontology
3. Utilitarianim
4. Ethics of care
5. Virtue ethics

Principlism

1. Respect for autonomy
2. Nonmaleficence (not to harm)
3. Beneficence (duty to do good)
4. Justice and equity

Principlism = a framework of moral analysis

The four principles:

- produce a framework of analysis through which we will identify and reflect on moral issues represents evaluation
- represents a starting point for moral deliberation and of a policy

1. **respect for autonomy**

Autonomy is**:**

- the foundation of the human dignity
- the process of development of self-identity,
- an autonomous person must develop his/her skills to choose/create a plan
- rational agents are involved in making informed and voluntary decisions.
- prerequisite: the patient has the capacity to act intentionally, with understanding, and without controlling influences
- this principle is the basis for the practice of " informed consent" in the physician/patient "transaction" regarding health care.

autonomy = freedom + rationality

Autonomy does not "explode" within human life!

It is achieved by careful practice.

Autonomy is not just accepting a random choice (doing "what I want")

BUT

full responsibility towards a set of well-defined values.

Rights ⟷ **Duties**

Autonomy is built on **moral responsibility**, commitment and concern for other human being.

A medical system which values patient's autonomy

1. Patients would want to take decisions
2. Patients would want to receive information
3. Physicians would be able and willing to offer the information
4. Patients would need to understand the information and remember it
5. Patients would appropriately debate the medical

"In these times I don't know what I want; perhaps I don't want what I know and want what I don't know"
(*Marsilio Ficino)*

When should autonomy be restricted?

In order to stop one person from hurting another one
In order to stop a person from hurting herself
In order to act in the best interests of that person while promoting the benefit to the society

2. Do not harm - Principle of Nonmaleficence

= main obligation of any doctor, sometimes more important than respecting patient's autonomy

- It also means not to harm an individual who could not object or even accept to be harmed.

- To do no harm = a constant duty
- Doing good = a limited duty

"Primum non nocere"

We do not intentionally harm or injury the patient, either through acts of commission or omission.

3. The duty to do good - Principle of Beneficence

Respect for autonomy + refrain from doing harm +
an active contribution to the well-being of the individual.

- the duty of health care providers to take prevent and to remove harm from positive steps to the patient

"With purity, holiness and beneficence I will pass my life and practice my art"
(The Hippocratic Oath)

4. Justice and equity

"Equals should be treated equally and unequals should be treated unequally" (Aristotel)

Justice:
- refers to what is appropriate, worthy and is right.
- It is valuable principle only when certain standards are met (political, social, or cultural).

Equity:
- the presumption that all citizens have the same political rights, equal access are treated equally by the law.

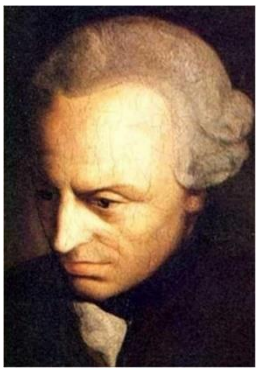
Universal Declaration of Human Rights
Article 1
"All human beings are born free and equal in dignity and rights."

- What is fair for one should be fair for all.
- Treating people equally may not mean treating them the same.

Deontology

- the morality of an action should be based on
- whether **that action is right or wrong**
- doing your duty,

rather than based on the consequences of the action.



Utilitarianism

"*To ignore consequences is to leave an ethical story half untold.*"
Sen A. On Ethics and Economics. Oxford: 1987

- what is morally acceptable - that what produces **the greatest amount of good for the greatest number of people** - social utility
- the moral character of an action is determined by its contribution to the general utility

Alternatives to the classical ethical theories: ethics of care

The ethics of care

• Theory that emphasizes the values that characterize interpersonal relationships: sympathy, compassion, fidelity, respect, love

• Giligan: there are two ways of moral thinking: the ethics of care and the ethics of rights/justice

• Involves a form of empathy — a value ignored in rights based theories

• Women - prone to an ethics of care, in contrast to men — who are rather attached to an ethics of rights and obligations

Virtue ethics

• Aristotle and Plato: cultivation of virtues - central features of moral life

• the ROLE of the motivational structure of the person's character

• Seeks to develop individual character

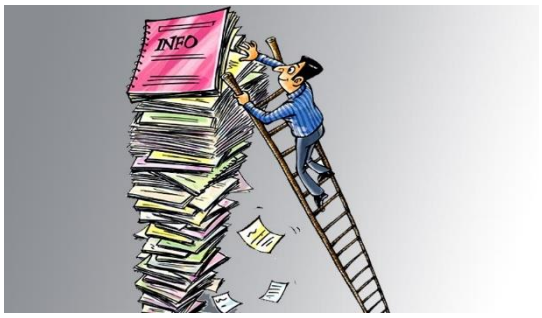
E.g.: therapists who fulfil their responsibilities only out of fear of not getting into a dispute -^ virtues such as compassion, conscientiousness, dedication are excluded

• Assumes good persons will make good decisions

• a person with moral virtues and motivations is more able to finalize an action with a moral ideal, than a morally incorrect or indifferent person.

• We make intellectual judgments based on knowledge and skills and moral judgments based on what we feel is right or wrong

Right to Information and Informed Consent



An appropriate person with decision-making capacity (COMPETENCE) given the required information in an understandable manner and, without coercion (VOLUNTARINESS) makes a decision to approve the course of action.

Purposes of informed consent (IC)

1. **respect individuality, self-determination, and autonomy**

• Promote autonomy

• Bodily integrity

• Individual preferences and priorities

2. **shape the relationship with the patient**

• Learn patient preferences/expectations

• Develop mutual confidence/respect

3. **enhance outcome**

• Realistic expectations

Enhance patient's ability to cooperate with and participate in care:

• Compliance,

• Spotting treatment errors,

• Understanding which side effects are significant and what to do in

Information component

A description, in a language the patient could reasonably be expected to understand, of the:

1. diagnosis;
2. nature and the risks of the proposed intervention/treatment;
3. anticipated results of the proposed intervention/treatment;
4. recognized possible alternative forms of treatment;
5. The recognized:
   - serious possible risks,
   - complications
   - anticipated benefits involved in the treatment
   - possible alternative forms of treatment, including non-prognosis of the disease without applying treatment

6. a decision on whether they still want to be informed if the information presented by the doctor would cause him sufferance.
7. the option NOT to be informed (about the test and/or about the results) and to choose another person to be informed in his place
8. the right to ask and get another medical opinion.
9. the patient has access to personal medical data.

Additional elements:

— Statistics

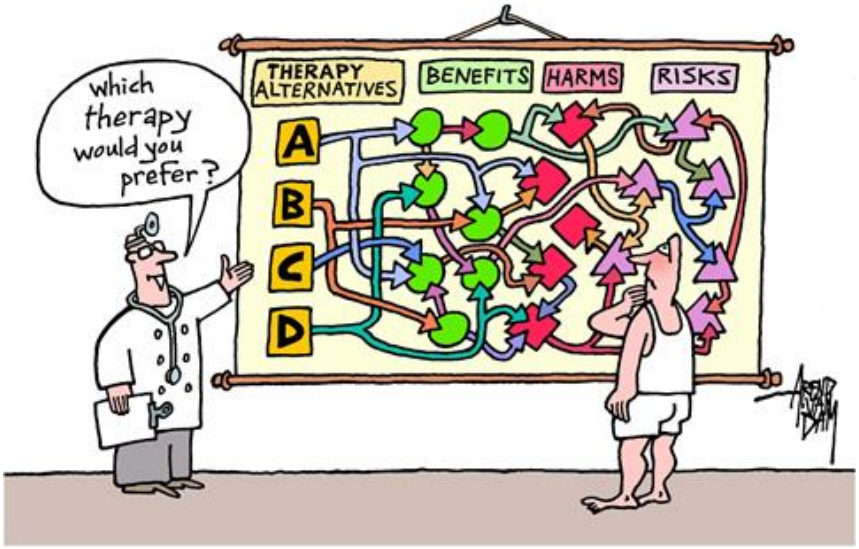
— Educational affiliations, information on the identity and professional status of health service providers,

— Conflicts of interest

— Financial relationships with drug/device companies

Presentation of information

- pay attention to disability - hearing, sight, etc./inability to read
- Need for translation
- Information at a reasonable understanding power of the scientific level for the patient
  - in mother tongue OR a language they know (if an interpreter is found)
  - in a language close to an international one



informed consent

Circumstances in which requirement have been exceptions to consent proposed or allowed

- Emergency
- Threat to community
- Contagious disease
- Dangerousness
- Criminal law enforcement
- Preservation of life/Life of Others
- Prevention of suicide/homicide

Informed consent is also mandatory for:

- collecting samples
- storage
- the use of all biological products taken from the body to establish the diagnosis or treatment with which they agree.

Refusal of an intervention

- The patient has the right to refuse or stop medical intervention by taking responsibility for their decision in writing;
- The consequences of refusing or stopping medical acts should be explained to the patient.

Particularities of informed consent

If healthcare providers consider that the intervention is in the patient's interest, and the legal representative refuses to give consent, the decision may be transferred to a specialized arbitration panel (depending on national legal regulations).

Confidentiality

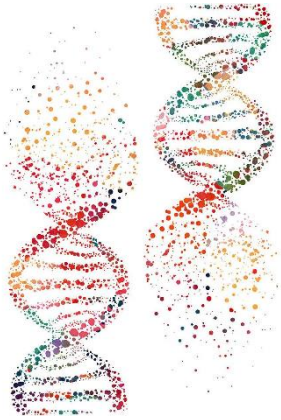
- All information on the patient's condition, the results of investigations, diagnosis, prognosis, treatment, personal data **are confidential** even **after their death**
- Confidential information may only be provided if **the patient gives explicit consent** or if **the law expressly requests** it (depending on national legal regulations).

(1) any interference in the private, family life of the patient is prohibited, unless this interference positively influences the diagnosis, treatment or care provided and only with the consent of the patient.

(2) The cases when the patient poses a danger to himself or to public health shall be considered as exceptions

- Relatives and friends of the patient can be informed about the progress of investigations, diagnosis and treatment, with the consent of the patient (depending on national legal regulations).

Ethical aspects of genetic testing



Defining elements of informed consent for genetic testing

- understanding the nature of testing and its potential implications
- knowing that the test is **voluntary**
- knowing that the patient can ask questions and get answers before making a decision
- knowing the degree of associated risk (of the procedure itself and its subsequent effects)
- who will **benefit** from testing
- what are the strategies implemented for random discovery associated with DNA analysis **(„incidental findings")**
- to know how **personal data** will be used, and subsequently protected, (possibly re-used for research)

• knowing that at any time they can **withdraw** their biological samples from the product bank, including the clinical data obtained

• finding out about any possibly associated commercial interest

• ensuring that any **minor involved**, for whom the parents have given their consent, will be asked to reconsider once he has reached the age of 18

• the right not to know

"It is our choices, that show what we truly are, far more than our abilities."

Genetic testing: reasons, benefits

1. Early diagnosis (± a gene transmission can be stopped)
2. Life decisions, future planning: follow up of a pregnancy, marriage, education, work, insurance
3. Removes the burden of uncertainty

Genetic testing: risks

• **May diagnose a disease for which there is no treatment.**

• Does not predict the severity of a genetic disorder (e.g. cystic fibrosis, polycystic kidney)

• breach of confidentiality: dissemination of information by telephone, mail etc.

• feelings of guilt, depression, suicide, marital instability, impairment of family relationships

• Genetic discrimination: people with genetic defects may be denied insurance (health, life), access to certain schools, services etc.

• Redefines the disease + implies a fatalistic attitude towards health and disease

• Risk of perpetuating racial and ethnic inequalities

• **Psychological consequences**: may affect family relationships and overall well-being

Fear of testing:

• is due to uncertainty related to loss of employment, education, the repercussions of testing: banking advantages etc.

• Studies show that testing was not associated with an increase in anxiety or depression.

Genetic testing-particular situations

1. prenatal testing
2. presymptomatic testing of children
3. genetic susceptibility testing

RIGHT TO KNOW

There is no greater force than the power of information!

Prenatal testing

• beneficial and recommended-in case of **treatable conditions**: e.g. PKU, congenital hypothyroidism

• reduced benefit-screening of carriers (autosomal recessive conditions)

• uncertain benefit

Prenatal screening for malformations (e.g. neural tube defects) or genetic syndrome (Down syndrome)

family factors should be considered: information, reproductive planning

Pre-implantation genetic diagnosis (PIGD)

Used for:

• Testing of monogenic diseases (cystic fibrosis, thalassemia, sickle cell anemia, muscular dystrophy)

• Testing of chromosomial abnormalities (Down Syndrome, trisomy 18)

• Detection of genes for late-onset diseases: Huntington's, family predipositions to cancer

• the benefit of another relative

• selection of sex (for stem cell production etc.)

Advantages of pre-implantation genetic diagnosis

• Allows couples to have a "healthy" child without going through a miscarriage

• Even in case of failure of embryo implantation — this is morally preferable than to end the life of a fully developed foetus

• Helps to emphasise the distinction between the destruction of life (termination of pregnancy) and the inability to save life

• Allows a greater capacity to choose:

• Which embryos to be implanted vs to terminate a pregnancy or not

Pre-symptomatic testing of children

• Also acceptable for late-onset diseases?

• Is there any way to intervene or track the disease, which could be beneficial for the child in the future?

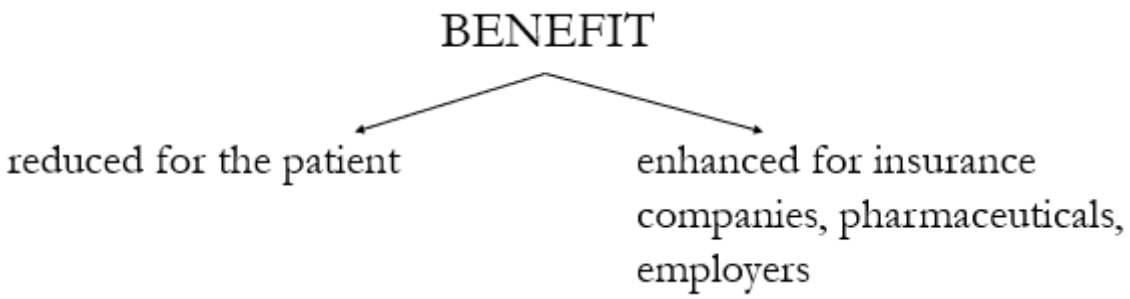
• Ignores the rights of the future adult individual to make an autonomous decision

• confidentiality — that is implied for any adult, it implicitly loses its value in the genetic testing of the child

• emotional and social consequences for the child

Testing genetic predisposition

E.g.: Testing genetic predisposition to cancers, diabetes, Alzheimer's Disease hypercholesterolaemia



- enables free screening programs
- behavioural changes
- pre-symptomatic medical therapies

Possible negative of genetic consequences testing

- genetic discrimination: people with genetic defects may be denied insurance (health, life), access to certain schools, services etc.
- involves a fatalistic attitude to health and disease
- risk of perpetuating racial and ethnic inequalities
- the decision to procreate will be made on the basis of genetic information
- encourages mating of people with "valuable "genes and discourages up to prohibition the people with "defective" genes"
- encourages abortion of foetuses with genetic abnormalities

Ethical issues in genetic testing for inherited cancer predisposition

- genetic information is, by definition, shared, at least in part, by multiple family members.
- In general, it is the responsibility of the patient to disseminate their relevant genetic information appropriately to their relatives.
- Clinicians may, and usually do, help to facilitate this by providing patients with a letter or leaflet to pass to relevant family members.

Most families do understand information with relatives, and the importance of sharing their outright refusal to inform relatives genetic is rare.

HOWEVER:

- patients do not always inform family members of their risk: barriers to disseminations:
- a desire to protect oneself and/or family from potentially distressing information,
- lack of contact or closeness with relatives
- poor understanding of the risks posed and its relevance to others

Case discussion 1

- What happened when one of the two identical twins, having a BRCA2 gene pathogenic variant present in her family, would accept to perform the predictive genetic testing but explicitly state that she would not inform the other sister about her result.

Points to be considered for case 1

• Relatives such as Sarah should not be 'forced' to know, or given without their consent, genetic test results relevant to them (**respect for autonomy**)

• Non-disclosure of the genetic test result to the twin sister could result in her undergoing unnecessary breast screening (if the BRCA2 pathogenic variant were shown not to be present), or not pursuing risk- reducing surgery (if the variant were shown to be present).

• However, non-disclosure of the test results could cause harm to the twin sister. (**non-maleficence**)

Case discussion 2

• What happen if a person suffering from endometrial cancer sample highly suggestive of Lynch syndrome decline the germline testing for the MSH2 and MSH6 genes in order to determine whether the loss of protein expression was caused by a pathogenic variant in one of the genes, causing Lynch syndrome.

+ also declined a referral for colonoscopy.

+ she said she would not share any of the information she had been given with her family.

Points to be considered for case 2

• Reinforce the importance of **physician —patient relationship** → hopefully later the person in case might her mind and agree to be tested, to her benefit and potentially to the benefit of her

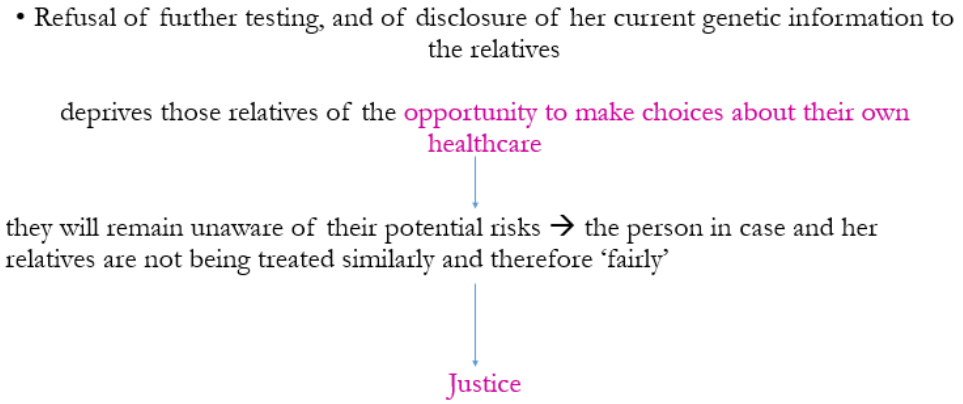
(Beneficence & Non-maleficence)

• Breaching confidentiality may cause mental distress → it should be avoided.

• Any patient has the right to control the dissemination of their medical data including genetic information (**Autonomy)**

Points to be considered for case 2

• All genetic information is inherently familial.



Ethical issues in genetic testing for inherited cancer predisposition

Joint Committee for Genomic Medicine (2019): where consent to inform relatives has been explicitly withheld, "it may be justified to break confidence where the avoidance of harm by the disclosure outweighs the patient's claim to confidentiality"

Respecting the patient's right to confidentiality may be consistent with:

• the principles of beneficence,

• non-maleficence and

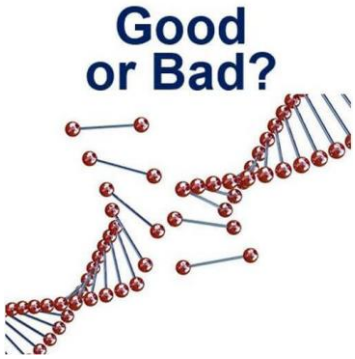
• autonomy as far as the patient is concerned,

Whereas

• failure to breach may result in harm to the relative (contravening the principle of non-maleficence).

• It may also deny the relative the right to potentially life-saving medical treatment and the chance to make decisions about their own health, breaching the principles of both beneficence and autonomy.

1. whether breaching the patient's confidentiality will irreparably damage the clinician-patient relationship.
2. the practical difficulties involved in tracing relatives, especially where the proband is unwilling to assist
3. whether knowledge that confidentiality may be breached so as to inform relatives of risks may act as a deterrent to people coming forward for genetic testing in the first instance



Genetics will change the definition of "the disease"

e.g.: a woman carrying a BRCA mutation who has lived for many years asymptomatically

Might be considered a sick person, while in fact she is not (yet)

Genetic testing means

Take home message

• In order to identify the ethical issues regarding genetic testing in oncogenetics, the health practitioner must understand and selectively apply the main ethical principles and theories:

- Principlism
- Deontology
- Utilitarianism
- Virtues ethics
- Ethics of care

The most sensitive ethical behaviour in oncogenetics stems from the use and application of:

- Informed consent
- Confidentiality

Health practitioners who identify cases related to familial disclosure should consider referral to a genetics service/expert for further discussion, before testing is undertaken.

Refusal of a patient to inform family members of a relevant genetic result may justify the clinician breaching patient confidentiality, however --> this should only be undertaken after attempts have been made to persuade the proband to disclose the information.

- the proband should be informed of the planned disclosure in advance.
- the risks and benefits of any proposed breach of confidentiality should be weighed up in advance
- discussion clearly documented

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PART II

II.1. The structure and the organization of the Department of Oncogenetics

Learning objectives

• definition, goals, objectives and attributions of the Department of Oncogenetics;
• principles of organization and specific activities;
• staff structure and attributions;
• the stages of carrying out the activities;
• performance indicators for achieving the objectives;

Department structure based on the Center Jean Perrin (Clermont Ferrand, France) expertise

Professeur Yves-Jean Bignon

With the contribution of Prof. Yves-Jean Bignon

• Founding Director of the Oncogenetics Department of the Jean Perrin Center

• Founding Director of the OncoGénAuvergne Medical Biology Laboratory at the Jean Perrin Center

• Chairman of the Allied Committee of the League Against Cancer

• Vice President of the Innovatherm regional cluster of excellence

• Pioneer of Oncogenetics in France in 1988, among the first in the world

Evolution of Oncogenetics

• Oncogenetics developed in the early 90s, with the discovery of major cancer predisposition genes (e.g. BRCA1 and BRCA2 for breast cancer or MMR genes involved in the development of colon cancer).

• The concept of cancer risk stratification has been developed along with the possibility of genetic testing and customized risk reduction solutions.

• In terms of prevention, oncogenetics has become a major economic solution for health systems in Western countries in the past years. Although the patients it reaches do not represent the majority in the cancer rate, oncogenetics can save lives in these patient groups.

• The effectiveness of oncogenetics has been demonstrated through a tendency to decrease the incidence of cancers, by improving risk prediction in families of patients with breast, ovarian or colon neoplasia.

Department of Oncogenetics

Definition: highly specialized and multidisciplinary service, with a role in detecting, specifying diagnosis, monitoring and applying preventive strategies in people with hereditary risk for breast, ovary and colorectal cancer.

The aim and objectives of the Oncogenetics Department

Aim: multidisciplinary evaluation of patients and their families, with a hereditary or familial risk of cancer and who require investigation through molecular genetics diagnosis testing.

Objectives:

- Identification of target groups consisting of at-risk patients (breast/ovary, colorectal), through Multidisciplinary Consultancy Group (GCP) meetings.

- Oncogenetic examination – a multidisciplinary action with the participation of qualified medical specialists in oncogenetics field.

- Testing the presence of mutation - molecular biology analysis of genetic risk changes.

- Implementation of oncogenetic monitoring strategies for patients at risk and their families, through personalized Oncogenetic surveillance programs (PSOP)

- Epidemiological studies on hereditary risk of cancer in various populations to establish its distribution characteristics by area, region, country, continent

Appropriateness of the Oncogenetics Department

To achieve the objectives, the work carried out in the Department of Oncogenetics must include three components:

- **clinical** - evaluation and monitoring,
- **molecular** - diagnosis
- **epidemiological** - interpretive.

Organisational Principles of Oncogenetics Department

•Highly specialised multidisciplinary clinical service in oncogenetics;

•Bio-information storage and information communication system;

•Interdisciplinary network of doctors who will put into practice a Personalized Oncogenetic Surveillance Program (PSOP);

• Accredited laboratory for molecular diagnosis of hereditary cancer risk.

Activities in the Department of Oncogenetics

1. Identification and patient recruitment through a network of medical specialists
2. Pre-diagnosis oncogenetic examination: information
3. Molecular diagnosis
4. Meetings of the Multidisciplinary Advisory Group (GCP).
5. Oncogenetic post-diagnosis examination: communication of the result
6. Oncogenetic monitoring of patients and their families (Personalized Oncogenetic Surveillance Programs-PSOP)

Addressability - who should come to the oncogenetics examination?

Common situations - dominant autosomal risk

- 3 or more cases in the same family line
- 2 or more cases in a nuclear family, or for a rare form of cancer
- 1 or 2 cases of cancer in young people
- Multiple cancers in the same person

Recommendations for a medical examination

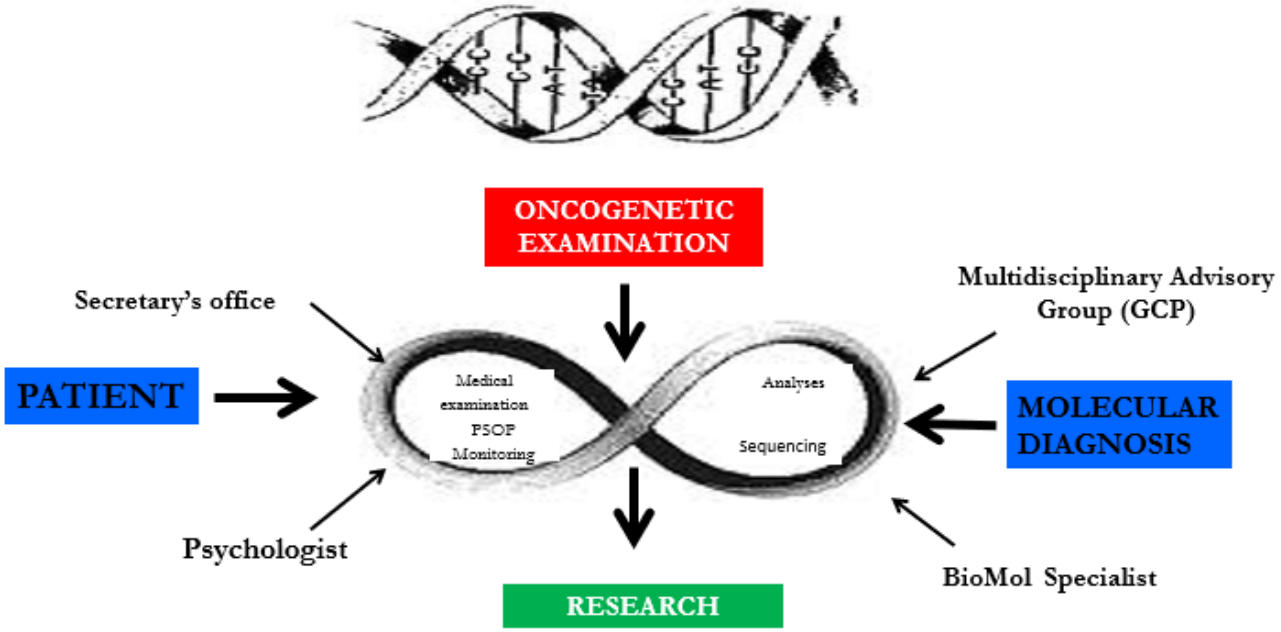
- Ovarian cancer in a woman < 60 years
- Colon cancer in a person < 40 years

• Breast cancer in a woman < 35 years
• Breast cancer in men
• Medullary or basal-like breast Cancer
• Digestive polyposis
• Multiple cancers
• Cancer in a monozygotic twin

Prospects of Oncogenetics Department's development

1. Interdisciplinary oncogenetic network at national and European level
2. Setting up of reference centers in oncogenetic molecular diagnosis
3. Extensive epidemiological studies of population oncogenetics
4. Oncogenetic research programs
5. Setting up of a European consortium of oncogenetics

Department of Oncogenetics - Oncogenetic chain



Structure of the Oncogenetics Department

1. The Secretary's Office of the department, where patients or their families come to make up a file consisting of documents especially designed for analysis and enrollment in the molecular diagnosis program.

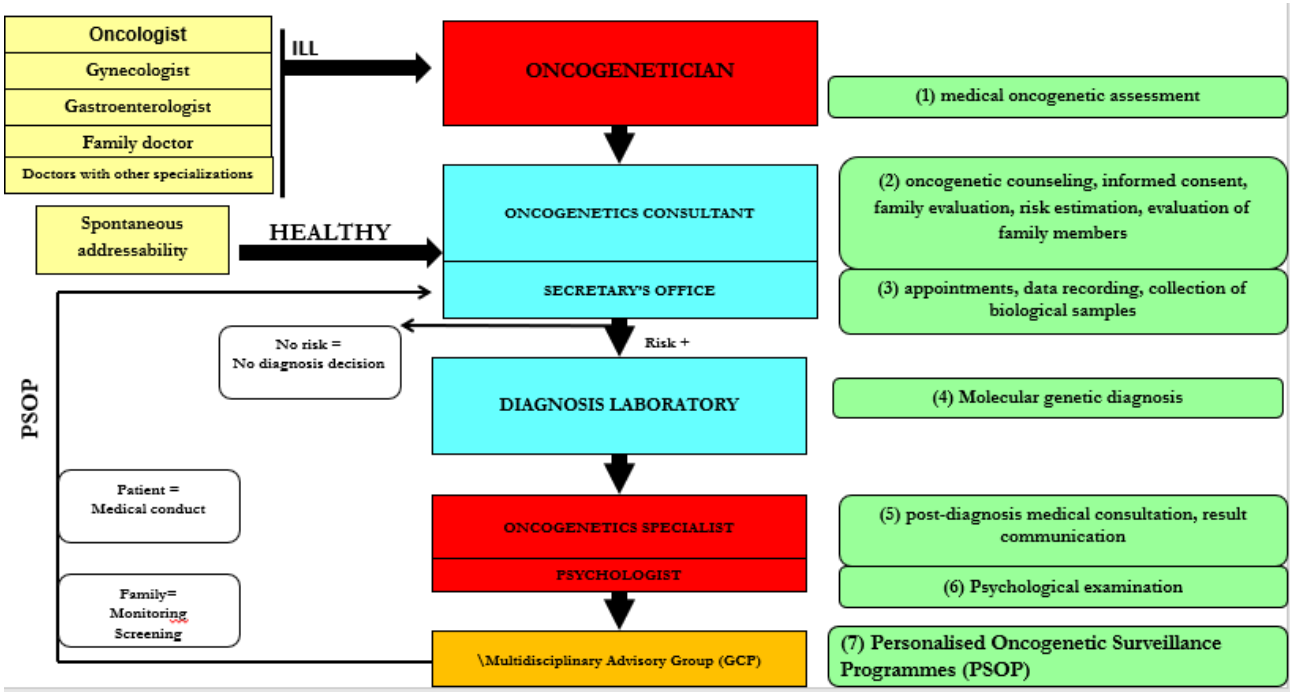
2. Multidisciplinary Advisory Group (GCP), consisting of medical specialists: epidemiologist, geneticist, oncologist, oncologist surgeon, gynecologist, gastroenterologist, endocrinologist, family doctor, ethics specialist, psychologist. The files made within the Secretary's Office of the Department are subject to GCP analysis who, through multidisciplinary consultation, will decide the evaluation of the hereditary risk by performing molecular testing to identify the mutation.

3. Oncogenetics consult (epidemiologist and geneticist) that will bring additional information on hereditary and non-hereditary risk factors.

4. Molecular testing will consist in analyzing the samples collected from the people for whom GCP decided to conduct this investigation and based on the results will establish the diagnosis of hereditary cancer.

5. Personalised monitoring that will include a personalized monitoring protocol applied to the patient identified with genetic mutation and to the family members with positive molecular test.

Structure of the Oncogenetic Department (examination algorithm)



Department of Oncogenetics - specialized staff

1. ONCOGENETICIST

Role: oncogenetic pre-and post-diagnosis examinations, oncogenetic monitoring of patients (Personalized Oncogenetic Surveillance Program – PSOP).

2. MONITORING OF THE FOLLOW-UP PROGRAM OF PATIENTS

Role: oncogenetic counseling, pre-and post-outcome of oncogenetic diagnosis

3. MOLECULAR BIOLOGY SPECIALIST Role: Molecular genetic diagnosis

4. PSYCHOLOGIST

Role: oncogenetic psychological counseling, pre - and post - result of oncogenetic diagnosis

5. SECRETARY

Role: interaction with patients, contact data collection and oncogenetic evaluation (family anamnesis, risk factors, etc.), appointments, data management through specialized software

Multidisciplinary Advisory Group (GCP). Structure

- Oncogeneticist
- Oncogenetics counselor
- Geneticist
- Oncologist
- Gynecologist
- Gastroenterologist
- Endocrinologist
- Surgical oncologist
- Molecular biology specialist – responsible for the diagnosis and molecular biology technician
- Epidemiologist
- Psychologist
- Bioethics specialist

Oncogenetic examination

The examination allows: identification of individuals with hereditary predisposition to cancer and establishment of effective personal risks, with the ultimate goal of prolonging the life of the person/patient by optimizing clinical monitoring.

The examinations are aimed at both cancer patients and healthy individuals with no history of cancer in their family, the recommendation of an oncogenetic check-up being based on the identification of the forms of familial cancer.

The Department of Oncogenetics – patient itinerary

ONCOGENTIC SURVEY – 2 distinct meetings

1. Pre-diagnosis examination

- Stage I: recording the information-secretary and psychologist

- Stage II: hereditary risk counselling - oncogenetics counselor and secretary

- Stage III: signing of informed consent + collection of biological samples -secretary and psychologist

2. Post-diagnosis examination

- Stage I : communication of the result - secretary and oncogenetics counselor - Stage II: monitoring the counselling program - oncogenetics counselor

- Stage III: counselling-psychologist

Stages of oncogenetics examination

• The oncogenetics examination is necessary when there is suspicion of the presence of a high-penetrating genetic disease, based on clinical criteria (e.g. rare cancers, associations of malignancies in the same patient, breast cancer in men) or family aggregation (e.g. 3 people with cancer in the family).

• The examination is addressed to the patient diagnosed with familial cancer aggregation risk, but also to their relatives. If the presence of a genetic mutation is detected, the next step is the construction of the gene tree, which enables the identification of all subjects with potential risk to be carriers of the mutation in question.

• The oncogenetics examination is performed only after obtaining the informed consent of the patient or their legal representative.

• After the preliminary evaluation of the patient's file by the oncologist, the medical examination is individual and is carried out by a multidisciplinary team. Following the consultation, the patient is given the results on the risk profile (hereditary/familial risk to which non-genetic factors are associated), the result of molecular diagnosis, if the patient wants to know it, and the briefing about the preventive measures and/or alternatives of regular customized tracking.

The consequence of detecting a mutation is the oncological record of the subject and the application of primary, secondary or tertiary prevention measures, such as:

- adjustment/modification of medical or surgical therapeutic measures (e.g. elimination of hormone replacement therapy in menopause);

- different application of screening and early diagnosis measures (onset in young ages, high frequency, special tests);

- individualized application of preventive measures: bilateral ovariectomy, bilateral mastectomy, thyroidectomy, ablation of polyps, use of contraceptives, etc.

Stages of molecular diagnosis

1. DNA extraction
2. Mutational pre-screening (recurrent mutations)
3. PCR amplification of regions of interest

4. Purification of PCR products
5. Amplicon sequencing
6. Purification of sequencing products
7. Capillary electrophoresis
8. Interpretation of results

Personalized Oncogenetic Surveillance Program – PSOP

An important step which corroborates the complex information that must contribute to the diagnosed person's understanding of the notion of hereditary risk with all its implications for both the person requesting oncogenetics consultant and for family members identified with a mutation.

Solutions offered by the Personalized Oncogenetic Surveillance Program (PSOP)

People with BRCA mutations

• **preventive surveillance for breast cancer** (BRCA), which consists of: Clinical Breast Examination every 6-12 months and mammography/MRI annually from 18 years old;

• **preventive surveillance for ovarian cancer (OVCA)**, consisting of annual dosing of CA-125 serum from the age of 25 years old, gynecological examination every 6 months and annual transvaginal ultrasound;

• **lifestyle changes** (diet, alcohol, etc.);

• **chemoprevention** (BRCA & OVCA) with tamoxifen, oral contraceptives;

• **bilateral prophylactic mastectomy**, which may reduce the risk of BRCA by >90%;

• **bilateral prophylactic ovariectomy,** which may reduce the risk of OVCA by > 95%, and in pre-menopause reduces the risk of BRCA by approximately 50%.

People with MMR, APC mutations

• **periodic monitoring** by repeating colonoscopy every 2-3 years, since adolescence;

• **the periodicity of testing** is adjusted according to the number, dimensions, characters of the polyps;

• **other risk reduction measures**: colectomy or proctocolectomy that can be proposed, given that the risk of cancer reaches 100% at the age of 50 and that the highest incidence of occurrence is in the 3rd decade of life.

Psychological counseling

• Oncogenetic counselling and testing are the intra-familial experiences the individual and the members of his family faces, which sometimes involves medical decisions, difficult to manage, such as: complex ethical, legal, and psycho-social issues.

• Individual and family psychological counseling is oriented to the development of adaptation and integration models for a better approach to oncogenetic risk, aiming to maintain a high level of the individual and their family's quality of life.

• The basic variables, which influence the person's decision, on the psychological level, of being involved in the process of testing and genetic counseling are: risk perception, expected benefit or limitations of genetic testing, the general or psychological stress or the prognosis of a cancer diagnosis at some point in their life, a lack of confidence in their emotional reactions when they are faced with a negative event, the level of expectation for family support and communication within the family.

• The psychological impact on the individual can be influenced by a number of factors, such as: anxiety issues, misunderstanding of the medical information or family issues that may occur in the various stages of the ***Personalized Oncogenetic Surveillance Program***, in the course of the

preparation of the family history (the compilation of the gene tree), pending the outcome of the test, at getting the result of the oncogenetic test or when the person choses to implement the recommendations for cancer prevention.

Multidisciplinarity

• The multidisciplinary approach proposed in this context aims at facilitating the understanding of genetic predisposition risk as well as the possibilities of medical management of this risk, without generating inappropriate anxiety.

• Geneticists and clinicians will transmit the information in a gradual manner, taking into account the difficulty of providing complex information in an emotional context combined with the the existence of a family or personal history.

• The perception of the disease development risk as well as the knowledge of the genetic aspects relevant in this context, without aggravating emotional concern, and by encouraging the choice between different options, such as making the decision to perform a test and then deal with the consequences of its outcome.

Indicators of achieving the objectives of the Department of Oncogenetics

1. Physical indicators

• total number of people evaluated for clinical and epidemiological determination of oncogenetic risk: number/year;

• number of patients with cancer and hereditary risk for whom the molecular diagnostic test is performed in order to identify the mutation: number/year;

• number of molecular tests performed for people without cancer, but eligible for oncogenetic monitoring, to identify the mutation: number/year;

• number of people as members of the patients families with cancer and hereditary risk for whom the molecular diagnostic test is performed in order to verify the presence of the mutation: number/year

• number of people included in the monitoring system by Personalized Oncogenetic Surveillance Program: number/year

2. Efficiency indicators

• average cost/person diagnosed with oncogenetic risk through clinical and epidemiological investigations (epidemiological screening test): value in national currency;

• average cost/person diagnosed for oncogenetic risk of breast cancer, ovarian cancer or colorectal cancer, through molecular investigations to identify mutation: value in national currency;

• average cost/person related to a genetically diagnosed patient through molecular tests for the risk of breast cancer, ovarian cancer and colorectal cancer, to verify the presence of mutation: value in national currency.

3. Result indicators

• an A% increase in the number of persons detected through epidemiological screening and presenting a potential risk for breast cancer, ovarian cancer and colorectal cancer;

• a B % increase in the number of people with breast cancer, ovarian cancer and colorectal cancer, detected through molecular diagnosis as carriers of gene mutations;

• a C % increase in the number of people in the families of cancer patients, detected through molecular diagnosis, as carriers of gene mutations by dominant autosomal transmission;

• decrease by up to D% of the number of persons diagnosed with breast cancer and ovarian cancer from families with oncogenetic risk, following monitoring by Personalized Oncogenetic Surveillance Program;

• decrease by up to E% in the number of people diagnosed with colorectal cancer from families with oncogenetic risk;

• selection, depending on the type of genetic mutation, of X% of people at genetic risk, who will receive prophylactic medicine or surgical therapy

Department management: expenses for carrying out activities

I. Equipment, functioning

• setting up of spaces with appropriate route plan (secretary's office, Oncogenetic examination room, sample collection room and a room for individual discussions with a psychologist or other specialists, molecular analysis laboratory with appropriate route plan)

• equipment, laboratory of molecular analysis

• maintenance of the Department (expenses with maintenance, repairs, additional equipment etc.)

II. Expenses for carrying out the activities

• expenses for services rendered by the oncogenetic specialist, molecular diagnosis officer, Multidisciplinary Advisory Group, secretary's office;

• expenses for molecular diagnosis activity consisting of paying for consumables and activities for molecular diagnostic techniques optimization (internal and external validation), operation and maintenance of the diagnostic laboratory;

• the costs related to law-abiding procurement of the office supplies necessary for the preparation of the documents in the monitored person's file, the methodology, and the printed informative materials on oncogenetic risk (for physicians, nursing staff, patients and their families);

• expenditure on printing methodological guides and informative materials on oncogenetic risk (for physicians, environmental health professionals, patients and their families);

• postage costs, telephone calls for scheduling patients and family members at an oncogenetic examination;

• expenses resulting from trainings: expenses for the organization of education and training courses, symposiums, conferences and congresses;

• expenses related to the activity of improvement, maintenance and data entry in the websites and the National Cancer Register.

Take home message

• Oncogenetics involves a multidisciplinary process which brings together geneticists, genetic counselors, oncologists, clinicians, and psycho-oncologists in order to better respond to the 3 dimensions of the primary education (need for information), help in decision making and psychological support (assistance in adaptation).

• Regular exchanges of opinion between professionals will allow the collection of information and the analysis of perceptions for a better overall understanding of expectations, values, choices and possible psychological difficulties. This process must be the guarantee of an approach aimed at the respect and autonomy of the patient in making their decision.

• To be acceptable, oncogenetic evaluation and monitoring must prove safety, as well as a reversal of the balance of "side effects", the result being in favor of improving the survival and/or quality of life of the individual.

• Through specific activities within specialized Departments Oncogenetics will contribute to reducing mortality also by early detection and the increase of the quality of life through personalized preventive or therapeutic actions.

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How to use the oncogenetic software

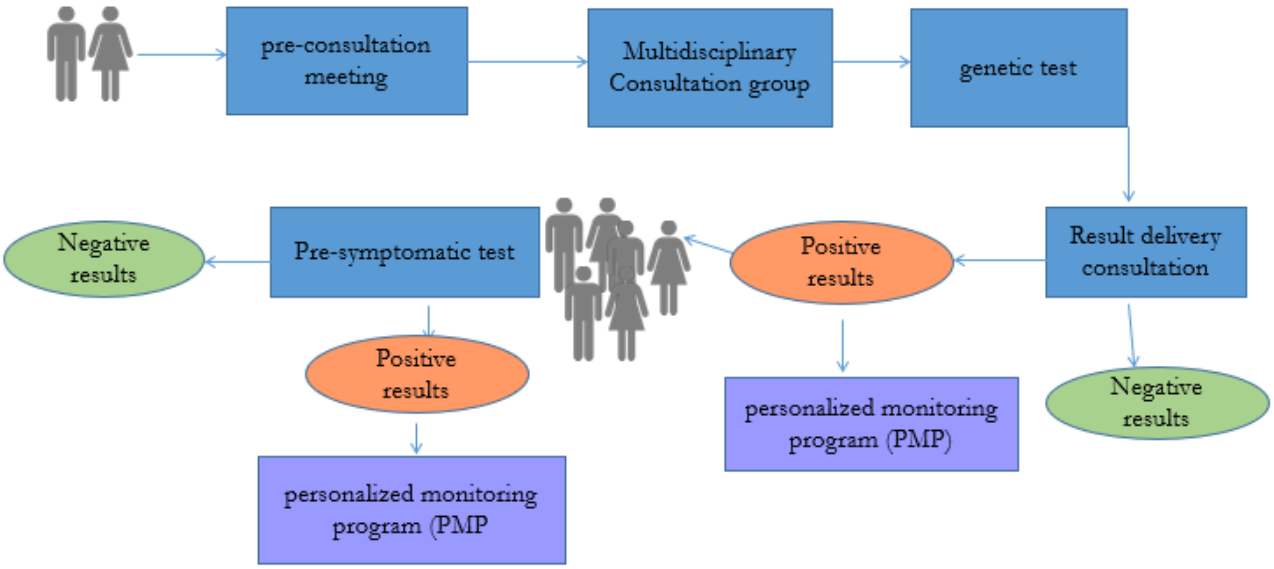
Learning objectives

• to know the general follow up of oncogenetical program

• to understand the role and implication of secretariat department in each step of oncogenetical program

• to be able to use oncogenetical software;

Case general follow-up presented in the context of hereditary cancer syndrome



The first oncogenetic consultation

• The first oncogenetic consultation have several stages.

• First, a pre-consultation meeting with a genetic counselor will be organized. During this meeting - the most complete as possible genealogic tree will be designed. More information will be asked:

- the personal data
- the questions about the diagnostic: -what is the clinical, imagistic or anathomopathological diagnostic ? Type of tumor, biochemical investigations, tumor markers etc.
- what was the age of diagnosis ?
- Which family members have cancer?
- How old were they at the time of diagnosis?
- Question about environmental and behavioral risk factors

• The investigation will thus focus on the cancer history of the two branches of the family, maternal and paternal. Medical records should be collected as far as possible. Hereditary transmission will be explained to the patient and more information and additional materials will be provided to the patient.

• In most of the cases, hereditary predisposition to cancer is at risk of transmission to one of two (50%) children from the next generation (so-called "autosomal dominant").

Multidisciplinary consultation group

All the files of the patients will be discussed in the Multidisciplinary Consultation group. This group will evaluate the cancer risk and decide about the opportunity of genetic testing . The index patients who are considered to be with high rick will be invited for the second consultation. During this consultation the patient will be advises on a possible hereditary predisposition to cancer, whether or not it offers a genetic test, and transmits the recommendations for care and follow-up of the patient and his or her close relatives, in the eventuality of positive result of genetic test.

The initial genetic analysis is performed on a blood test taken from the affected family member with the highest probability of being mutated (= index case).

Patients will sign an informed consent (mandatory) which will notify them about: • the right to refuse to have the result communicated,

• total anonymity of results

• the relevance of a positive or negative result,

• the importance of this result for the rest of his family, the intellectual property rights over the test results

Psychological counseling is systematically offered. Except for specific indications of urgency, the time between the first consultation and the outcome report is variable and can be long (at least 12 months).

The result delivering consultation

• Once the genetic analysis is completed, the patient receives an email or a phone call informing about the availability of the results. Consultation is essential for the delivery of these results, their explanation by the oncogenetician, and consideration of any possible follow-up.

• If no genetic abnormality is identified the analysis is called negative, meaning that the genes involved do not seem to be responsible for the family history. Depending on the family history, the doctor will recommend appropriate medical supervision. Other genes may be tested.

• If a genetic mutation is identified, such mutation is therefore responsible for an increased risk of developing cancer in the family: in those relatives who have been inherited the mutation. Regular monitoring and specific care of individuals at genetic risk will then be proposed for early screening [Personalized Monitoring Program (PMP);].

• Psychological counseling is systematically recommended.

Pre-symptomatic test

Disease causing mutation is passing down from generation to generation: so the carrier must inform his/her relatives - at best directly, or through the oncogenetician – all the branch of heredity affected (if it can be specified) so that they could made also a test to determine whether or not they carry the same mutation. They will return in this case as part of a pre-symptomatic test.

Can determine the status of a tumor-free individual regarding to a significant mutation previously identified in their family.

It has 3 stages.

STAGE STEP 1: Consultation with the oncogenetician

• This is an information consultation that explains the characteristics of this anomaly. It answers questions such as:

• How is this anomaly transmitted?

• How likely are you to have inherited it?

• What risk will this create?

• What monitoring will be proposed?...

• It is also possible that this preliminary information may be transmitted by the index case or the relative in charge of the family information.

STEP 2: time for reflection

A time for reflection is indispensable. When the decision is made, a new meeting is scheduled that allows the molecular investigation to be initiated as soon as possible because the risk of being a carrier is 50%. The psychologist's intervention, which is very useful and complementary to that of the medical team, must be involved in the reflection time.

STEP 3: receiving the result

• The time between the first consultation and the result rendering is usually a maximum of 2-3 months, and the oncogenetician deliver the results in an additional consultation.

- If results are negative - the absence of the family mutation (statistically one of two), then the person has a cancer risk comparable to the general population.

- if the mutation is present then the person has a higher risk than the general population to develop cancer.).

• The follow-up of newly identified hereditary predisposed cancer patients is discussed during multidisciplinary consultation meetings (MCM).

PMP- personalized monitoring program

• Usually presented during the consultation aimed at molecular results announcement.

• Several other documents can be given to the patient during this consultation (information leaflet on genetic predispositions, program presentation booklet, personalized follow-up notebook ...) and, if patient agrees to be included into the follow-up program, a specific agreement is necessary. It can be signed at the end of this consultation or after a period of reflection

• This is generally accepted follow-up scheme to use an already scheduled consultation to hand over the PMP program to the patient. However, it is only applicable to the people with very high risk of newly identified cancer, prospectively.

• Different approaches need to be adopted to solicit the agreement and then hand over the PMP program to the people with molecular diagnosis and high risk of cancer identified in other institutions.

• They are initially contacted by phone or mail so the possibility to follow-up a patient management program is presented to them. The next step is to propose a consultation and patients must allow the updating of individual and family data. It can be accompanied by a clinical assessment including, if it is necessary, an additional examination. At the end of this stage, patient must sign the informed consent. Therefore follow-up program would be discussed later, during multidisciplinary consultation meetings (MCM), and the developed PMP could be delivered by mail or presented during a new consultation.

• It should be noted that the first letter sent may contain, in addition to the presentation of the program, a questionnaire to update the patient`s data and the consent to participate to the program. In this case, all preliminary consultations can be skipped and the patient will meet the team for the first time at the consultation aimed for prepare and deliver the PPFU program.

Internal tracking

• If the person agrees to be followed within the institutions associated to the program, he/she will benefit of several advantages: **multidisciplinary** (involving all the partners likely to be solicited as part of its follow-up) and **facility** (consulting will be done in a single day and in the same place if possible).

• New interterm organizations need to be found to streamline the circuits, promoting the program's adherence and ensuring long-term follow-up.

The programs have carried out one or more of the following actions:

• setting up joint operations between the different services involved in the monitoring;

• creation of secretariats or monitoring centers to ensure centralized and coordinated planning of surveillance consultations and reviews;

• creation of a single point of contact (unity of place) facilitating the patient`s access to multidisciplinary facilities;

• scheduling, where possible, all examinations and consultations : gynecological examinations, radiological monitoring, endoscopic procedures, biological assessments, consultation and genetic counseling, psychological support in one or two days (unity of time)

External tracking

• The person may also decide to be followed outside the institutions associated with the program, near his place of residence. In this context, the program aims to structure a network of professionals (mainly gynecologists, gastroenterologists and radiologists) practicing in the nearest city in public or private health institutions .

• A few different approaches have generally been adopted to encourage professionals to participate in this network:

-contact all professionals in the region by mail, telephone or at information meetings;

- demand the regional cancer networks, the departmental screening structures or associations, this approach could make possible to reach a wide range of specialists;

- ask directly to the patient to disseminate the information to some chosen referring doctors

Compendium of follow-up review results

Since the patient starts to be monitored, the program must collect the results of the surveillance and examinations. Different data retrieval methods can be proposed, especially when monitoring is made in the external manner:

• direct request of the monitored person,

• collaboration/contact with the referring doctor,

• possible appointment for a summary consultation.

The tracking software, capable to generate alert messages when the results of the examinations have not been received (the planned consultation not performed or data not transmitted), can be used . But without this kind of software the secretariat department will send a reminder message to the patient and/or his doctor with a pre-defined periodicity, by various ways (emails via secure messaging, phone calls etc.).

When receiving the reports, the quality and the results of the examinations are uploaded in the patient`s personal file:

• if the follow-up program is respected then the next exams are automatically programmed according to the periodicity established in the PMP;

• if any undercurrent events have occurred (special radiological monitoring, pregnancy, any cancer sign occur) or if preventive surgery has been performed then surveillance is discussed again during a new multidisciplinary consultation and the PMP may be updated

Data computerization and launching the monitoring

• The consent signature of the given by the patient marks the real beginning of its monitoring coordinated by the PMP program.

• The patient`s file will be created and saved in a software which give the possibility to trigger scheduling the appointments, alert messages, reminders etc, ensuring that the monitoring is in line with the PMP program recommendations (i.e. planned examinations, achievement dates, frequencies etc.).

• There are several types of software, and they all include two monitoring mandatory features: editing appointment, reminders and alert messages, and collecting the results of follow-up examinations.

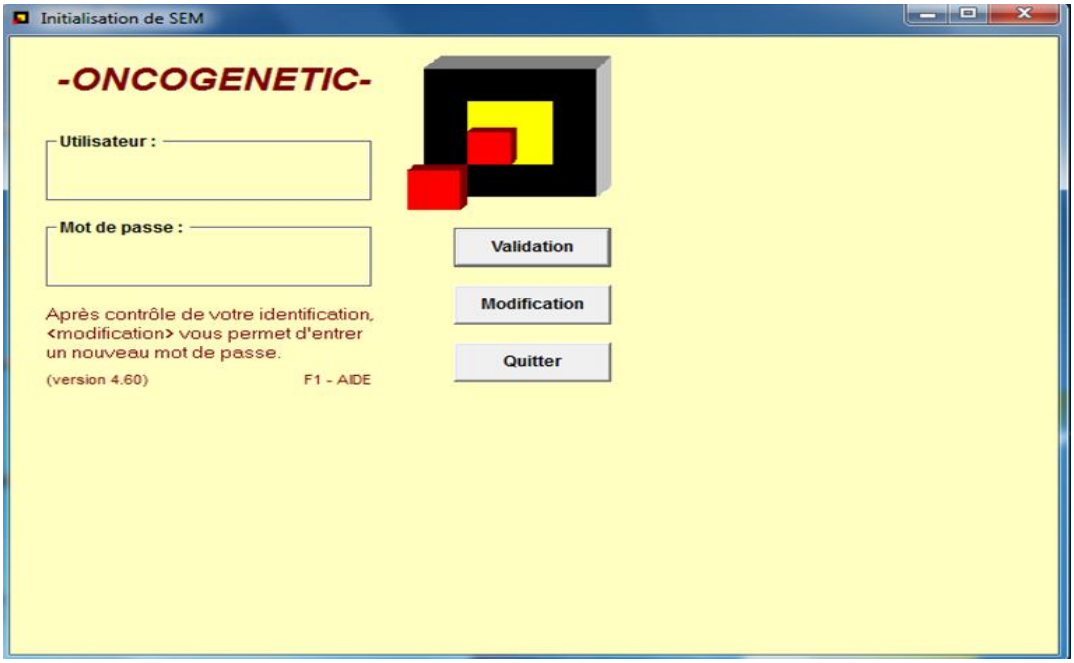
IT resources

• For all patients considered to be in a high risk a personal file is created in a database using one of available software. In our Department the patient`s database is managed using a modified **SEM (Statistics Epidemiology Medicine) software – named GATACA .** The software is created at Centre Jean Perrin from Clermont-Ferrand, France , and was given to us for use.

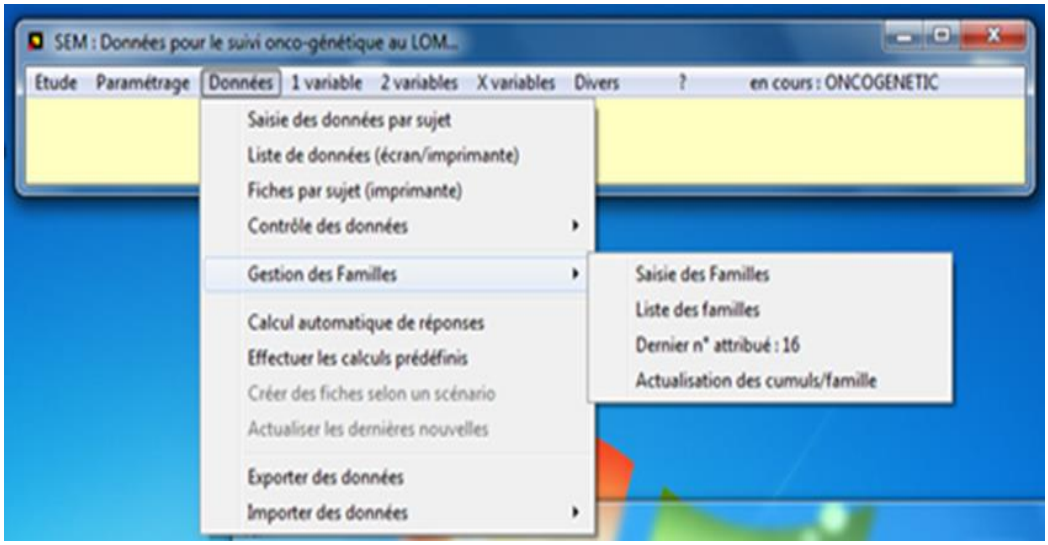
• It's a flexible medical and biological data management tool. In this new version, this management is based on a standard database manager (Microsoft ACCESS and SQL-Server are supported). All data types are supported, especially textual data such as comments. SEM allows you to manage a medical file.

• SEM is particularly efficient for the management of trials (therapeutic or other) and surveys: to do this, nearly half of the programs perform tasks allowing the creation of questionnaires, the printing of cards, labels, controlled seizure answers, randomization, extractions, lists, imports/exports, mergers, etc., while offering functions to ensure the confidentiality of data

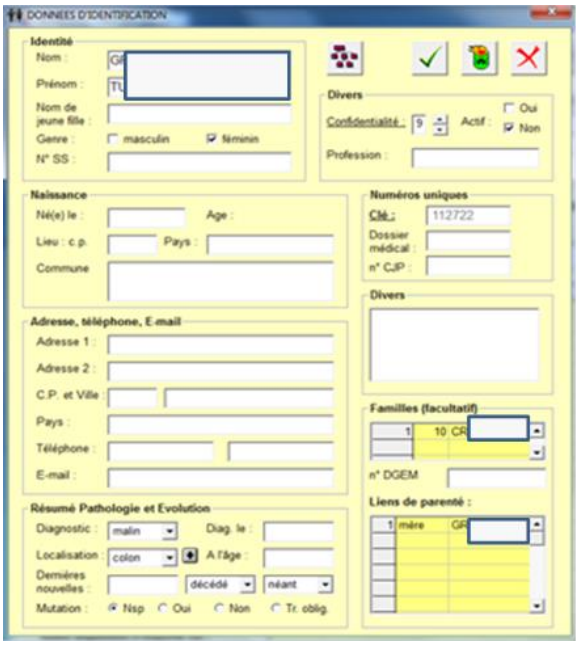
• software that also has a module dedicated to consultations and even oncogenetics laboratories, and is therefore able also to manage the clinical and biological annotations, genetic data (family trees, family histories...), genetic tests and their results



SEM homepage, user identification



Database management



Patient`s personal file

• Oncogenetic Consultations

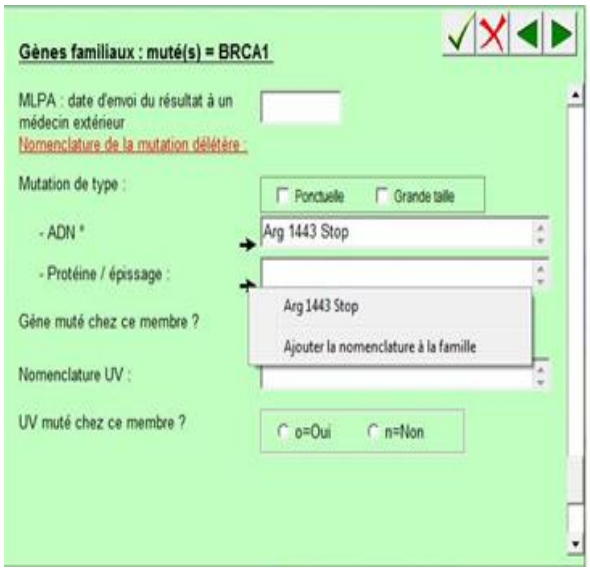
• Management of patient and related records, management of family records, construction of family trees, genetic testing requirements, scheduling of appointments

• Oncogenetics Laboratories

• Management of patient and family records, genetic tests, results, editing of reports

• Collection, recording and archiving of data and/or monitoring reports

Entering tracking forms with configurable content | Digital archiving of test records within the person's file



Genetic testing file

Record data about genetic alteration identified after genetic testing for index case and the family members

• Once the PMP is prepared and submitted, the programs must ensure that the monitoring of the patient is carried out, regardless of the location of his/her care. To do this is necessary the use of monitoring software dedicated only to this surveillance and presenting at least two essential functionalities for monitoring people at very high risk of cancer:

1) sending reminders of appointments/warning messages and
2) collecting the results of follow-up examinations.

Take home message

ØThe oncogenetical program is a both logical and complex follow up procedure that must be implemented in the Department of Oncogenetic

ØThe secretariat department activity consist of patient`s personal data collection, monitoring program management and the result of the monitoring program storage, but also joint operations between the different services involved in the monitoring and the patient`s family

ØThere are several type of used software to help this activities, but all of them as a minimum offer two features that are mandatory for monitoring: editing appointment reminders and alert messages, and collecting the results of follow-up examinations.

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II.3.1. The selection criteria. Difficulties and challenges in hereditary colorectal cancer

Learning objectives

• understanding the characteristics of hereditary colorectal cancers
• understanding who should come to the oncogenetics consultation
• criteria for identifying hereditary risk in digestive cancers (HNPCC + polyposis)
• clinical criteria
• medical necessity criteria for specific genetic tests
• molecular testing strategies in hereditary colorectal cancer

Introduction

Approximately 5 to 10 percent of colon cancer is hereditary.
The major hereditary colon cancer syndromes are:
• Lynch syndrome - previously known as Hereditary Non-Polyposis Colorectal Cancer (HNPCC)
• Familial Adenomatous Polyposis (FAP)

Over the past few decades, the expansion of familial cancer registries and advancement in genomics have led to the development of clinical diagnostic criteria for specific hereditary syndromes as well as the discovery of multiple genes in which germline mutations predispose individuals to syndrome-associated neoplastic manifestations.

Based on an individual's personal and family history of cancer, the oncogenetic counselors can:
• identify the level of risk
• determine if genetic testing is appropriate
• provide guidance for an early detection and prevention strategy.

Characteristics of cancers with hereditary predisposition

• Family history = the best and easiest way to suspect mutation carriers at predisposition genes
• General clinical features of cancers with hereditary predisposition: - family agglomeration (≥ 3 cases of cancer in the same family)
- age at early diagnosis, compared to cancers in the general population - multifocal/bilateral cancers
- multiple cancers

Selection criteria for predisposition to colon cancer

• Colon cancer diagnosed at < 50 year of age
• Multiple colonic malignancies present, either synchronous or metachronous
• Multiple primary cancers diagnosed, either colonic or extracolonic
• Over a lifetime, ≥ 10 adenomas present or ≥ 2 histologically characteristic hamartomatous polyps
• Colon cancer in > 1 generation of the individual's family
• Clustering of extracolonic cancers in family members

Who should come to the oncogenetics consultation?

• Common situations - autosomal dominant risk - 3 or more cases in the same family line
- 2 or more cases in a small family, or for a less common form of cancer
- 1 or 2 cases of cancer in young people

- multiple cancers in the same person

• Consultation guidelines for the identification of hereditary colon cancer: - colon cancer in an individual under 40 years old

- digestive polyposis - multiple cancers

- cancer in a monozygotic twin

Criteria for identifying hereditary risk in digestive cancers (HNPCC + polyposis)

• The Oncogenetics Department frequently receives patients from the oncology services:

• Digestive Oncology

• Gastroenterology

• General surgery

• **Gynecology - !!! 5% of uterine (endometrial) cancers can be hereditary, under Lynch syndrome**

- information is needed about: histology of bioptic curettage, age, other cancers, hereditary-collateral history of tumours associated with Lynch syndrome

- Patients with endometrioid, undifferentiated or clear cell histo-types, younger than 60 years or with at least one related to Lynch syndrome-related tumours are contacted after completing their primary treatment and invited to schedule an appointment at the OD.

Selection criteria for genetic testing in hereditary colon cancer

• HNPCC and FAP syndromes cause 2-4% and 1% respectively of colorectal cancer cases

• **Lynch syndrome**

- Bethesda criteria

- Amsterdam criteria

• **Familial Adenomatous Polyposis (complete/attenuated form)** - clinical diagnosis (colonoscopic - number of polyps)

• For 20-25% of CRC cases that have suggestive features for hereditary cancers, responsible genetic abnormalities are not yet clearly described and are known as family CRCs

- the strategies of monitoring the individual and the family are based on clinical criteria

Criteria for identifying hereditary risk in colorectal cancers (HNPCC + polyposis)

3 essential questions:

• Do you have a first-degree relative who developed colorectal cancer before age 50?

• Did you develop colorectal cancer before the age of 50?

• Do you have at least 3 relatives who have developed colorectal cancer?

These 3 questions identify:

• 77% of persons at high risk of hereditary predisposition

• 95% of Lynch families with MMR mutations

Clinical criteria for determining the increased risk of hereditary colorectal cancer in HNPCC

• were established on the basis of four expert meetings in Amsterdam (2) and Bethesda (2)

• the clinical elements of the four guides are complementary

• meeting the criteria is sufficient to suspect the familial character of colorectal cancer and justify directing the patient or family to the Department of Oncogenetics

Amsterdam criteria I	Amsterdam criteria II	Bethesda criteria (modified)
At least three relatives with colorectal cancer	At least three relatives with HNPCC-associated* cancer	A case with colorectal cancer at age < 50 years
One case should be first degree relative of the other two	One of the cases must be first degree relative	Presentation of synchronous and metachronous colorectal cancers, or other HNPCC-associated tumours
Presentation of cancer in two successive generations	Presentation of the different types of cancer in two successive generations	Presentation of one case of microsatellite instability-associated colorectal cancer at age < 60 years
One of the cases at age < 50 years	One of the cases occurs at age < 50 years	A first-degree relative with colorectal or HNPCC-associated* cancers at less than age 50
Exclude cases of familial adenomatous polyposis	Exclude cases of familial adenomatous polyposis	At least two first or second degree relatives with colorectal or HNPCC-associated* cancers, of any age

HNPCC = hereditary non-polyposis colorectal cancer
* The HNPCC-associated cancers: Colorectal, endometrial, stomach, ovary, ureter/renal pelvis, brain, small intestine, hepatobiliary duct, and cutaneous

Medical necessity criteria for specific genetic tests

Hereditary non-polyposis colorectal cancer (HNPCC)/Lynch syndrome (LS):

• genetic testing for HNPCC (MLH1, MSH2, MSH6, PMS2, EPCAM sequence analysis) medically necessary for members who meet any of the following criteria:

- Member meets Amsterdam II criteria or revised Bethesda guidelines (see appendix); *or*
- Member is diagnosed with endometrial cancer before age 50 years; *or*
- Member has a 1st- or 2nd-degree relative with a disease confirmed to be caused by a HNPCC mutation (genes MLH1, MSH2, MSH6, PMS2, EPCAM) upon testing of the 1st- or 2nd-degree relative; *or*
- Member has ≥5% risk of LS on a validated mutation prediction model (e.g., MMRpro, PREMM[MMR]predict).

Lynch Syndrome - summary

Lynch syndrome should be suspected:

• patients with synchronous or metachronous colorectal cancer (CRC)
• CRC prior to 50 years of age
• multiple Lynch syndrome associated cancers (e.g., CRC and endometrial, ovarian, stomach, small intestine, or renal pelvis/ureter)
• familial clustering of Lynch syndrome associated cancers.

Candidates for genetic evaluation:

• all newly diagnosed patients with CRC (alternatively, those diagnosed < 70 years);
• endometrial cancer < 60 years;
• first-degree relative of those with known MMR/EPCAM gene mutation;
• individuals with a CRC with > 5 percent chance of an MMR gene mutation by prediction models;
• family cancer history meeting Amsterdam criteria or revised Bethesda guidelines.

Medical necessity criteria for specific genetic tests

Adenomatosis polyposis coli (APC):

• genetic testing medically necessary for either of the following indications:

• members with greater than 10 colonic polyps; *or*

• members with a desmoid tumor, hepatoblastoma, or cribriform-morular variant of papillary thyroid cancer; *or*

• members with 1st-degree relatives (i.e., siblings, parents, and offspring) diagnosed with familial adenomatous polyposis (FAP) or with a documented APC mutation. The specific APC mutation should be identified in the affected 1st-degree relative with FAP prior to testing the member, if feasible. Full sequence APC genetic testing is considered medically necessary only when it is not possible to determine the family mutation first.

Risk prediction models for Lynch syndrome

To guide the guidance for further assessments, in the case of people without a personal history of colorectal cancer, but with AHC suggestive of sdr Lynch, a risk prediction model can be used.

• **PREMM 5 model** = clinical prediction algorithm that estimates the likelihood of an individual carrying a MLH1, MSH2, MSH6, PMS2 or EPCAM gene mutation.

• **MMRpro** - BayesMendel Lab - Projects at Harvard = statistical model, associated with a software, to evaluate the likelihood of an individual carrying a mutation of the MLH1, MSH2 and MSH6 MMR genes

Necessary:

• information about the evaluated individual/personal or family history of colorectal cancer, endometrial (uterine) cancer, or other cancers associated with Lynch syndrome

• types of cancer and age at diagnosis of first-degree relatives from the affected part of the family (parents, siblings, children)

• types of cancer and age at diagnosis of relatives of the second degree from the affected part of the family (grandparents, aunts, uncles, grandchildren)

Molecular testing strategies in hereditary colorectal cancer (CRC)

Risk category	Eligibility	Test
Family history of CRC	- Moderate-risk or high-risk family history	dMMR/pMMR
	- Amsterdam criteria families where MMR testing is not possible	Panel testing of affected individuals or unaffected testing
CRC	Universal testing	dMMR/pMMR and subsequent testing
Early onset CRC (EOCRC)	Diagnosis of CRC at 30 years and under	Panel testing determined by MMR status
Linch-like syndrome	dMMR tumours without hypermethylation/BRAF pathogenic variant and no constitutional pathogenic variant in MMR genes	Somatic testing panel
Multiple colorectal adenoma (MCRAs)	MCRAs under 60 years of age with ≥ 10 adenomas, or patients over 60 years of age with ≥20 adenomas, or ≥10 with a family history of multiple adenomas or CRC	Gene panel testing
dMMR, MMR proficient; MMR, mismatch repair; pMMR, MMR deficient		

Source: Monahan KJ, *et al. Gut* 2019; 0:1–34. doi:10.1136/gutjnl-2019-319915

Selection criteria for genetic testing in colorectal cancer predisposition

I. Suspicion of Lynch syndrom (MLH1, MSH2, MSH6, PMS2, EPCAM)

II. Adenomatous polyps/polyposis (APC, MUTYH, POLE, POLD1)

III. Hamartomatous polyposis (STK11, BMPR1A, SMAD4,PTEN); clinical phenotype for Peutz-Jeghers syndrome, juvenile polyposis, Cowden disease

Absence of multiple adenomatous polyps/polyposis

• ***Indications based on individual characteristics***

- Any tumor of the Lynch spectrum, (including cutaneous) of MMR phenotype

- If tumor phenotype not available: any Lynch spectrum tumor diagnosed at age <41 years; 2 Lynch spectrum tumours, the 1st diagnosed at an age of <51 years; 3 Lynch spectrum tumours, regardless of age at diagnosis

Note: 1 advanced adenoma (> 1 cm and/or high grade dysplasia) can replace one (and only one) tumor in the case of tumours multiple primitives phenotype

• ***Indications based on family history***

Familial aggregation of cancers of the Lynch syndrome or POL3 spectrum validating the Amsterdam criteria or at least 2 of the 3 criteria Amsterdam

Multiple adenomatous polyps or adenomatous polyposis

• 15 colorectal adenomas regardless of age, characteristics of adenomas ("advanced" or not) and family history

• From 5 to 14 colorectal adenomas and 2 of the following secondary criteria:

- ≥ 2 advanced adenomas
- all adenomas occurred at age <51 years
- personal history of CRC diagnosed at age <61 years
- personal history from other cancer of the Lynch syndrome spectrum (extra-colorectal)
- CRC with KRAS G12 C variant regardless of age at diagnosis
- history of CRC or multiple colorectal adenomas (> 5) in siblings diagnosed at age <61 years - ≥1 duodenal or ampullary adenoma or adenocarcinoma
- profuse glandulocystic gastric polyposis
- multiple sebaceous lesions
- consanguinity

• Gastric adenomatous polyposis

Difficulties/challenges

• Not all families with increased hereditary risk of colon cancer come to Oncogenetics consultation.

• **Causes:**

- poor communication in the family
- family members who do not want to expose their risks
- families with too few members for a sufficient number of cancer cases to occur - the emergence of new mutations
    - it will take several generations before the model is recognized in the family
- change of penetrance over time
    - older generations may be less likely to develop cancer or develop a different tumor pattern

Take home message

The major hereditary colon cancer syndromes are Lynch syndrome (HNPCC) and Familial Adenomatous Polyposis (FAP); HNPCC and FAP syndromes cause 2-4% and 1% respectively of colorectal cancer cases

Family history is the best and easiest way to suspect mutation carriers at predisposition genes

Oncogenetics consultation can identify the level of risk, determine if genetic testing is appropriate, provide guidance for an early detection and prevention strategy

Common situations when hereditary colorectal cancer can be suspected: 3 or more cases in the same family line, cases of cancer in young people, multiple cancers in the same person, cancer in a monozygotic twin, digestive polyposis

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II.3.2. The selection criteria. Difficulties and challenges in breast and ovarian cancer

Learning objectives

• To know which are the selection criteria for BRCA genetic testing
• To know which are the strategies for early detection of cancer in BRCA mutation carriers
• To know which are the screening recommendations in BRCA mutation carriers
• To know which are the risk-reduction and therapeutic strategies in BRCA mutation carriers
• To know the management of women without identified BRCA mutations

Hereditary breast and (HBOC): ovarian cancer syndrome introduction

• ≈ **7 %** of all **breast cancers** (BC) and
• **11–15 %** of **ovarian cancers** (OC)
- inherited predisposition (germline mutations in high penetrance **BRCA1/2 genes**)
• mean cumulative BC risk at age 70 years = **57 %** for **BRCA1** and **49 %** for **BRCA2**;

Other forms of hereditary cancer

Syndrome	Gene mutation	Associated cancers
Li Freumeni syndrome	TP53	Breast (female), sarcomas (connective tissue cancers), bone, adrenal gland, brain, leukemia, pancreatic, colon, liver
Cowden syndrome	PTEN	Breast (female), thyroid, uterine, colon, kidney, melanoma
Peutz-Jeghers syndrome	STK11	Breast (female), uterine, ovarian, colon, gastric, small bowel, pancreatic, lung, and cervical
Lynch syndrome	MLH1, MSH2, MSH6, PMS2, EPCAM	Colon, uterine, ovarian, gastric, urinary tract, small bowel, CNS
	RAD51C, RAD51D	Ovarian
	CHEK2	Breast (female and male), prostate, and colon
	PALB2	Breast (female)
	CDH1	Gastric and breast (female)

HBOC families associated to BRCA1 or BRCA2 germline mutations present:

- autosomal dominant hereditary pattern
- early ages of cancer onset
- bilaterality
- male breast cancer.

Criteria for BRCA genetic testing

• **Based on personal and family history** to estimate a minimum 10 % detection rate

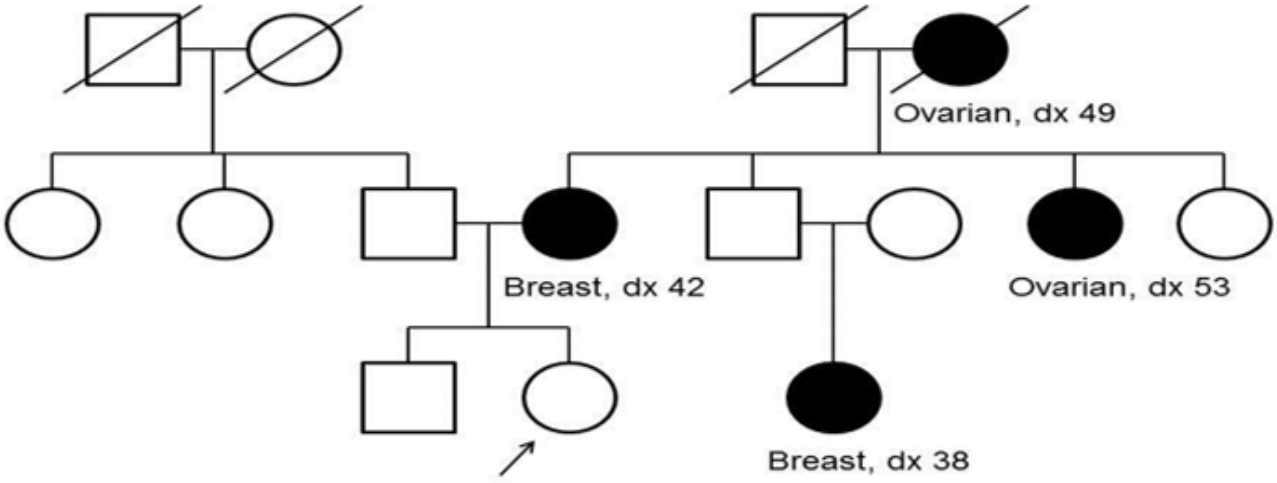
• BRCA 1 and 2

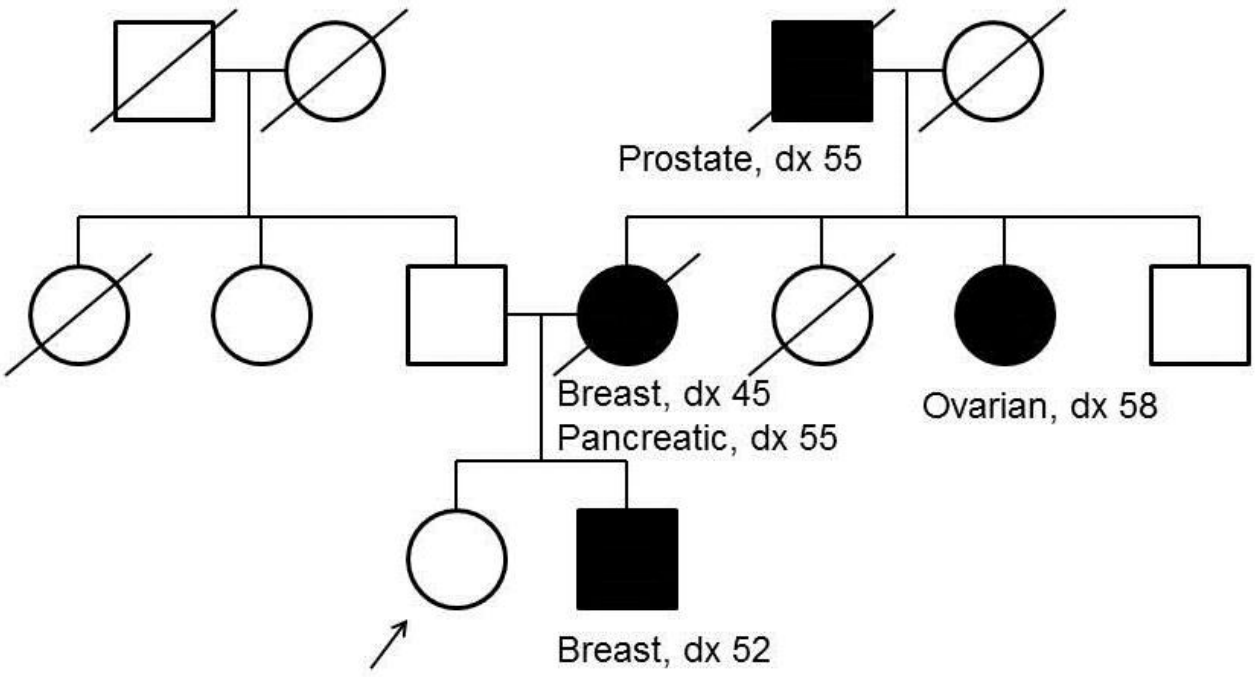
• If multiplex testing is considered for HBOC, include **TP53, PALB2, RAD51C, RAD51D, EPCAM, MLH1, MSH2, MSH6, PMS2**

• **CDH1** and **PTEN** - based on *familial phenotype* (bilateral lobular BC\50, Cowden-like features)/ when *specific criteria for the hereditary cancer syndrome* are present (is not systematic, depending on the laboratories)

• **Genetic counselling**: pre- and post-germline genetic testing.

BRCA1 pedigree example





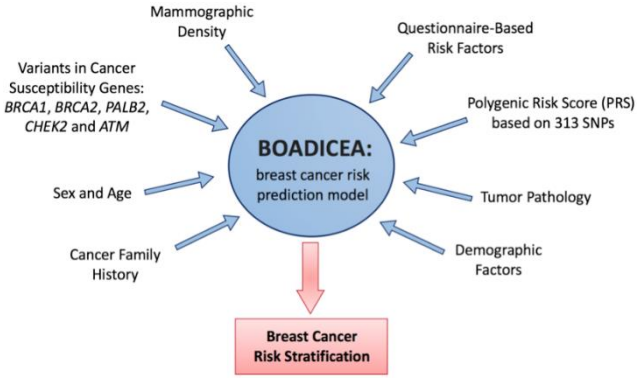
Selection criteria for BRCA genetic testing

Regardless of family history:
Women with synchronous or metachronous BC and OC
BC ≤ 35 years (or ≤ 40 years in case of uninformative family[a])
Bilateral BC (the first diagnosed ≤ 40 years)
Triple-negative BC ≤ 50 years
High-grade epithelial non-mucinous OC (or fallopian tube or primary peritoneal cancer)
2 or more first degree relatives[b] with any combination of the following high-risk features:
Bilateral BC + another BC < 50 years
Male breast cancer
BC + OC
Two cases of BC diagnosed before age 50 years
3 or more direct relatives[b] with BC and/or OC:
≥ 3 BC ± OC

BC breast cancer, OC ovarian cancer
[a] Less than 2 women who have lived until age 45 or older in each side of the family
[b] In the same side of the family

Risk calculation scores

- Eisinger score
- Manchester score
- BOADICEA (Breast and Ovarian Analysis of Disease Incidence and
- Carrier Estimation Algorithm)



Eisinger score

• Familial mutation of BRCA	5
• Breast cancer < 30	4
• Breast cancer 30-39	3
• Breast cancer 40-49	2
• Breast cancer 50-70	1
• Breast cancer man	4
• Ovarian cancer < 70	4

Score > 5: excellent indication, score 4 or 3 possible indication, score 1 or 2 low medical utility

Manchester scoring system

Age of onset	BRCA1	BRCA2
FBC < 30	6	5
FBC 30–39	4	4
FBC 40–49	3	3
FBC 50–59	2	2
FBC > 59	1	1
MBC < 60	5 (if BRCA2 tested)	8

MBC > 59	5 (if BRCA2 tested)	5
Ovarian cancer < 60	8	5 (if BRCA1 tested)
Ovarian cancer > 59	5	5 (if BRCA1 tested)
Pancreatic cancer	0	1
Prostate cancer < 60	0	2
Prostate cancer > 59	0	1

• Scores are added for each cancer in a direct lineage. FBC, female breast cancer; MBC, male breast cancer.

• High risk score ≥ 16

Surveillance and strategies for early detection of cancer in mutation carriers

• **breast MRI** as an adjunct to mammography significantly ↑ the sensitivity of screening in women with BRCA1 and BRCA2 mutations as compared with mammography alone (specificity is significantly reduced)

• **Women** screening for BRCA1 and BRCA2 mutation carriers**:**

- annual breast MRI from age 25, with a synchronous **annual mammography** added after age 30 until age 70

- women without prophylactic salpingo-oophorectomy may follow:

• **Ca125** and **transvaginal ultrasound** since age 35 (they should be informed that early detection of ovarian cancer is not guaranteed)

• **Men**: mammography at age 40 years, especially if gynaecomastia or in BRCA2 carriers (IIIC).

Screening recommendations in BRCA mutation carriers

	Age	Evidence and recommendation
Women		
Breast self awareness	Starting at age 18 years	IIA
Clinical breast exam every 6-12 months	Starting at age 25 years	IIA
Annual breast MRI	25-70 years	IIA
Annual mammogram	30-35 to 75 years	IIA
Transvaginal ultrasound and Ca 12.5 every 6-12 months	30 years	IIC
Men		
Breast self awareness	Starting at age 35 years	IIIC
Annual clinical breast exam	Starting at age 35 years	IIIC
Basal mammogram	40 years (individualised)	IIIC
Annual Prostate Cancer screening	Starting at age 40 years	IIIB
Men and women	Consider individualised screening based on cancers in the family	IIIC
Pancreatic and melanoma	Starting at 40 years or younger if family history	IIB
Colorectal cancer screening, especially in BRCA1		

*prostate cancer – only for BRCA2 mutation carriers

Risk-reducing surgery: Bilateral salpingo-oophorectomy

• **80 % reduction** in the risk of ovarian, fallopian tube or peritoneal cancer in BRCA1 or BRCA2 carriers

• 77 % reduction in all-cause mortality

• 1–4.3 % residual risk of a primary peritoneal carcinoma (some studies)

• should be offered to women with a BRCA1 or BRCA2 mutation, **between 35 and 40 years and after completion of childbearing**, or individualised based on the earliest age of ovarian cancer diagnosed in the family (at the latest 45 for BRCA2 mutation)

• PALB2 as BRCA1 but no ovarian cancer screening

• RAD51C and D- bilateral salpingo-oophorectomy at 45-50 years old

Risk-reducing surgery: Prophylactic mastectomy

• **bilateral risk reduction mastectomy (BRRM)** decreases breast cancer risk by at least 90 % in BRCA1 and BRCA2 mutation carries

• an option for **healthy BRCA1 and BRCA2 mutation carriers**, as well as contralateral mastectomy for **young patients with a prior BC diagnosis**

Chemoprevention

• Adjuvant **tamoxifen** reduces the risk of a second breast cancer in patients with a **BRCA mutation and a prior BC**

• No demonstrated benefit for primary chemoprevention of breast cancer in BRCA1 or BRCA2 mutation carriers

Treatment strategies in BRCA carriers

• **Platinum salts** – neoadjuvant setting and in the metastatic setting among patients with BC and a BRCA mutation

• **Alkylating and DNA-damaging agents** - for patients with ovarian cancer

• **Poly(ADP-ribose) polymerase inhibitors (PARPi):** Olaparib - maintenance therapy in patients with relapsed platinum-sensitive high-grade serous ovarian cancer

Risk reduction and therapeutic strategies in BRCA mutation carriers

• **Adjuvant tamoxifen** reduces the risk of contralateral breast cancer (IIA)

• Benefit of tamoxifen for primary prevention is not demonstrated in BRCA mutation carriers (IA)

• **Oral contraceptives** protect against ovarian cancer (IIB), but caution should be used when considering use of oral contraceptives in mutation carriers (conflicting results on their effect on breast cancer risk)

• **Platinum salts** might be considered in neoadjuvant setting (IC) and in the metastatic setting (IA)

• **Bilateral Salpingo-oophorectomy** should be recommended at 40 years for BRCA1 and between 40-45 for BRCA2

• **Bilateral prophylactic mastectomy** reduces the risk of breast cancer by at least 90 % (IIB), and is an option for **healthy BRCA1 and BRCA2 mutation carriers**, as well as **contralateral mastectomy for young patients with a prior breast cancer diagnosis** (IIB)

• **PARPi** are recommended as **maintenance therapy** in patients with high-grade serous ovarian cancer and for breast cancer (after first line of treatment)

Management of women without identified BRCA mutations (BRCAX)

- Women with a **breast cancer family history** and an **inconclusive BRCA genetic test** have a **higher risk of developing breast cancer** but **no increased ovarian cancer risk**
- **Monitoring:**

- breast awareness from age 18
- clinical breast examination every 6 months since age 25
- annual mammography from age 40/10 years before the youngest case of breast cancer in the family
- annual breast MRI from age 25 when the BC lifetime risk is over 20–25 % (predictive models as BRCAPRO, BOADICEA or Tyrer-Cuzyck).

Take Home Message

✓ 7% of all breast cancers and 11–15% of ovarian cancers present inherited predisposition (germline mutations in high penetrance BRCA1/2 genes)

✓ The screening strategies for BRCA1 and BRCA2 mutation carriers include: annual breast MRI from age 25 with a synchronous annual mammography added after age 30 until age 70

✓ For women without prophylactic salpingo-oophorectomy may include: Ca125 and transvaginal ultrasound

✓ There are different risk-reduction and therapeutic strategies in BRCA mutation carriers, but also for people without BRCA mutations

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II.3.3 The selection criteria. Difficulties and challenges in endocrine tumours

Learning objectives

• to recognize the major clinical signs of hereditary endocrine cancers in early stages.

• to be able to identify patients with endocrine tumours likely to be hereditary upon specific clinical features.

• to integrate the medical history of the family with particular characteristic of each subject with endocrine tumor in order to exclude a possible hereditary form.

Introduction

• Despite the progress made in the diagnosis and treatment of hereditary endocrine tumours, their recognition in early stages is extremely difficult and at the same time their differentiation from sporadic endocrine tumours poses major problems.

• Differentiating between hereditary and non-hereditary endocrine tumours is extremely important both for the investigative subject and for the need for family screening.

• There are several criteria that help us to select possible patients with hereditary endocrine tumours: age at diagnosis, associated tumours, presence of germline mutations, sex, multicentric tumours, bilateral tumours, aggression, tumour localization, mixed tumours, clinical heterogeneity and genetics.

Content

• Age at diagnosis

• Gender of the patients

• Associated tumour

• Tumour characteristics: multicentric, bilateral, aggressive

• Tumoral localization

• Take home message

• Table 1. Characteristics of primary hyperparathyroidism in sporadic, men1 and men2a patients.

• Table 2. Clinical features suggestive for hereditary pheochromocytoma (VHL= Von Hipple-Lindaw, PGL=paraganglioma)

Age at diagnosis

• Hereditary endocrine tumors appear at younger ages than sporadic ones.

• Primary hyperparathyroidism (by adenoma/parathyroid hyperplasia) in MEN1 is generally diagnosed around the age of 25-30 years. On the contrary, sporadic forms of primary hyperparathyroidism appear after the age of 50 years.

• Thyroid medullary carcinoma from MEN2 can be diagnosed at ages 5 to 10 years, especially in families where family members have been screened for mutations of the RET proto-oncogene. Extremely aggressive forms of thyroid medullary carcinoma caused by mutations of codon 918 occur in the first year of life. On the contrary, sporadic forms are diagnosed at older ages (20-30 years).

• In patients with pheochromocytoma carrying twin type mutations SDHA, SDHB, SDHC, SDHD, RET the onset of the disease occurs around the age of 20-30 years while sporadic forms of pheochromocytoma manifest themselves in the fifth decade of life.

• Pituitary tumors from MEN1, Carney syndrome and isolated familial forms (FIPA) are usually diagnosed in the second decade of life although they may occur in children. At the opposite pole are the sporadic pituitary adenomas that manifest around the age of 30-40 years. Gigantism (excess GH in young people, before closure of growth cartilages) is more common in FIPA and Carney syndrome than in patients with sporadic GH-secreting tumors.

Gender

Because hereditary endocrine tumors are autosomal dominant, the gender distribution is 1:1. Contrary to the sporadic forms of endocrine tumors (eg parathyroid tumors), the gender ratio is 3:1 in favor of the female (especially in the middle-aged ones).

Associated tumors

Patients with more than one endocrine tumor are potential candidates for a hereditary tumour syndrome and must be investigated very closely in order to detect a possible mutation, to fall into a nozological category and of course a therapeutic approach accordingly.

Mutations that are transmitted hereditary (germline type)

• The signature of tumours that are transmitted hereditary is given by the presence of a germline mutation in the gene that determines the disease.

• For example, the presence of the germline mutation of the RET gene in cases of thyroid medullary carcinoma, pheochromocytoma or hyperparathyroidism defines them as tumors with hereditary transmission.

• Almost all hereditary endocrine tumors are autosomal dominant, meaning that the first-degree relatives of the princeps patient have a 50% risk of carrying the mutation. As a result, they inherit a predisposition to a particular type of tumor.

Multicentric tumors

• Hereditary endocrine tumors are in most cases multicentric.

• In cases of MEN2 in general thyroid medullary carcinoma, pheochromocytoma and parathyroid tumors are multicentric.

• In sporadic forms, the same tumors appear as unique formations.

Bilateral tumors

• Patients with hereditary endocrine tumors have an increased risk (over 60%) of presenting with a contralateral tumor, as in MEN2 or VHL-related feo.

• Both thyroid lobes are affected in medullary carcinoma of MEN2 and both adrenal glands may have tumors (pheochromocytoma) in MEN2. Even other tumors (which may be associated with hereditary endocrine tumor syndromes), such as renal carcinoma, are also bilateral.

• Bilateral tumors do not appear simultaneously in all cases!

• Sporadic thyroid carcinoma and sporadic pheochromocytoma usually occur unilaterally. However, in the presence of tumors that may be part of a hereditary endocrine tumor syndrome, careful follow-up is necessary in order to detect early possible contraceptive locations.

Aggressivity

• MEN1 pituitary tumors are more aggressive than sporadic ones.

• Hyperparathyroidism in MEN1 has much earlier and more severe renal and bone complications than sporadic forms.

• Metastasis to cervical lymph nodes in cases of thyroid medullary carcinoma of MEN2 has been described in children up to 3 years of age (in those with mutations codon 918 RET gene).

• Tumor aggression may be associated with resistance to specific therapy (mutations of codon 804 RET gene present in patients with thyroid medullary carcinoma resistant to therapy with tyrosine kinase inhibitors).

Tumour localization

• The localization of hereditary endocrine tumours differs from the sporadic ones.

• Sporadic gastromas are mainly located in the pancreas while gastromas in MEN1 are located in the duodenum.

• Sporadic gastromas are a single pancreatic tumor, while multiple pancreato-duodenal gastromas are documented in 80% of patients with MEN1.

• Parathyroid tumours in the sporadic forms of primary hyperparathyroidism are localized to a single gland, whereas in MEN1 hyperparathyroidism all 4 parathyroids are affected by the risk of tumour proliferation and in supranumerary or ectopic parathyroid tumors.

Mixed tumors

• Mixed tumours are common in hereditary endocrine tumours and extremely rare in sporadic forms.

• Different types of pituitary adenomas secreting from MEN1 or FIPA have been reported in the same patient and in a single family. Mixed pituitary adenomas (eg GH and prolactin) are common in FIPA and MEN1.

• MEN pancreatic tumors can secrete insulin and gastrin.

• Sporadic pituitary adenomas are less commonly mixed.

Clinical heterogeneity

• Patients with hereditary endocrine tumours with the same type of germline mutation and belonging to the same families may have a high degree of clinical variability.

• Patients with MEN1 may have 20 different types of tumours, with no genotype-phenotype correlation.

MEN1 mutational analysis in clinical practice

1) confirmation of the clinical diagnosis

2) identification of family members who harbor the MEN1 mutation and require screening for tumor detection and early/appropriate treatment

3) identification of 50% of family members who do not harbor the familial germline MEN1 mutation

Indications for MEN1 mutational analysis

• **an index case** with two or more MEN1-associated endocrine tumors (i.e. parathyroid, pancreatic, or pituitary tumors);

• **asymptomatic first-degree relatives** of a known MEN1 mutation carrier;

• **a first-degree relative of an MEN1 mutation carrier** expressing familial MEN1 (i.e. having symptoms, signs, biochemical or radiological evidence for one or more MEN1-associated tumors);

• **in patients with suspicious or atypical MEN1**, which includes individuals with parathyroid adenomas occurring before the age of 30 yr; or multigland parathyroid disease, gastrinoma, or multiple pancreatic NET at any age; or individuals who have two or more MEN1-associated tumors that are not part of the classical triad of parathyroid, pancreatic islet, and anterior pituitary tumor (e.g. parathyroid tumor plus adrenal tumor)

When to perform genetic test for MEN1 in primary hyperparathyroidism (PHPT)?

• earlier onset, age 20 to 30 versus 50 to 75 of sporadic PHPT

• the youngest patient with MEN1-PHPT was 12 years old

• Most cases occurring after the age of 10 years were asymptomatic and detected by biochemical screening

• In keeping with the autosomal dominant transmission, there is an equal gender distribution, and, above all, the disease is characterized by multiglandular asymmetric involvement

Indications for MEN1 mutational analysis

• **When should testing be undertaken?**

As early as possible (e.g. before the age of 5 for asymptomatic individuals).

• **Where should test be performed?**

• n accredited department/laboratory undertaking DNA testing for **MEN1 gene**.

Indications for MEN2 mutational analysis in case of PHEO/PPGL

• A positive family history or syndromic presentation in patients with PHEO/PPGLs not only indicates a high priority for genetic testing, but also may direct targeted germline mutation testing.

• The MEN2 syndromes are usually characterized by distinct clinical stigmata that directs targeted testing of ***RET* genes**.

Multiple endocrine neoplasia type 2A	Medullary thyroid cancer, primary hyperparathyroidism, and cutaneous lichen amyloidosis
Multiple endocrine neoplasia type 2B	Medullary thyroid cancer, mucocutaneous neuromas, skeletal deformities (e.g. kyphoscoliosis or lordosis), joint laxity, myelinated corneal nerves, and intestinal ganglioneuromas (Hirschsprung disease)

Decisional tree: clinical features suggestive for hereditary pheochromocytoma

Pheochromocytoma
- Sudden death, particularly at a young age
- Hypertension or stoke, particularly at a young age or during pregnancy
- Hypertensive response to anesthesia

VHL
- Kidney or pancreatic cysts or cancer
- Testicular mass or cyst in children
- Early onset of deafness
- Early onset of blindness
- Central nervous system tumours

MEN2
- Thyroid cancer, goiter
- High blood calcium or kidney stones

PGL1 and PGL4
- Head and neck tumours with signs and symptoms mainly related to their location (dysphonia, dysphagia, etc.) more than excessive production of catecholamines

Abdominal tumours

Clinical features may lead to gene mutations

• Different mutations in the RET gene produce varying phenotypes for the disease, including age of onset.

• Approximately 85% of patients with MEN2 have a mutation of **exon 11 codon 634**, whereas mutations in codons 609, 611, 618, and 620 account for 10% to 15% of cases.

• Particularly early aggressive behavior and metastasis in MEN2A and MEN2B are associated with **C634 and M918T mutations**, respectively, requiring early intervention.

Decisional tree: characteristics of primary hyperparathyroidism in sporadic, men1 and men2a patients

Characteristics	Sporadic pHPT, n = 467	MEN1-pHPT, n = 52	MEN2-pHPT, n = 16
Female, n	357 (76%)	33 (63%)	9 (56%)
Age, y, median (range)	63 (20 to 88)	33 (11 to 62)	39 (20 to 66)
Symptoms at first presentation, n	467 (100%)	42 (81%)	12 (75%)
Fatigue	188 (40%)	16 (31%)	4 (25)
Renal stones	115 (25%)	14 (27%)	3 (19%)
Osteoporosis	73 (16%)	0	4 (25%)
Gastrointestinal symptoms	67 (14%)	7 (14%)	0
Neuropsychiatric	42 (9%)	7 (14%)	1 (6%)

Twigt et al., Orphanet Journal of Rare Disease 2013, **8**:50
http://www.ojrd.com/content/8/1/50

When to perform genetic test for MEN2 in primary hyperparathyroidism (PHPT)?

• is characterized by an earlier onset as compared with sporadic PHPT (40 and 50-75 years, respectively) and by a multiglandular disease involvement;

• is often mild and asymptomatic.

Take Home Message

• Hereditary endocrine tumors occur at young ages.

• Both sexes present the same risc to develop hereditary endocrine tumors.

• Hereditary endocrine tumors they are most often multicentric, bilateral and extremely aggressive.

• Mixed secretion (more than one hormone) of only one gland is more frequent in hereditary endocrine tumors.

• There is no correlation genotype-phenotype in hereditary endocrine tumors, clinical picture being highly variable.

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Learning objectives

• Evaluate the advantages and disadvantages of each method used in molecular diagnostic;
• Adapt the laboratory workflow to the available samples;
• Integrate specific population mutational data to the diagnostic methodology;
• Choose appropriate methods for targeted mutational pre-screening;
• Improve efficiency of molecular oncogenetic diagnostic;
• Develop further research projects from molecular oncogenetic diagnostic results.

Complete Sanger gene sequencing - the complexity and the problems

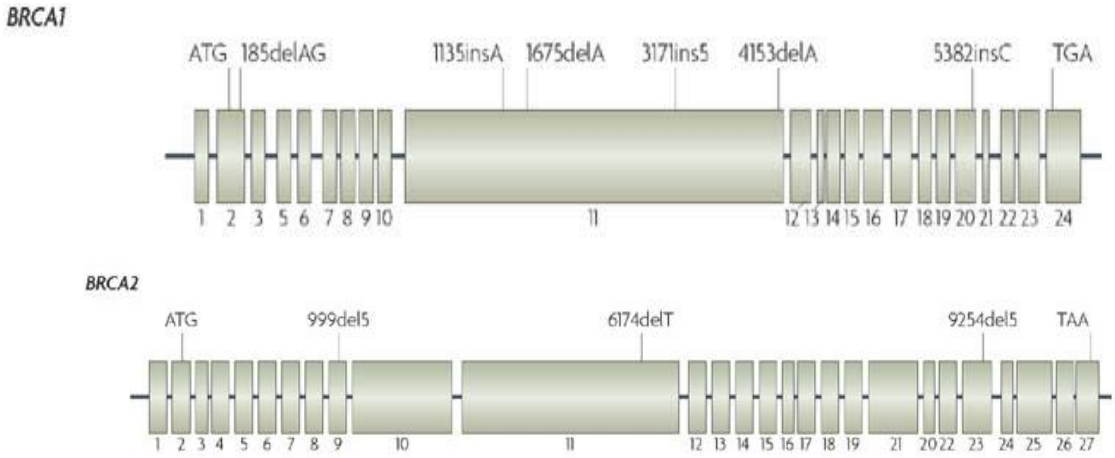
1. Very long genes (thousands of nucleotides), numerous exons
2. Thousands of different mutations already identified
3. About 100.000 nucleotides to "read" for a "simple" BRCA test
4. Numerous benign common polymorphisms present in those genes
5. About 50% of identifies sequence variants are not pathogenically clear (unclassified variants)
6. The continuous danger of false-positives/false-negatives

- Responsible of the diagnostics – highly specialized + responsibility
- Big cost
- Long to interpret
- Coherent organization of the workflow

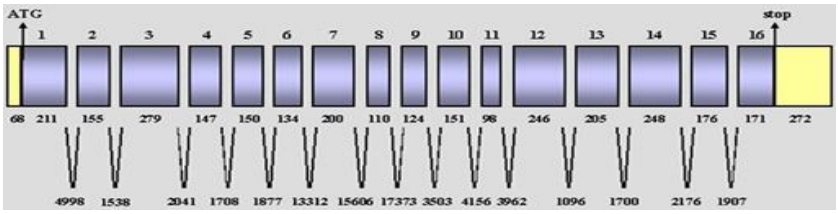
SANGER > gene by gene analysis
NGS > analyses multiples genes in the same time and more sensitive

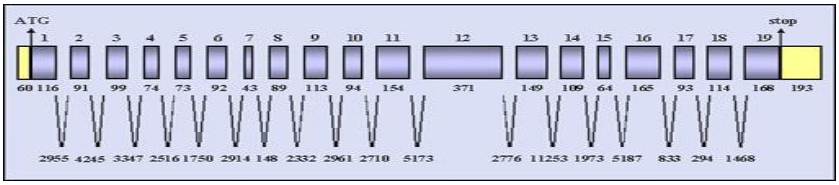
Genetic material that needs to be decrypted

HBOC :
***BRCA1*: >2000 germ-line mutations identified.**
Several recurrent/founder mutations.
Distribution among all 22 exons. 32 amplicons
***BRCA2*: >2000 germ-line mutations identified.**
Few recurrent/founder mutations.
Distribution among all 26 exons.

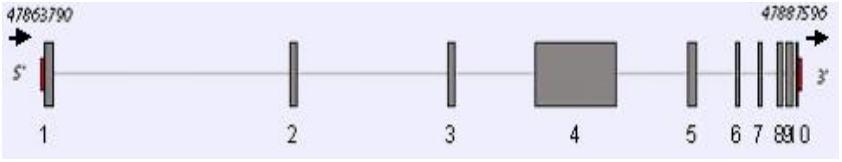


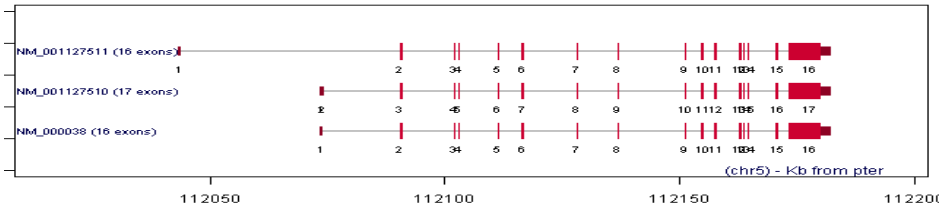
HNPCC :
MSH2 **: >300 germ-line mutations identified.**
No recurrent/founder mutations.
Distribution among all 16 exons. 16 amplicons
MLH1 **: >300 germ-line mutations identified.**
Few recurrent/founder mutations.
Distribution among all 19 exons. 19 amplicons
MSH6 **: few germ-line mutations identified.**
No recurrent/founder mutations.
Distribution among all 10 exons. 15 amplicons





FAP :
APC **: >300 germ-line mutations identified.**
No recurrent/founder mutations.
Distribution among all 16 exons. 36 amplicons



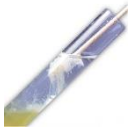


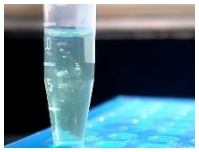
Complete Sanger gene sequencing. The complexity and the problems

PROBLEM:
- Forward + Reverse sequencing of coding regions + exon/intron boundaries
- Long Exons are sub-divided in small amplicons (e.g. 15 fragments for B2-e11)
- The genes are very very long !!! (kb)
- Any identified variation must be verified on a second independent sample

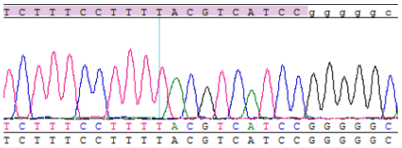
IN FACT: The genetic material to be decrypted :
HBOC : 15849 nucleotides = 76 amplicons = 48 exons
HNPCC : 9999 nucleotides = 50 amplicons = 45 exons
PAF : 8538 nucleotides = 36 amplicons = 16 exons
Forward + Reverse sequencing, all work doubles !!!!! (supposing 100% success/efficiency – în reality 60-70%)

Molecular oncogenetic diagnostics



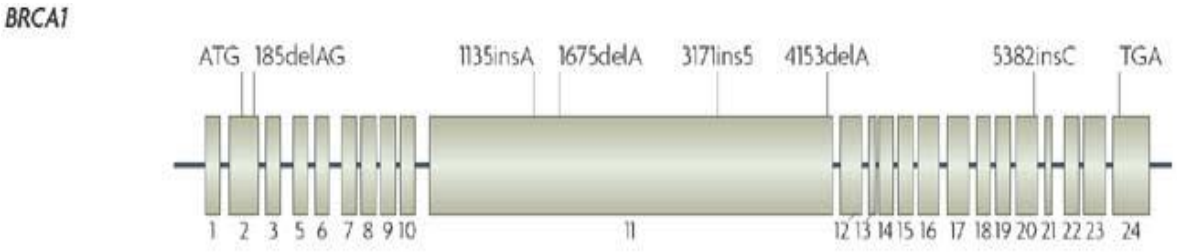


1. DNA EXTRACTION
2. MUTATIONS PRE-SCREENING
3. PCR AMPLIFICATION (REGIONS OF INTEREST)
4. PURIFICATION OF PCR PRODUCTS
5. SEQUENCING OF AMPLICONS
6. PURIFICATION OF SEQUENCING PRODUCTS
7. CAPILLARY ELECTROPHORESIS
8. INTERPRETATION OF THE RESULTS (most important !!!)

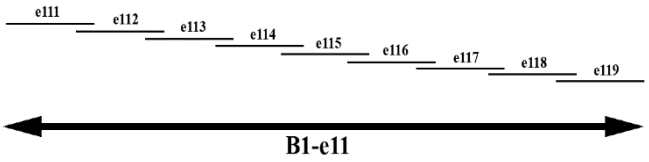


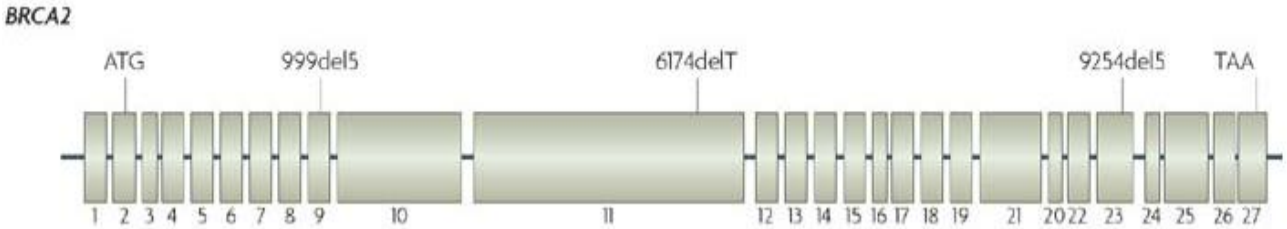
Sequencing of BRCA genes

BRCA1: >2000 germ-line mutations identified.
Several recurrent/founder mutations.
Distribution among all 22 exons. 32 amplicons

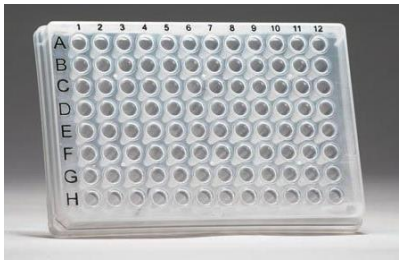


BRCA2: >2000 germ-line mutations identified.
Few recurrent/founder mutations.
Distribution among all 26 exons. 44 amplicons

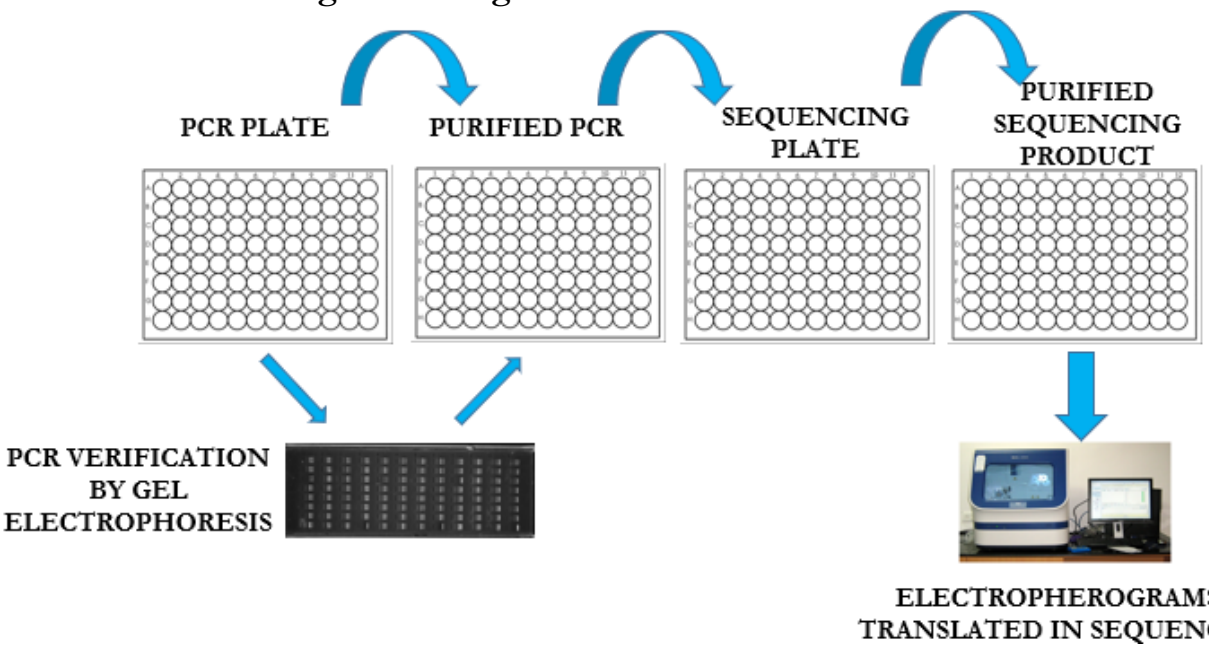


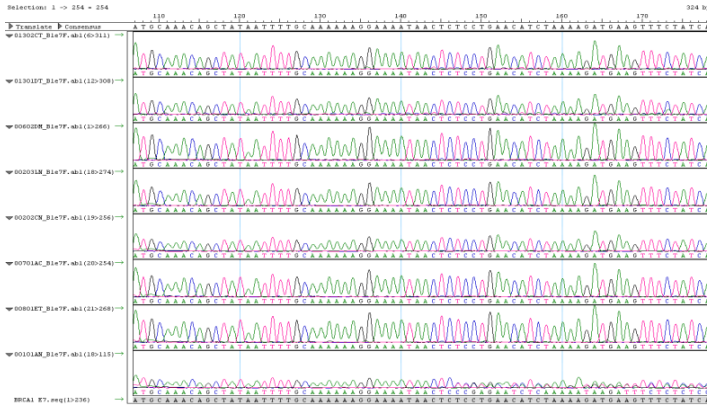


Molecular oncogenetic diagnostics



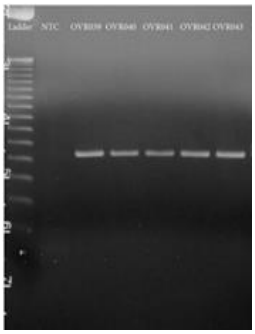
Molecular oncogenetic diagnostic – the workflow





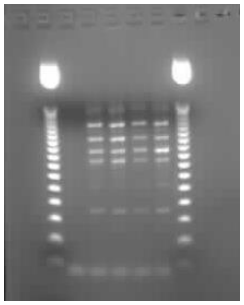
PCR – what can we observe ?

GOOD PCR :
- Unique amplicon
- Efficiency
- No contamination



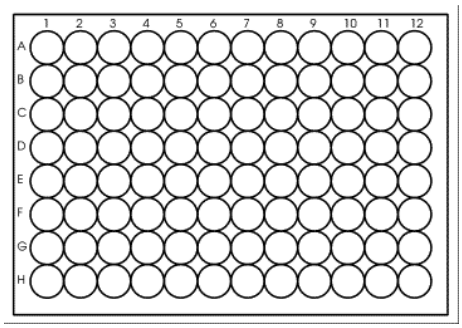
BAD PCR :
- No amplicon
- No Efficiency
- Troubleshooting !!

VERY BAD PCR:
- No specificity
- Contamination
- Troubleshooting !!!



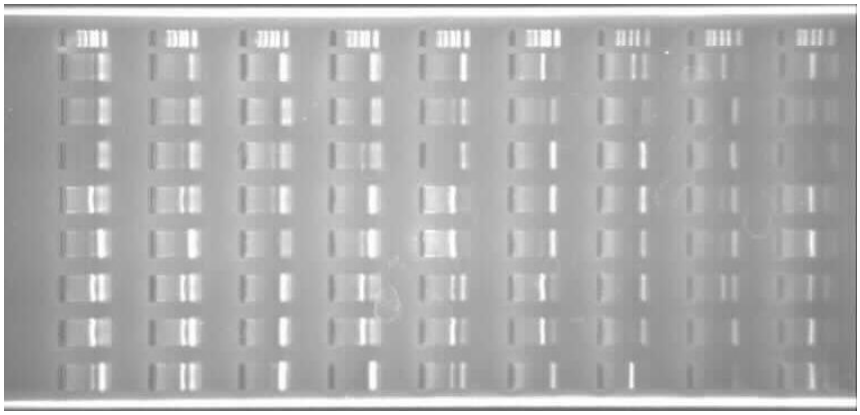
Molecular oncogenetic diagnostic

If all wells contain the same PCR reaction from different samples, the PCR conditions are the same and efficiency will be good for all samples.

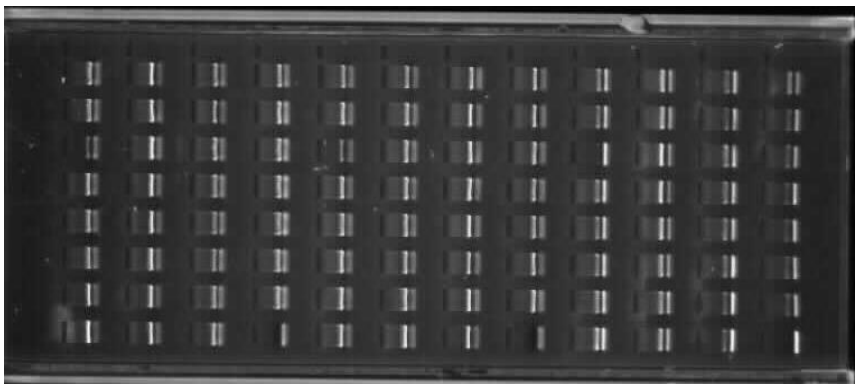


1 PLATE = 1 EXON
95 PATIENTS

	H	G	F	E	D	C	B	A
1	Pacient 8	Pacient 7	Pacient 6	Pacient 5	Pacient 4	Pacient 3	Pacient 2	Pacient 1
2	Pacient 16	Pacient 15	Pacient 14	Pacient 13	Pacient 12	Pacient 11	Pacient 10	Pacient 9
3	Pacient 24	Pacient 23	Pacient 22	Pacient 21	Pacient 20	Pacient 19	Pacient 18	Pacient 17
4	Pacient 32	Pacient 31	Pacient 30	Pacient 29	Pacient 28	Pacient 27	Pacient 26	Pacient 25
5	Pacient 40	Pacient 39	Pacient 38	Pacient 37	Pacient 36	Pacient 35	Pacient 34	Pacient 33
6	Pacient 48	Pacient 47	Pacient 46	Pacient 45	Pacient 44	Pacient 43	Pacient 42	Pacient 41
7	Pacient 56	Pacient 55	Pacient 54	Pacient 53	Pacient 52	Pacient 51	Pacient 50	Pacient 49
8	Pacient 64	Pacient 63	Pacient 62	Pacient 61	Pacient 60	Pacient 59	Pacient 58	Pacient 57
9	Pacient 72	Pacient 71	Pacient 70	Pacient 69	Pacient 68	Pacient 67	Pacient 66	Pacient 65
10	Pacient 80	Pacient 79	Pacient 78	Pacient 77	Pacient 76	Pacient 75	Pacient 74	Pacient 73
11	Pacient 88	Pacient 87	Pacient 86	Pacient 85	Pacient 84	Pacient 83	Pacient 82	Pacient 81
12	Pacient 96	Pacient 95	Pacient 94	Pacient 93	Pacient 92	Pacient 91	Pacient 90	Pacient 89



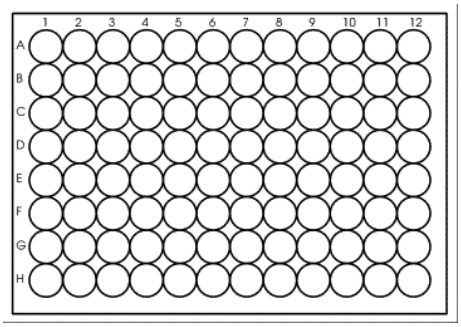
Before optimization, efficiency is poor (specific amplicon not visible on all lanes).



After optimization, efficiency is good (specific amplicon visible on all lanes).

EFICIENCY ≈ 90%
LESS EXPENSIVE, LESS TIME CONSUMMING

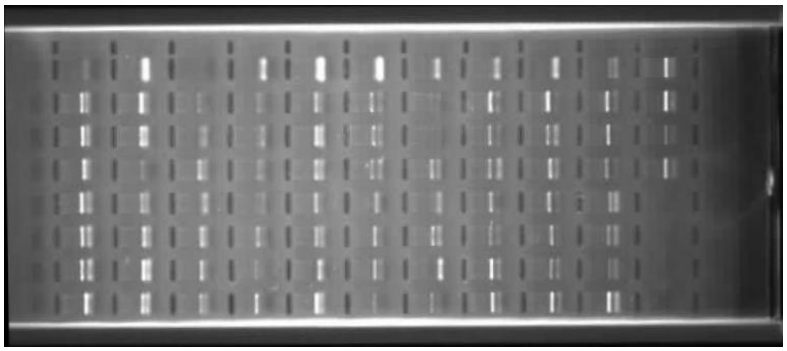
If in each well we have a different PCR reaction, even if the DNA sample is the same, the PCR conditions are different and efficiency will be different in each well. Specific amplicons are visible only in some lanes. Co-amplification of many targets within the same conditions is hard to achieve, even after optimization.



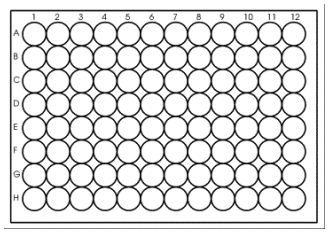
	H	G	F	E	D	C	B	A
1	B1 e10	B1 e9	B1 e8	B1 e7	B1 e6	B1 e5	B1 e3	B1 e2
2	B1 e118	B1 e117	B1 e116	B1 e115	B1 e114	B1 e113	B1 e112	B1 e111
3	B1 e1116	B1 e1115	B1 e1114	B1 e1113	B1 e1112	B1 e1111	B1 e1110	B1 e119
4	B1 e16	B1 e15	B1 e14	B1 e13	B1 e12	B1 e1119	B1 e1118	B1 e1117
5	B1 e24	B1 e23	B1 e22	B1 e21	B1 e20	B1 e19	B1 e18	B1 e17
6	B2 e10a	B2 e9	B2 e8	B2 e7	B2 e5/6	B2 e4	B2 e3	B2 e2
7	B2 e1105	B2 e1104	B2 e1103	B2 e1102	B2 e1101	B2 e10d	B2 e10c	B2 e10b
8	B2 e1113	B2 e1112	B2 e1111	B2 e1110	B2 e1109	B2 e1108	B2 e1107	B2 e1106
9	B2 e16	B2 e15	B2 e14-2	B2 e14-1	B2 e13	B2 e12	B2 e1115	B2 e1114
10	B2 E25	B2 E24	B2 E23	B2 E21	B2 E20	B2 e19	B2 e18	B2 e17
11	-	-	-	NTC	B2 E22	B2 E27b	B2 E27a	B2 E26
12	-	-	-	-	-	-	-	-

1 PLATE = 1 PATIENT
76 AMPLICONS

If in each well we have a different PCR reaction, even if the DNA sample is the same, the PCR conditions are different and efficiency will be different in each well. Specific amplicons are visible only in some lanes. Co-amplification of many targets within the same conditions is hard to achieve, even after optimization.



EFFICIENCY ≤ 40% VERY EXPENSIVE AND TIME CONSUMING

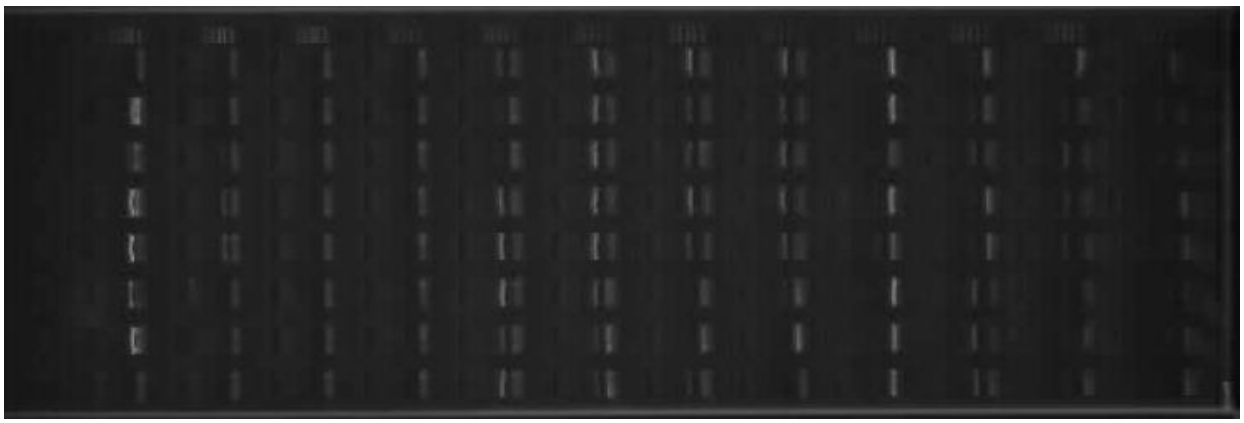


	1	2	3	4	5	6	7	8	9	10	11	12
A	032 – 01 CM – a	039 – 01 BR – a	045 – 02 CL – a	032 – 01 CM – a	039 – 01 BR – a	045 – 02 CL – a	032 – 01 CM – a	039 – 01 BR – a	045 – 02 CL – a	032 – 01 CM – a	039 – 01 BR – a	045 – 02 CL – a
B	033 – 01 RI – a	039 – 02 AV – a	045 – 03 CM – a	033 – 01 RI – a	039 – 02 AV – a	045 – 03 CM – a	033 – 01 RI – a	039 – 02 AV – a	045 – 03 CM – a	033 – 01 RI – a	039 – 02 AV – a	045 – 03 CM – a
C	034 – 01 DA – a	040 – 01 SS – a	046 – 01 CV – a	034 – 01 DA – a	040 – 01 SS – a	046 – 01 CV – a	034 – 01 DA – a	040 – 01 SS – a	046 – 01 CV – a	034 – 01 DA – a	040 – 01 SS – a	046 – 01 CV – a
D	035 – 01 CG – a	041 – 01 MM – a	047 – 01 AD – a	035 – 01 CG – a	041 – 01 MM – a	047 – 01 AD – a	035 – 01 CG – a	041 – 01 MM – a	047 – 01 AD – a	035 – 01 CG – a	041 – 01 MM – a	047 – 01 AD – a
E	036 – 01 DJ – a	043 – 01 DA – a	049 – 01 IA – a	036 – 01 DJ – a	043 – 01 DA – a	049 – 01 IA – a	036 – 01 DJ – a	043 – 01 DA – a	049 – 01 IA – a	036 – 01 DJ – a	043 – 01 DA – a	049 – 01 IA – a
F	036 – 02 ND – a	044 – 01 VC – a	051 – 01 LM – a	036 – 02 ND – a	044 – 01 VC – a	051 – 01 LM – a	036 – 02 ND – a	044 – 01 VC – a	051 – 01 LM – a	036 – 02 ND – a	044 – 01 VC – a	051 – 01 LM – a
G	037 – 01 PM – a	044 – 02 BV – a	052 – 01 DV – a	037 – 01 PM – a	044 – 02 BV – a	052 – 01 DV – a	037 – 01 PM – a	044 – 02 BV – a	052 – 01 DV – a	037 – 01 PM – a	044 – 02 BV – a	052 – 01 DV – a
H	038 – 01 UA – a	045 – 01 AM – a	NTC	038 – 01 UA – a	045 – 01 AM – a	NTC	038 – 01 UA – a	045 – 01 AM – a	NTC	038 – 01 UA – a	045 – 01 AM – a	NTC

B1-e B1-e B1-e B1-e

COMPROMISE 1 PLATE = 23 PATIENTS 4 AMPLICONS

The compromise would be to regroup in the same plate a few different reactions with similar amplification conditions. After optimization, good efficiency can be achieved for all amplicons.



EFFICIENCY ≈ 75% MIDDLE EXPENSIVE AND TIME CONSUMMING

Molecular oncogenetic diagnostics - Solutions

QUESTIONS >

• Do we really have to sequence the entire genes ?

• Are there exonic mutation hotspots ? NOT for BRCA1/2 (although they exist for other genes included in oncogenetic diagnostic)

• Are there mutations appearing more frequently ? YES !

Molecular oncogenetic diagnostics – mutation distribution

Western Europe: no pre-screening needed

– Complete status : **Few recurrent/founder mutations.**

(Excepted for isolated populations like Iceland or Ashkenazi jewish)

– Important proportion of **unique/familial mutations**

– Thousands of different mutations reported in databases

– Classical haplotypes

– Oncogenetic follow-up is current medical practice

Central and Eastern Europe: Pre-screening is mandatory

Complete status: Poland [*Gorski et al., 2000*], Slovakia [*Ciernikova et al., 2006*],

Czech Rep. [*Machackova et al., 2001*], Hungaria [*Van der Looij et al., 2000*], Slovenia [*Krajk et al., 2008*], Greece [*Kataki et al., 2005*], Turkey [*Yazici et al., 2000*], etc…

– Few unique/familial mutations, the vast majority of HBOC families are due to a few recurrent/founder mutations (**eastern mutations**)**:**

BRCA1 185delAG, 5382insC, 300T>G, BRCA2 6174delT

– Very few information about haplotypes

– Oncogenetic follow-up medium implemented

Molecular oncogenetic diagnostic – Pre-screening

• Is Pre-screening justified in Romania ?

• If yes, in what proportion ? (What would it economize for the molecular diagnostic, in terms of money, time, human resources ?)

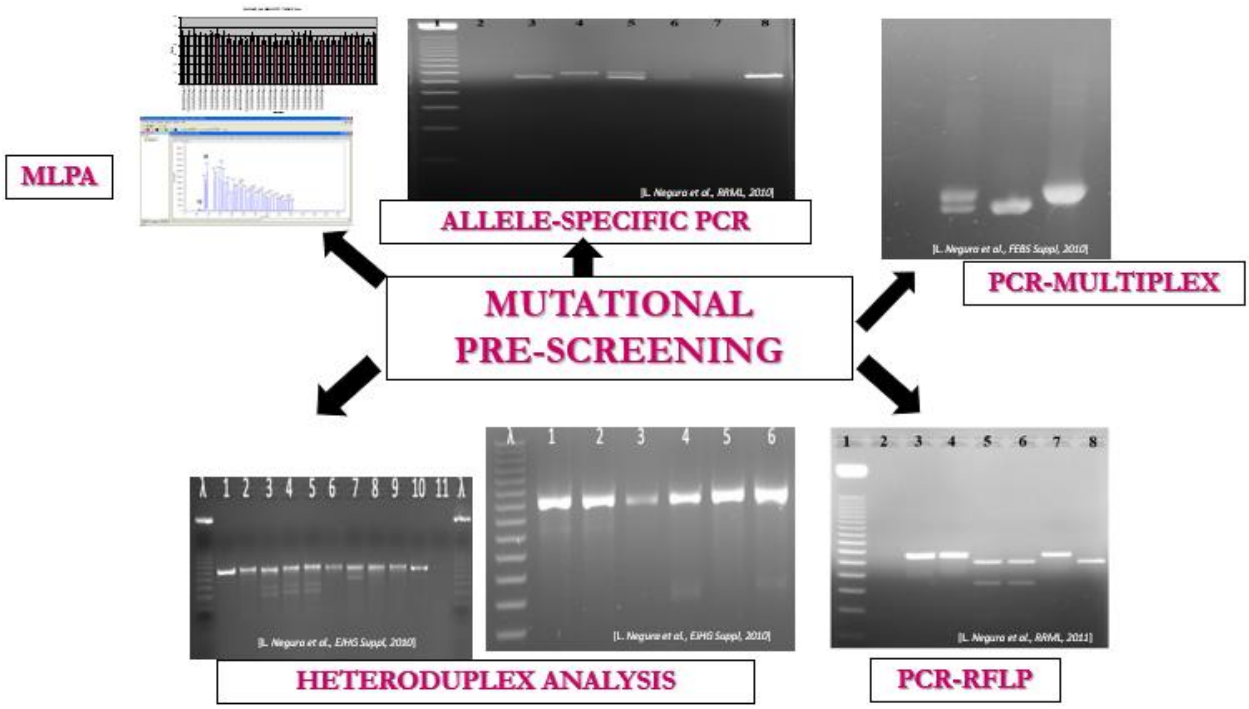
• For what mutations in the pre-screening justified ?

Founder eastern mutations

Recurrent eastern mutations

Founder/recurrent "local" mutations
Other mutations...
What should be, in this context, the locally adapted diagnostic approach ??

Romanian mutation spectrum:
½ known, predictable mutations : pre-screening justified
½ unknown, novel mutations : pre-screening not justified → sequencing

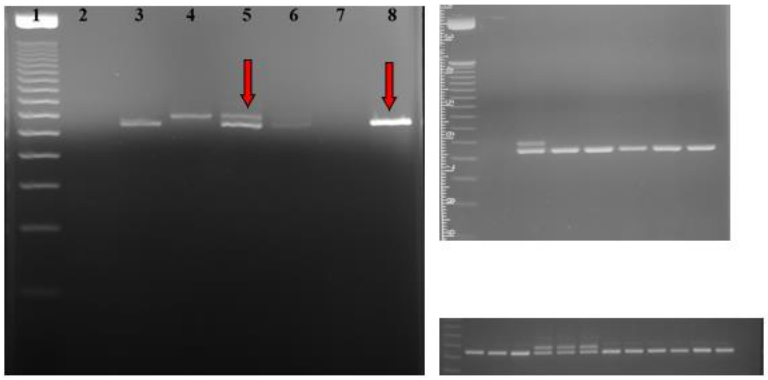


Specific mutations can be screened by rapid and cheap PCR-based methods. Advantage: many patients can be rapidly screened.

Disadvantage: One only specific known mutation is screened for at one time.

Allele-specific multiplex PCR. Detection of *BRCA1* 5382insC mutation

Using wild-type and mutation specific primers in the same PCR reaction, differential electrophoretic profile can be observed from genetically different patients. In this example, heterozygous mutation carriers appear as "double-bands", while wild-type appear as "single-bands".

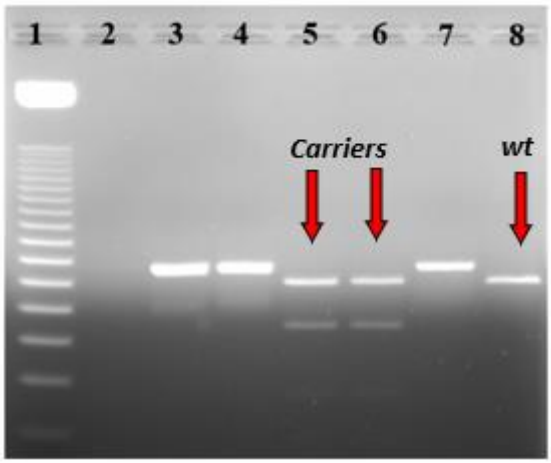


Left arrow: heterozygous mutation carrier; **right arrow:** wild-type patient

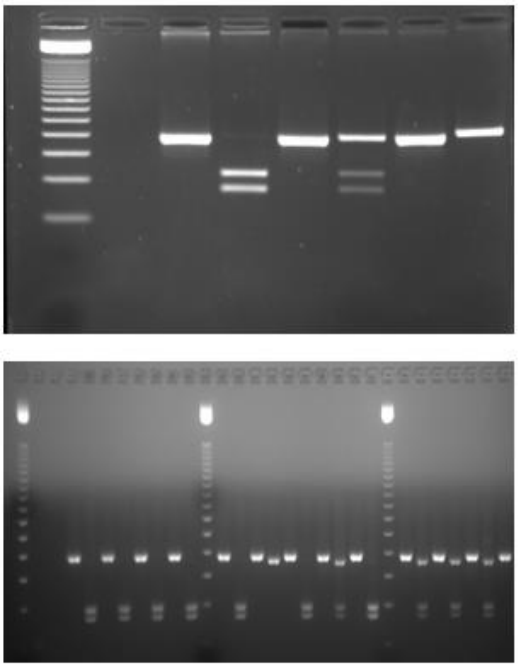
(Source: L. Negura et al., RJME, 2015)

RFLP (Restriction Fragment Length Polymorphism). Detection of BRCA2 c.8680C>T mutation

Using specific restriction enzymes, we obtain differential digestion profiles from genetically different patients. In this example, heterozygous mutation carriers appear as "double-bands", while wild-type appear as "single-bands".



(Source: L. Negura et al., RRML, 2011)

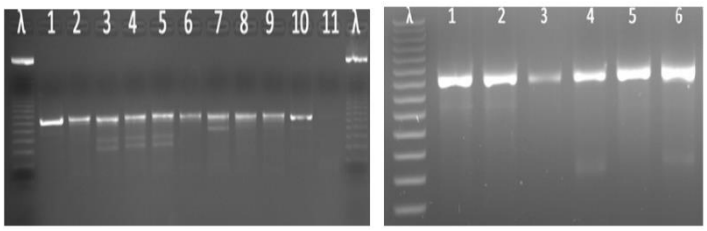


(Source: L. Negura et al., 2015)

Molecular oncogenetic diagnostics

Heteroduplex Analysis (HA)

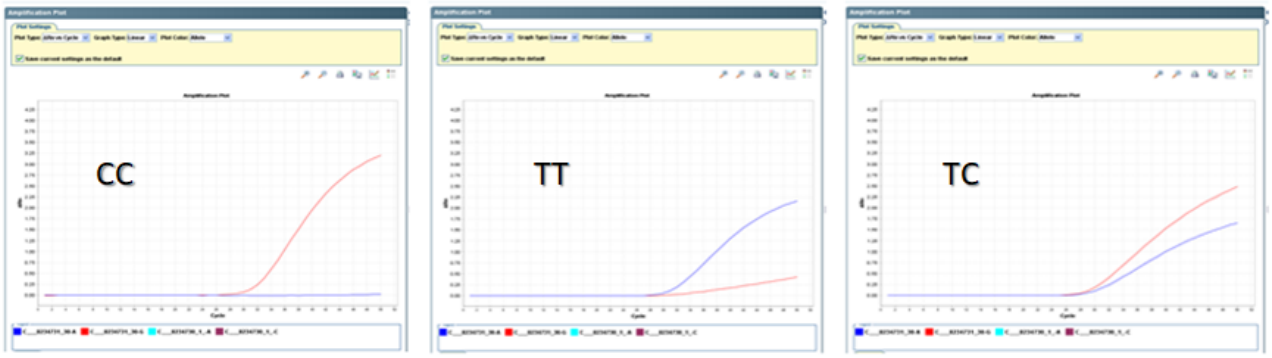
A mismatch specific endonuclease will differentially cut amplicons originated from wild-type and mutant samples. With this technique, a wider range of variants may be detected in the same amplicon. However, the exact nature of the mutation cannot be known without DNA sequencing.

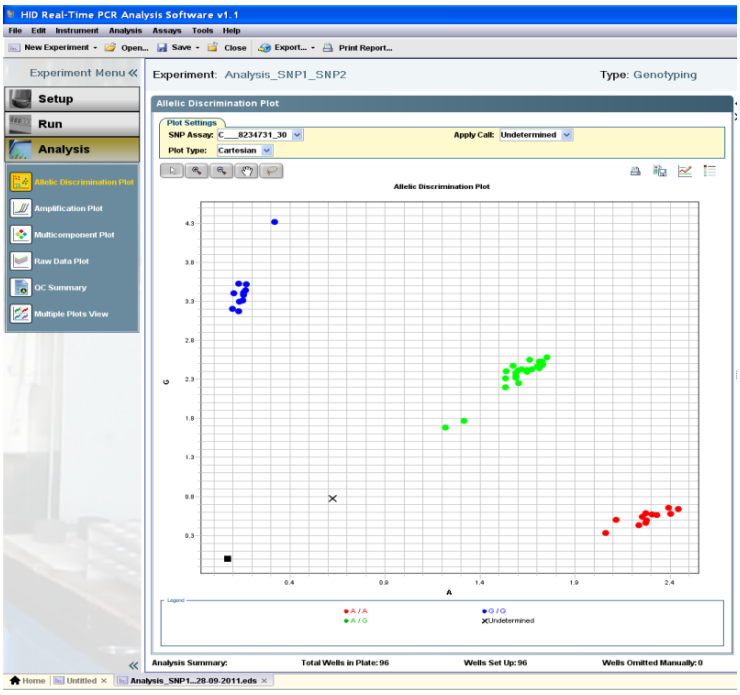


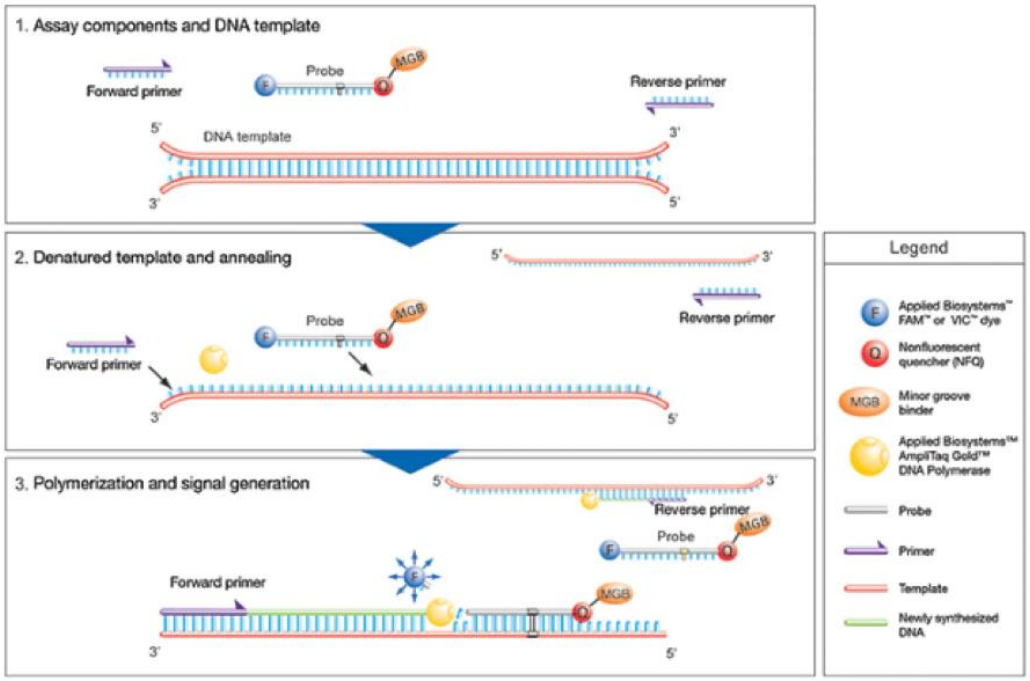
(Source: L. Negura et al., GMBUAIC, 2011)

SNP Genotyping : TaqMan® Assay Allelic discrimination

If different fluorescence is used for specific wild-type and mutation-specific probes, differential fluorescence can be used to discriminate between genotypes.





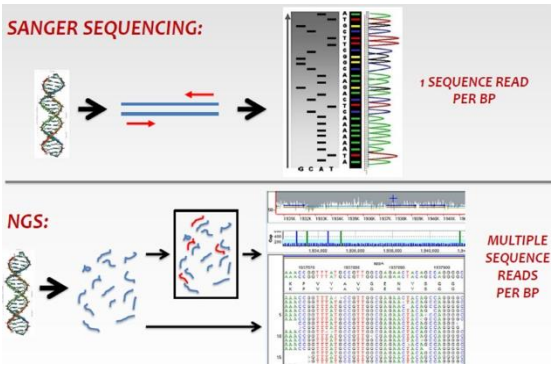


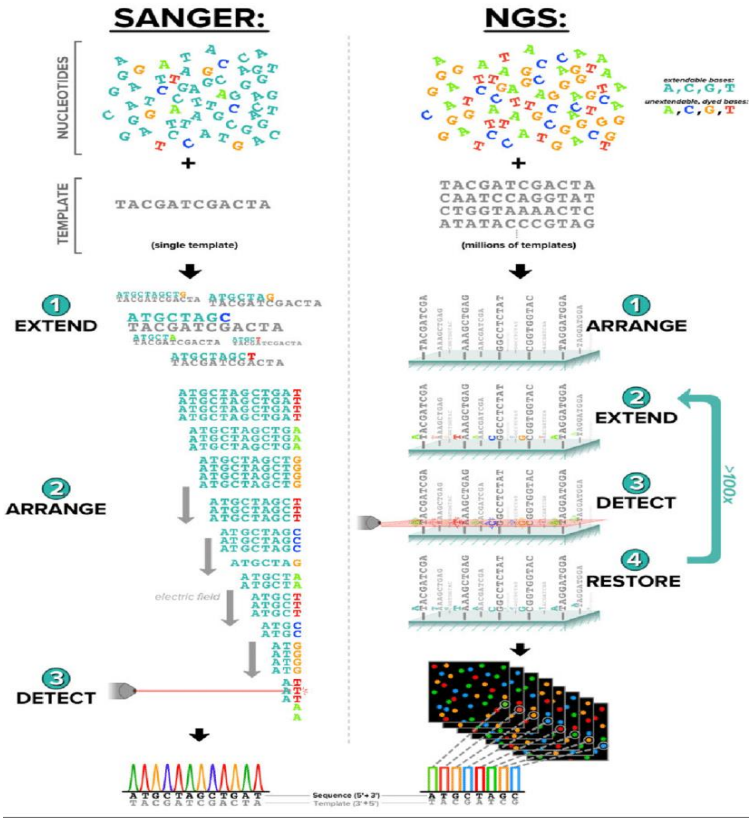
NGS (the future)

SANGER > gene by gene analysis

NGS > analyses multiples genes in the same time and more sensitive

Features	Sanger	NGS
Generation	1st	2nd-3rd
Year	Late 1990s-early 2000s	2006-Current
Sequencing Samples	Cloning, PCR	DNA Libraries
Preparation Steps	Simple	Complex
Data Collection	96-384 well plates	1-16 slides
Data	1 Read/Sample	10^3-10^6 Reads/Sample
Whole genome effort/cost	Hundreds of Scientists $3 billion/10 years Large machines	1-2 Scientists $1000/Hours Counter-top machine





Molecular oncogenetic diagnostics – NGS gene pannels

Molecular oncogenetic diagnostics – NGS gene pannels

Sanger: one gene ; NGS : 2 to > 500 genes

- Genetic tests to look at dozens of genes related to cancer
- Similar cost and turn around time as gene specific testing
- Higher risk of uncertain results

Gene tested	Comprehensive Cancer Panel (32 genes)	High/Moderate Risk Panel (23 genes)	Lynch/Colorectal High Risk (7 genes)	Colorectal Cancer (19 genes)	Breast Cancer High/Moderate Risk (8 genes)	Breast/Ovarian Cancer (20 genes)
SDHD						
SMAD3						
SMAD4		X		X		
SMARCA4						
SMARCB1						
STK11		X		X		
SUFU						
TERT						
TGFBR1						
TGFBR2						
TMEM127						
TP53		X		X	X	X
TP53BP1						
TSC1						
TSC2						
VHL		X				
WT1						
XRCC2						X

Myriad Hereditary Cancel Panel Tests

Gene tested	myRisk (27 genes)	Pancreatic (12 genes)	Colorectal High Risk (7 genes)	Colorectal and Polyposis (17 genes)	Breast and ovarian (17 genes)	Breast cancer (8 genes)
ALK			X			
APC						
AKT1						
APC	X			X		
ATM	X	X			X	X
ATR						
AXIN2						
BAP1						
BARD1	X					
BLM						
BMPR1A	X			X		
BRCA1	X	X			X	X
BRCA2	X	X			X	X
BRIP1	X					
CDH1	X			X	X	X
CDK4	X					
CDKN2A	X			X		
CHEK1						
CHEK2	X			X	X	X
CTNNA1						
DICER1						
EGFR						
EPCAM	X	X	X	X	X	
FAM175A						
FANCC						
FANCP						
FH						
FLCN						
GALNT12						
GATA2						
GEN1						

Genes included in next-generation sequencing multigene cancer panels

Cancer type	No. of genes	Gene list*
Breast cancer	17	ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, MRE11A, MUTYH, NBN, NF1, PTEN, RAD50, RAD51C, RAD51D, TP53, PALB2
Colorectal cancer	17	APC, BMPR1A, CDH1, CHEK2, EPCAM, GREM1, MLH1, MSH2, MSH6, MUTYH, PMS2, POLD1, POLE, PTEN, SMAD4, STK11, TP53
Paragangliomas/ Pheochromocytomas	12	FH, MAX, MEN1, NF1, RET, SDHA, SHAF2, SDHB, SDHC, SDHD, TMEM127, VHL
Renal cancer	19	MLH1, MSH2, MSH6, PMS2, PTEN, TO53, VHL, EPCAM, FLCN, TSC2, TSC1, SDHB, MET, MITF, SDHC, SDHD, SDHA, FH, BAP1,
Pancreatic cancer	13	APC, ATM, BRCA1, BRCA2, CDKN2A, EPCAM, MLH1, MSH2, MSH6, PMS2, STK11, TP53, PALB2
Ovarian cancer/ Uterine cancer	24	ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PMS2, PTEN, RAD50, RAD51C, RAD51D, STK11, TP53, PALB2, SMARCA4

CancerNext, T.B. (2016). Title : Genetic Cancer Susceptibility Panels Using Next Generation Sequencing Professional Institutional.

The Journal of Molecular Diagnostics, Vol. 18, No. 6, November 2016

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the Journal of Molecular Diagnostics
jmd.amjpathol.org

Sanger Confirmation Is Required to Achieve Optimal Sensitivity and Specificity in Next-Generation Sequencing Panel Testing

Wenbo Mu, Hsiao-Mei Lu, Jefferey Chen, Shuwei Li, and Aaron M. Elliott

From Ambry Genetics, Aliso Viejo, California

shape panels can be developed and adapted

NGS – the workflow

1. **Library Preparation**: Technology determines type of sequencing
   Non-targeted: Whole Genome Sequencing (30x-60x)
   Targeted: Exome and Gene Panel sequencing (>100x)
   ***Multiplexing:** barcoded adapters to sequence more than one sample in a single run
1. **Cluster Amplification**
2. **Sequencing:**
   Single-End: only provides forward sequence

***Paired-End:** provides forward and reverse sequence

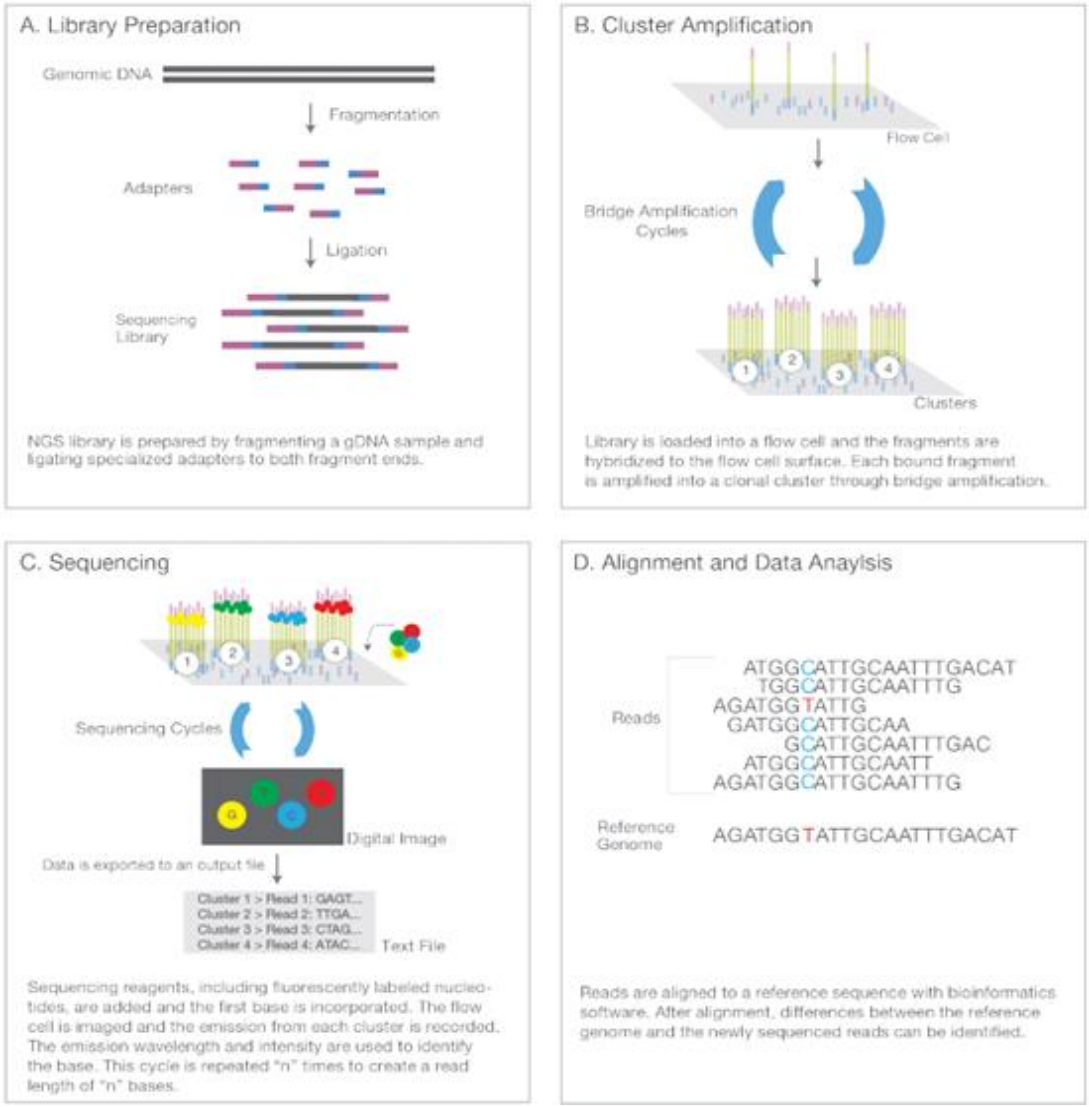
Primary Analysis: FASTQ file

1. **Alignment and Data Analysis**: can be performed by different bioinformatic platforms

Secondary Analysis: BAM – VCF files

Data clean up, variant calling, some variant interpretation

***Improving scalability**



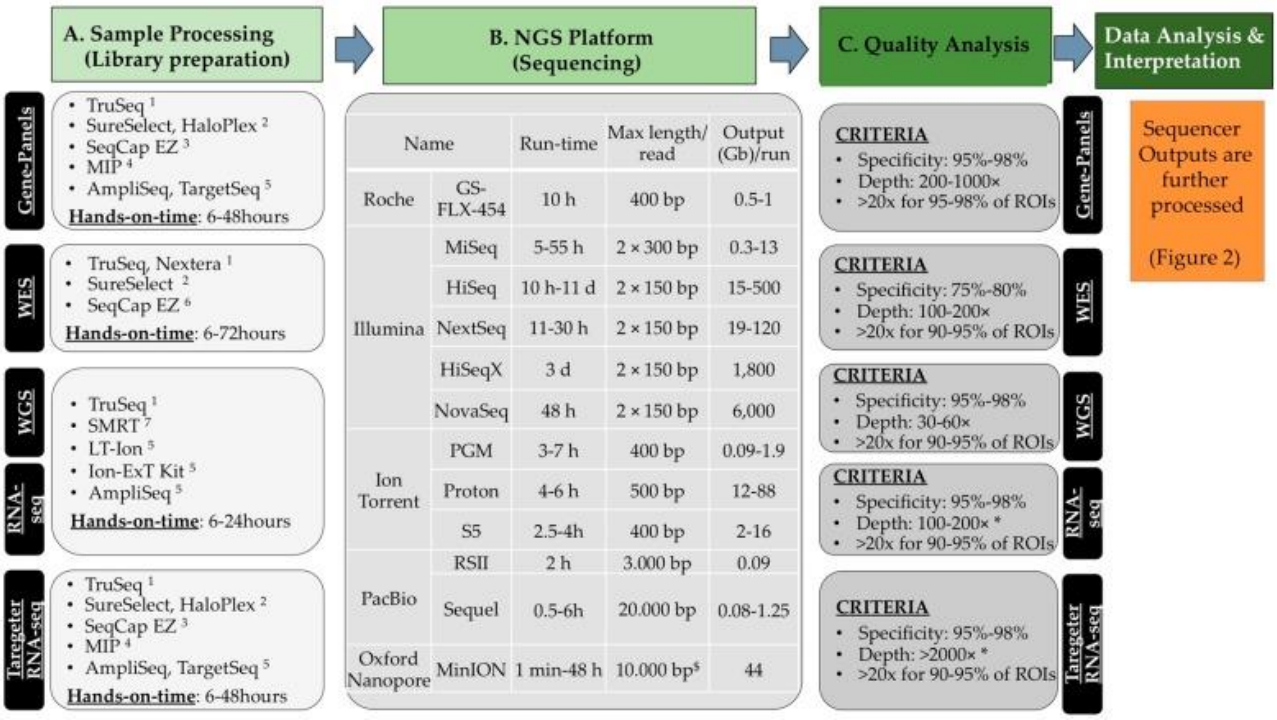
NGS – the systems

Sequencing system[a]	Estimated system cost	Consumable cost per single-end run (paired end run)	Read Length per single-end run (paired-end)	Gigabases sequenced per single-end run (paired-end)	Run-time per single-end run (paired-end)	Raw accuracy
454 Genome Sequencer FLX	$500,000[b]	n/a[c]	250-300 bp (2 X 110 bp)[d]	0.1 Gb[e] (0.1 Gb)	7.5 hours (7.5 hours)	99.5%
Illumina Genome Analyser	~$400,000	$3000 (n/a)[f]	36 bp[g] (2 x 36 bp)	1.5 Gb (3.0 Gb)	2.5 days (5 days)	> 98.5%
ABI SOLiD™ System	$525,000	$3390[h] ($4390)	35 bp (2 X 25 bp)[i]	3 Gb[I] (4 Gb)	5-7 days[k] (10 days)	99.94%
Helicos Heliscope	n/a	n/a	25-35 bp[I]	7.5-10 Gb	3-7 days	>99%

… and also… "Next-Next-" or "Third"-Generation Sequencing Technologies

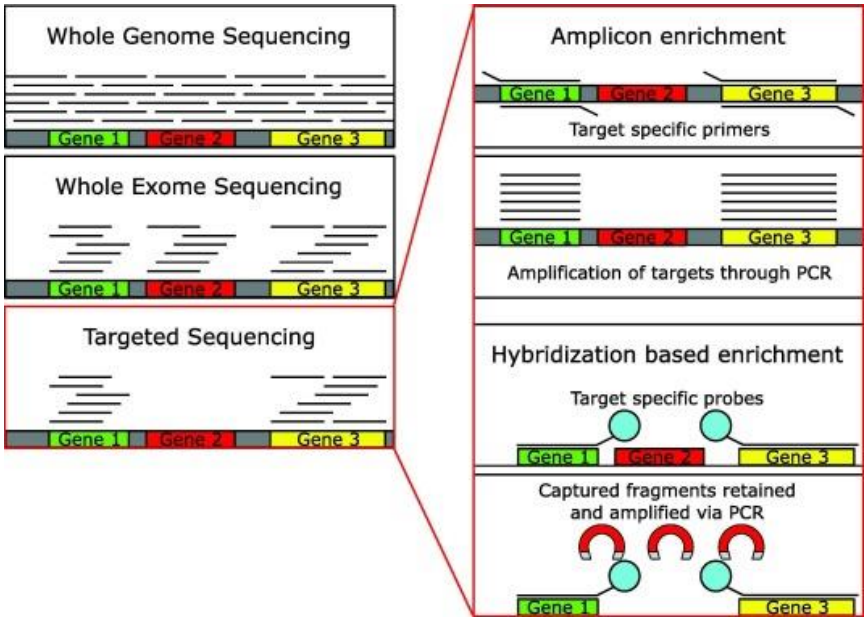
Pacific Biosciences
Oxford Nanopore
Ion Torrent
Others…

Molecular oncogenetic diagnostics - NGS



Kamps R, Brandão RD, Bosch BJ, et al. Next-Generation Sequencing in Oncology: Genetic Diagnosis, Risk Prediction and Cancer Classification. *Int J Mol Sci.* 2017;18(2):308. Published 2017 Jan 31. doi:10.3390/ijms18020308

Molecular oncogenetic diagnostics – targeted NGS



Conclusions

- The diagnostic algorithm should be adapted to the local mutation spectrum
- Pre-screening PCR-bases techniques can rapidly identify known mutations
- The overall diagnostic efficiency, cost/effectiveness and duration can be significantly improved
- Next-generation technologies are the future gold standard in molecular diagnostic

Take Home Message

- The methodology for molecular oncogenetics diagnostic is various in complexity and cost/effectiveness
- Various techniques target known precise mutations and SNPs
- Complete gene sequencing is expensive and time-consuming
- Overall methodology should be adapted to local mutation spectrum
- Gene sequencing should be preceded by adapted mutational pre-screening

References (selective)

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II.5. Interpretation of the molecular diagnosis results: from the laboratory test to the clinical decision

Learning objectives

• Define the scope, benefits and limits of oncogenetics;

• Describe the context of genetic testing, the most frequently used laboratory methods, as well as their advantages and disadvantages;

• Evaluate individual risk criteria (according to current guidelines) and identify clinical indications for genetic testing

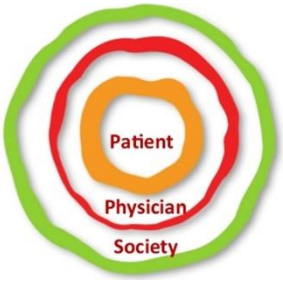
• Define, classify and interpret possible test results;

• Demonstrate an understanding of the basic elements of oncogenetic counselling;

• Summarize available scientific data regarding effectiveness of risk assessment, genetic counseling, and genetic testing in reducing cancer incidence and mortality (focus on BRCA1/2-related cancers);

• Assess the multi-dimensional impact of genetic testing on patients and their families.

Genetic assessment



• Implications of genetic assessment are reflected at multiple levels

Patients (education; psychological and medical risk management; financial, logistic and temporal burdens)

Physicians (education; resource allocation)

Society (testing and monitoring costs; workforce attrition)

Introduction: Context and rationale of genetic assessment

• Cancer is a (the) genetic disease by excellence…

…as we all have in our genes the predisposition for developing it.

• We know at least some inherited genetic lesions (BRCA, MMR, MSI…) imply great oncological risk for their carriers…

…and we now have the tools to highlight them before the symptomatic outbreak or even before the occurrence of the malignant lesion.

• Despite many therapeutic advances, cancer remains a deadly disease, unless discovered early in its clinical course…

…and prevention (e.g., by oncogenetic testing/counselling/interventions) is easier and more effective than treatment.

Genetic risk assessment, testing and counselling

• Cancer genetic risk assessment and genetic counseling is a multi-step process of identifying and counseling individuals at risk for familial or hereditary cancer

• Cancer genetic risk assessment is a dynamic process involving combined use of pedigree analysis with available risk assessment models to determine whether a family history is suggestive of sporadic, familial, or hereditary cancer

evaluation of an individual's absolute risk for breast and/or ovarian cancer

estimation of the likelihood that the individual has a heritable pathogenic or likely pathogenic variant in his/her family

Genetic risk assessment, testing and counselling are included by more and more specialists in the field under an umbrella term designating a new medical specialty:

ONCOGENETICS

"Oncogenetics": What does it mean?

• Some think it is a waste of time (and money)…

• Others think it is yet another term for carcinogenesis…

• Most think it is a synonym for genetic counseling, *i.e.*

"…the process by which patients or relatives at risk of a disorder that may be hereditary are advised of the consequences of the disorder, the probability of developing or transmitting it and the ways which this may be prevented, avoided or ameliorated."

• …but it actually is a major part of the genomic medicine of the (near) future, by which we will be able to…

"…document the drivers of an individual cancer and delineate the gain-of-function mutations giving rise to growth-promoting proteins that in turn induce oncogene 'addiction', in which a cancer is dependent on such proteins, […] also determine the loss-of-function mutations that deprive cancer cells of the proteins that direct DNA repair and/or provide directions to the cell death pathway. […] we will match the validated mutations of a particular cancer to an appropriate and scientifically determined targeted drug array."

Nathan DG et al. (2009)

"Oncogenetics": What does it do?

Putative main elements of oncogenetics:

Documentation of family information and pedigree construction

Knowledge of the cancers (within a syndrome) to which a predisposition may exist, their clinical features and diagnosis

Recognition of inheritance patterns and risk estimation

Communication and empathy with the probands/their families

Provision of information on available options and further measures

Support in decision-making and for decisions made

Arrangements of care for those at high risk

"Oncogenetics": What does it NOT do?

• The onset of multiple cancers in the same family is NOT necessarily a sign of a genetic predisposition (chance, shared exogenopredisposition gene does NOT "mandatorily" confirm onset of the cancer concerned

• Not discovering a predisposition us factors...)

• The discovery of a gene in a person belonging to a "high risk" family does NOT eliminate cancer risk, or the need for usual screening

• Technical capabilities have evolved before the tests were actually proven to be useful and innocuous, and before human subject protection and "education" to face such discoveries was established

Today, predictive genetic testing does not seem discriminative, but the technique is not perfect yet and the concerned population is still too small. And tomorrow?

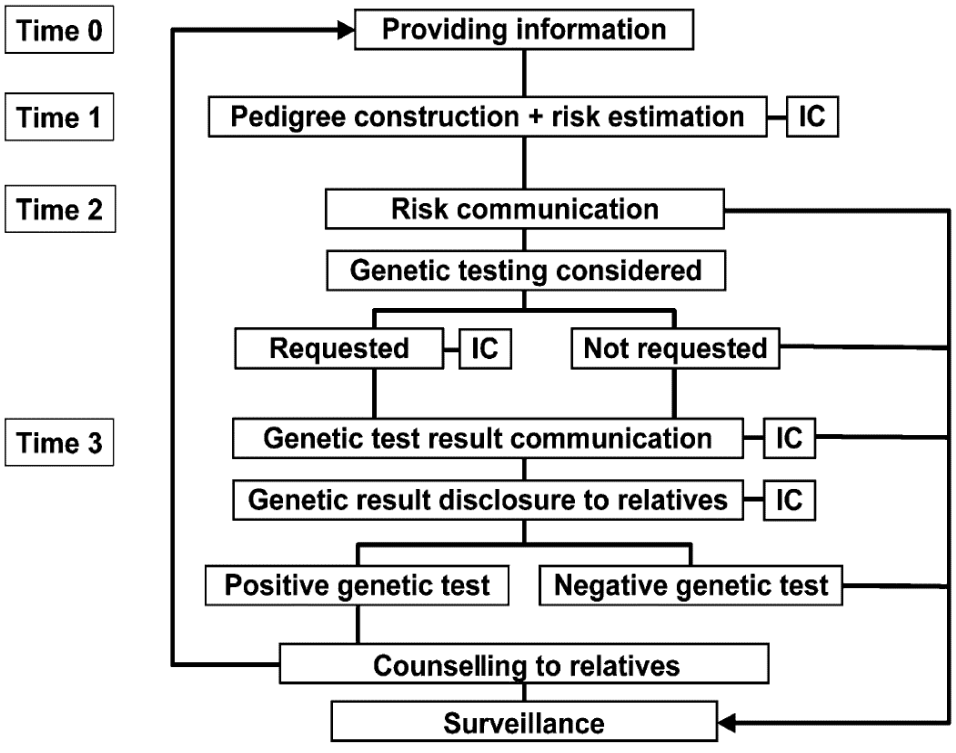
Who can and should be a guarantor of data usage and social integration, which is part of the respect of the human subject's integrity?

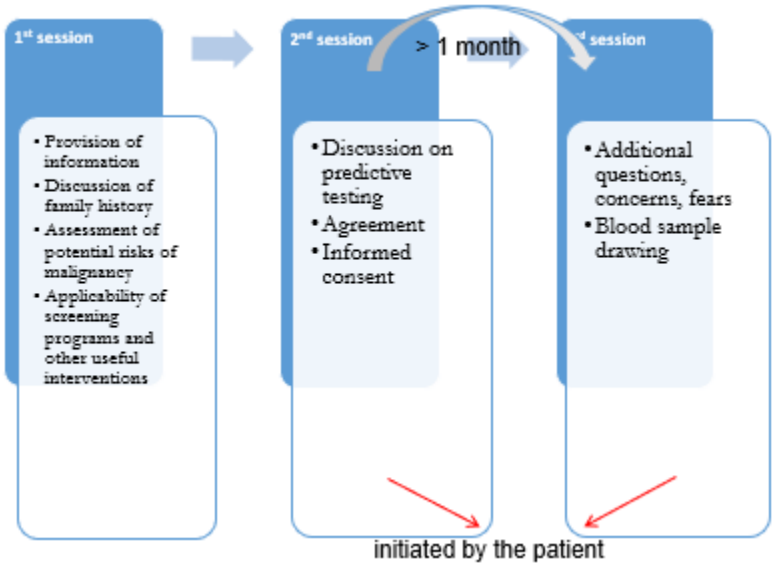
Oncogenetics approach: steps, actions & people

Step		Description	Professionals involved
T0	Providing information	Information/education about sporadic, familial and hereditary breast cancers. Information about risk assessment procedures. Information/education about preventive strategies, lifestyle implications and health-promoting behaviour. Collection of personal history, histological report.	Oncologist counsellor; psychologist (psychiatrist at the Catanzaro unit)
T1	Pedigree construction	Pedigree construction for at least three generations.	Oncologist counsellor.
	Risk estimation	Analysis of pedigree acquired. The risk profile is defined as individual, familial and inherited (Claus, Modena and Frank models).	Oncologist counsellor; geneticist (when requested)
T2	Risk communication	Communication about individual and/or familial and/or inherited risk	Oncologist counsellor; psychologist (psychiatrist at the Catanzaro unit)
		Communication about the implication of the risk estimation for the user and for the user's relatives.	
	Genetic testing considered	Genetic test offered in case of suspected inherited risk.	
		Discussion about advantages and limits of genetic testing.	
T3	Genetic test result communication	Communication of the results and discussion about implications.	Oncologist counsellor; psychologist (psychiatrist at the Catanzaro unit)
	Genetic results disclosures to relatives	The proband informs his/her relatives about genetic test results and informs them about counselling.	
	Genetic results for relatives	Relatives interested in counselling contact the unit for an appointment.	Oncologist counsellor; psychologist (psychiatrist at the Catanzaro unit)
	Surveillance	Surveillance measures modelled on different levels of risk. Discussion of preventive measures available, including chemoprevention and/or prophylactic surgery.	Oncologist; surgeon; gynaecologist; radiologist

Oncogenetics approach: Visit protocol

The process of predictive genetic testing should be delivered through a protocol requiring 3 sessions of counseling before communication of the results.





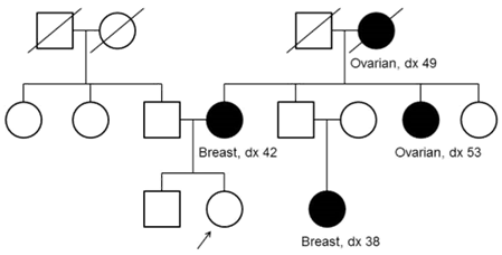
Genetic risk assessment, testing and counselling: Key elements

- **Evaluation of needs and concerns**
- **Clinical evaluation**
  - Detailed family history
  - Medical and surgical history
  - Focused physical examination
- **Genetic testing and interpretation**
- **Communication of test results**
- **Genetic counseling**
- **Passive/active follow-up and/or treatment** (the HBOC example)

Family history

- Involves development of an expanded pedigree

Starting with the individual diagnosed with cancer and proceeding outward to include first-, second-, and third- degree relatives on both the maternal and paternal sides
cancer diagnoses by primary site
age at diagnosis
bilaterality (when appropriate)
current age or age at death
Standardized pedigree nomenclature
Unaffected family members (living/deceased) also included
Cancer diagnoses in the family verified whenever possible (medical records, pathology reports, death certificates)
Other predisposal/medical conditions noted
Graphical representation.



Bennett RL et al. (2008)

Classic BRCA1 pedigree

Factors that limit the informativeness of the pedigree:

- small family;
- small number of individuals of the susceptible gender for sex-limited cancers;
- reduced penetrance;
- early deaths (hence not developing adult diseases);
- "prophylactic" surgery (e.g. hysterectomy for uterine fibroma also removing the ovaries);
- inaccurate or incomplete information on family members (e.g. adoption);

Source: Berliner JL et al. (2007); Calzone CA et al. (2008)

Medical and surgical history & Physical examination

- Estimates contribution of other risk factors that interact with or modify family history
    - Examples of relevant information:
        - Reproductive variables
        - History of previous breast biopsies and pathology results (especially if diagnostic for atypical hyperplasia or lobular carcinoma in situ)
        - History of salpingo-oophorectomy
        - Exposure to iatrogenic carcinogens (radiation, oral contraceptives)
- Physical examination performed by a qualified clinician

- Should also assess organs/areas of the body known to be affected in specific hereditary breast and/or ovarian syndromes (e.g., Cowden's syndrome: dermatologic examination, measurement of head circumference, and palpation of the thyroid).

Berliner JL et al. (2007); Calzone CA et al. (2008)

Genetic testing: Benefits & Drawbacks

Benefits

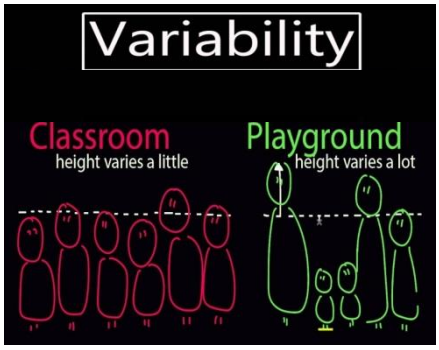
- Can end uncertainty

descriptive AND predictive for the disease risk

- May relieve anxiety
- Clarify cancer risks for an individual
- Clarify cancer risks for relatives
- Aid in medical decision making
- Aid in lifestyle decision making

Risks/Limitations

- Can initiate uncertainty
    NOT binary
    NOT predictive of disease severity, if any
- May induce anxiety
- Negative test results may be uninformative or falsely reassuring
- Most cancers are a result of the interactions between genes and environment
- Timing of testing may not be optimal
- Patient may prefer not to know his/her genetic status/future cancer risks
- Family dynamics
- Concerns about genetic discrimination
- Concerns about confidentiality



Genetic testing: Methodology

Molecular techniques can be performed on a variety of clinical specimens:
- fresh/snap-frozen tissue;
- formalin-fixed paraffin-embedded tissue;
- cytology specimens – fresh/fixed fine-needle aspirations (FNA) samples;
- blood;
- bone marrow;
- buccal swabs;

• Typically performed on a blood sample;
• Multiple laboratories available ;
• Depending of patient's personal and family history, testing target can consist of:
  - single site aberrations (Sanger sequencing);
  - founder mutations (Sanger sequencing);
  - targeted deletion/duplication analysis (CGH);
  - gene panel testing ("targeted" NGS);
  - large-scale sequencing (WGS, WES, "systematic" NGS);

comparative genomic hybridization (**CGH**)
next generation sequencing (**NGS**)
whole genome sequencing (**WGS**)
whole exome sequencing (**WES**)

Specimen requirements for molecular diagnosis

✓ Specimen requirements are related to the **type of the disease** and of the **chosen molecular technique** for analysis.

✓ RNA molecules are less stable than DNA molecules

✓ These molecules are very easy degraded by a variety of ribonuclease enzymes from the cell and its environment

✓ Only fresh/frozen samples – universally accepted for RNA-based testing

✓ Hematologic specimens (blood and bone marrow) – collection in the presence of anticoagulants (EDTA/ACD, but no heparin, which inhibits PCR amplification)

✓ Conventional cytologic analysis – fresh tissue

✓ Fluorescence in situ hybridization (FISH) – frozen sections, touch preps, paraffin-embedded tissue, cytology slides

✓ Formalin-fixed paraffin-embedded tissue samples – poor quality RNA, used for less applications

✓ The amount of tissue required depends on:
  - purity of the tumor sample
  - sensitivity of the technique

Related molecular abnormalities for each technique

Applications of various molecular samples

Molecular technique	Molecular abnormalities	Examples
Cells from buccal swabs	Detection of germline mutations	RET mutations in familiar medullary thyroid carcinoma
Blood and bone marrow biopsy material	Detection of chromosomal rearrangements	Hematologic malignancies
Tumor tissue samples	Somatic mutations	KRAS point mutations in colorectal cancer SYT/SSX rearrangements in synovial sarcomas EGFR mutations in lung adenocarcinomas
Fresh/snap-frozen tissue	Any type of molecular analysis – excellent quality of DNA, RNA, protein	Detection of somatic mutations Chromosomal rearrangements Gene expression arrays miRNA profiling
Formalin-fixed paraffin-embedded tissue	Do not provide well-preserved nucleic acids – mostly DNA testing	Breast cancer molecular subtypes (ESR1, ERBB2)
Fixed cytology specimens	Do not provide well-preserved nucleic acids – mostly DNA testing	Breast cancer molecular subtypes (ESR1, ERBB2)

Common techniques for molecular testing

Molecular technique	Principle	Applications
Polymerase chain reaction (PCR)	Exponential and bidirectional amplification of DNA sequences with oligonucleotide primers Subsequent gel electrophoresis	Detection of small deletions/insertions Microsatellite instability LOH (loss of heterozygosity)
Reverse transcription PCR	Modified standard PCR technique for mRNA amplification	Gene rearrangements Gene expression
Real-time PCR (rtPCR) **Quantitative PCR (qPCR)**	Similar main principles as PCR, but detects and quantifies in real time the PCR product, using fluorescently labeled molecules Without subsequent gel electrophoresis Variation of real-time PCR	Point mutations Single nucleotide polymorphism Gene expression levels/gene copy numbers
Restriction fragment length polymorphism analysis	Is based on the ability of restriction enzymes (endonucleases) to cut DNA at specific sites	Point mutations Single nucleotide polymorphism Separation of two amplified sequences with similar nucleotide composition
Single-strand conformation polymorphism analysis	Post PCR technique for mutations not limited to a single hot spot	Point mutations Small deletions/insertions
Allele-specific PCR **Allele-specific hybridization (dot-blot analysis)**	The amplification of target DNA is performed in two reactions The PCR products are directly spotted on the nylon membrane	Point mutations Sequence polymorphism
DNA sequencing analysis	It uses chain-terminating dideoxynucleoside triphosphates labeled with fluorescent dyes	For automated platforms for detection of various mutations
Fluorescence in situ hybridization (FISH)	Fluorescently labeled DNA probes that bind to homologous chromosomal regions and can assess interphase nuclei	Gene rearrangements Chromosome deletion and amplification Numerical chromosomal abnormalities

Interpreting results: Human genetic variant databases

• Researcher-submitted information about human genetic differences to document the evidence supporting links between such variants and a disease or condition, i.e. informed assessments of their

correlation (or lack thereof) based on the current state of knowledge

• Multiple private (mostly laboratory/pharma-owned) or public registries and databases (e.g., ClinVar, PROMPT, COSMIC, DECIPHER etc.) available

• Support the aggregation, curation, clinical interpretation, and sharing of data on disease-associated variants

• Designed to support increase of understanding of the relationship between genotypes and medically important phenotypes

• **Pathogenicity class is NOT automatically computed!**

Source: Ellard S, et al. ACGS (2019)

Database organization and results should be standardized and documented, including:

- **Standard Operating Procedures (SOPs)**, policies or other documents on:
  - general database operation
  - data confidentiality, privacy, integrity and security
  - curation, (re)evaluation
- **Validation studies** and **documentation of the qualifications** of evaluators
- **Data preservation plan**
- **Conflict of interest policies** and disclosures
- **Commitment to make publicly accessible** (via weblinks) all recommended documents
- **Disclaimer on clinical validity** of genetic data, including possibility of re-classification
  - *e.g.*, "This result does not confirm a genetic diagnosis of disorder X and should not be used in isolation for clinical decision making" *or* "The clinical significance of this variant is uncertain, and it should not be used in isolation for clinical decision making".

https://www.fda.gov/medical-devices/precision-medicine/fda-recognition-public-human-genetic-variant-databases

Ellard S, et al. ACGS Best Practice Guidelines for Variant Classification 2019. https://rebrand.ly/m3ea90

Interpreting results: Terminology

• **mutation** = permanent change in the nucleotide sequence

• **polymorphism** = variant with a frequency above 1%

  • incorrect assumptions of pathogenic and benign effects, respectively → both terms to be replaced by the term "**variant**"

• **"likely" (pathogenic/benign)** = (arbitrarily) greater than 90% certainty of a variant either being disease-causing or non-disease-causing (ACMG recommendation)

  • IARC guidelines suggest a 95% level of certainty (less tolerable by patients/clinicians?)

• Strict evidence-based rules must be applied to determine if a variant in a gene with definitive role in a Mendelian disorder may be pathogenic for that disorder, in general (independently of interpreting the cause of disease in an individual patient)

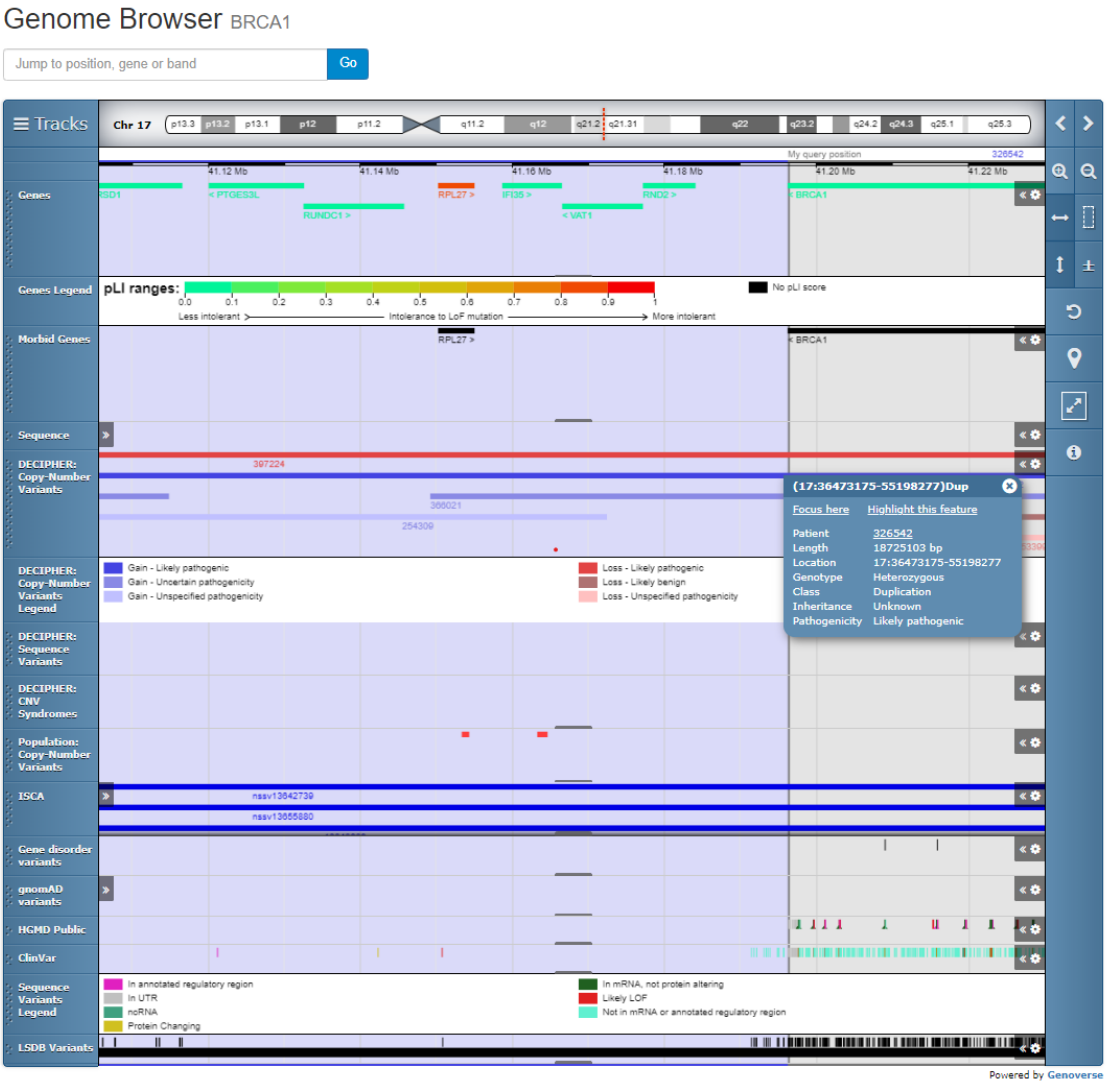
• All assertions of pathogenicity (including "likely pathogenic") should be reported with respect to a specific condition and inheritance pattern.

Interpreting results: Pathogenic/likely pathogenic variants

• (Likely) pathogenic gene variants disrupt protein function and increase the risk of developing cancer;

• Deleterious mutations: premature termination of protein synthesis, frameshift, alteration of essential amino acids, alteration of splicing sites.

Presence of a (likely) pathogenic variant does NOT imply a definitive risk of cancer



Example of query results for a likely pathogenic BRCA1 variant
from the DECIPHER online database

https://decipher.sanger.ac.uk/browser#q/BRCA1/location/17:41105676-41230676

Interpreting results: Benign/likely benign variants

• (Likely) benign gene variants are alterations that do not imply phenotypic modifications (common polymorphisms) – but may be (yet unknown) risk modifiers

• There is a continuous danger of false-positive/false-negative results

- **manipulation errors** (less frequent)

– contamination

– mixing/inverting samples
– coding errors
  - **false-positives** (very rare)
– identifying a non-existing mutation
– "over-evaluating" an UV
  - **false-negatives** (quite often)
– not identifying an existing mutation
– "sub-evaluating" an UV

Presence of only benign/ likely benign variant(s) does NOT imply a definitive lack of cancer risk

Interpreting results: Variants of unknown significance (VUS)

- **VUS** <= available evidence insufficient to ascertain pathogenicity (~50% of variants!)
- Mutations with unknown *a priori* effects: premature terminations at terminal sites, alteration of non-essential amino acids, splicing site alterations, del/ins, substitutions
- Major challenge in genetic counseling and clinical management
  - *US Supreme Court*: Assoc. for Molecular Pathology v. Myriad Genetics, 569 U.S. 576 (2013)
  - individually rare (2.1% in major US labs, after 20 years & 1 mil. samples tested), but collectively frequent (especially in people of African, Asian, or Middle Eastern descent)
  - exposure to VUS increased when testing for a larger number of genes
  - most likely benign (patients overestimate VUS significance!) and some (most?) re-classified over time (patients need to be notified if management altered …years later?!)
  - patient management based on personal and family history
  - genetic labs to agree on clinically clear and uniform format for reporting BRCA test results to non-geneticists (ACMG criteria)
  - clinical research essential

NOT NECESSARILY BAD, BUT NOT NECESSARILY GOOD EITHER

Vos J (2012), Culver JO et al. (2013), Cartwright-Smith L. (2014), https://supreme.justia.com/cases/federal/us/569/576/

Reporting results: Guidelines

- **ACMG / AMP / CAP standard description of gene variants** (Mendelian disorders):
  - ***Class 5***: "pathogenic variant"
  - ***Class 4***: "likely pathogenic variant"
  - ***Class 3***: "variant of uncertain significance"
  - ***Class 2***: "likely benign variant"
  - ***Class 1***: "benign variant"

Should NOT be used for genetic counselling

ACMG, American College of Molecular Genetics; AMP, Association for Molecular Pathology; CAP, College of American Pathologist

- **Classification mostly based on in-silico analysis**
- **No data for quantitative assignment of variant certainty to any class**

- **Forms of evidence** (+/– multifactorial model):
  - population data
  - pedigree (segregation) analysis
  - evolutionary data
  - computational predictions
  - functional observations from clinical/laboratory material

(Publications)
Public/NHS databases
Laboratory databases

Richards S, et al. *Genet Med.* 2015 May;17(5):405-24.
Ellard S, et al. ACGS Best Practice Guidelines for Variant Classification 2019.
https://rebrand.ly/m3ea90

Reporting results: ACMG criteria for classifying pathogenic variants

Very strong evidence of pathogenicity

PVS1 Null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease

Strong evidence of pathogenicity

PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change

PS2 *De novo* (both maternity and paternity confirmed) in a patient with the disease and no family history

PS3 Well-established in vitro/in vivo functional studies showing damaging effect on the gene/gene product

PS4 Variant prevalence in affected individuals is significantly increased vs. prevalence in controls

Moderate evidence of pathogenicity

PM1 Located in mutational hotspot and/or critical, well-established functional domain (e.g. active site of enzyme) without benign variation

PM2 Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes or ExAC

PM3 For recessive disorders, detected in trans with a pathogenic variant

PM4 Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants

PM5 Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before

PM6 Assumed *de novo*, but without confirmation of paternity and maternity

Supporting evidence of pathogenicity

PP1 Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease

PP2 Missense variant in gene with low rate of benign missense variation and where missense variants are a common mechanism of disease

PP3 Multiple lines of computational evidence prove deleterious effect on gene/gene product (conservation, evolutionary, splicing impact)

PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology

PP5 Reputable source recently reports variant as pathogenic but evidence not available to the laboratory for independent evaluation

Richards S, et al. *Genet Med.* 2015 May;17(5):405-24.

Reporting results: ACMG criteria

ACMG criteria organization by type of evidence and strength of the criteria for a benign (left side) or pathogenic (right side) assertion.

	Benign		Pathogenic			
	Strong	**Supporting**	**Supporting**	**Moderate**	**Strong**	**Very Strong**
Population Data	MAF is too high for disorder *BA1/BS1* **OR** observation in controls inconsistent with disease penetrance *BS2*			Absent in population databases *PM2*	Prevalence in affecteds statistically increased over controls *PS4*	
Computational And Predictive Data		Multiple lines of computational evidence suggest no impact on gene /gene product *BP4* Missense in gene where only truncating cause disease *BP1* Silent variant with non predicted splice impact *BP7*	Multiple lines of computational evidence support a deleterious effect on the gene /gene product *PP3*	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before *PM5* Protein length changing variant *PM4*	Same amino acid change as an established pathogenic variant *PS1*	Predicted null variant in a gene where LOF is a known mechanism of disease *PVS1*
Functional Data	Well-established functional studies show no deleterious effect *BS3*		Missense in gene with low rate of benign missense variants and path. missenses common *PP2*	Mutational hot spot or well-studied functional domain without benign variation *PM1*	Well-established functional studies show a deleterious effect *PS3*	
Segregation Data	Non-segregation with disease *BS4*		Co-segregation with disease in multiple affected family members *PP1*	Increased segregation data →		
De novo Data				*De novo* (without paternity & maternity confirmed) *PM6*	*De novo* (paternity & maternity confirmed) *PS2*	
Allelic Data		Observed in *trans* with a dominant variant *BP2* Observed in *cis* with a pathogenic variant *BP2*		For recessive disorders, detected in *trans* with a pathogenic variant *PM3*		
Other Database		Reputable source w/out shared data = benign *BP6*	Reputable source = pathogenic *PP5*			
Other Data		Found in case with an alternate cause *BP5*	Patient's phenotype or FH highly specific for gene *PP4*			

Richards S, et al. *Genet Med.* 2015 May;17(5):405-24.

BS, benign strong; BP, benign supporting; FH, family history; LOF, loss-of-function; MAF, minor allele frequency; path., pathogenic; PM, pathogenic moderate; PP, pathogenic supporting; PS, pathogenic strong; PVS, pathogenic very strong.

Reporting results: Rules for combining criteria to classify sequence variants

PATHOGENIC	**(i) 1 very strong (PVS1)** *AND* a) ≥1 strong (PS1-4) *OR* b) ≥2 moderate (PM1-6) *OR* c) 1 moderate (PM1-6) and 1 supporting (PP1-5) *OR* d) ≥2 supporting (PP1-5) *OR*
	(ii) ≥2 strong (PS1-PS4) *OR*
	(iii) 1 strong (PS1-PS4) *AND* a) ≥3 moderate (PM1-6) *OR* b) 2 moderate (PM1-6) AND ≥2 supporting (PP1-5) *OR* c) 1 moderate (PM1-6) AND ≥4 supporting (PP1-5)
LIKELY PATHOGENIC	**(i) 1 very strong (PVS1)** *AND* **1 moderate (PM1-6)** *OR*
	(ii) 1 strong (PS1-4) *AND* **1-2 moderate (PM1-6)** *OR*
	(iii) 1 strong (PS1-4) *AND* **≥2 supporting (PP1-5)** *OR*
	(iv) ≥3 moderate (PM1-6) *OR*
	(v) 2 moderate (PM1-6) *AND* **≥2 supporting (PP1-5)** *OR*
	(vi) 1 moderate (PM1-6) *AND* **≥4 supporting (PP1-5)**

BENIGN	**(i) 1 stand-alone (BA1)** *OR*
	(ii) ≥2 strong (BS1-4)
LIKELY BENIGN	**(i) 1 strong (BS1-4)** *AND* **1 supporting (BP1-7)** *OR*
	(ii) ≥2 supporting (BP1-7)
UNCERTAIN SIGNIFICANCE	**(i) other criteria shown above not met** *OR*
	(ii) criteria for benign and pathogenic contradictory

Communicating test results

- **Informed consent is a dominant consideration.**
- Genetic counselling is time intensive and depends on accurate data gathering.
  - It is vital that sufficient time is allocated and that a full discussion takes place.
- The motivation of the individual for attendance should be assessed.
  - It is important to consider the timing of the visit (may have been provoked by cancer-related events that have occurred within the family in the past).
- Peoples' perception of risk varies and their wishes regarding the definition of risk and how it is to be conveyed should be determined.
- Children should not be involved in the process unless this is specifically indicated where there is a risk of cancer onset in childhood.

- In layman's terms, possible results provided by molecular genetic tests may be dubbed:

Positive (i.e., ACMG *class 4 "pathogenic"* or ACMG *class 5 "likely pathogenic"*)
Unclear (i.e., ACMG *class 3 "variant of uncertain significance, VUS"*)
Benign (i.e., ACMG *class 2 "likely benign"* or *ACMG class 1 "benign"*)
Negative (i.e., no relevant variant identified whatsoever)
true negative
uninformatively negative

- **"Positive" results (classes 4 & 5) MUST be confirmed** using an orthogonal method:

re-extraction of the sample and re-testing
testing of parents
restriction enzyme digestion
sequencing the area of interest a second time
using an alternate genotyping technology

Richards S, et al. *Genet Med.* 2015 May;17(5):405-24.
Ellard S, et al. ACGS Best Practice Guidelines for Variant Classification 2019.
https://rebrand.ly/m3ea90

Communicating test results: *Positive test meaning*

= An expected (known to be associated with an inherited cancer susceptibility syndrome) genetic variant was found.

A positive test result may:

• **confirm the inherited genetic component of the cancer** (for a person already diagnosed) and possibly assist therapeutic decision

• **indicate an increased risk of developing cancer** and the necessity of oncogenetic team case management to lower that risk

• **help other family members decide whether to have genetic testing** to detect that, or any other inherited variant

Communicating test results: *"Unclear" test meaning*

= An UNexpected genetic variant was found (NOT commonly seen, but also NOT known to be associated with an inherited cancer susceptibility syndrome).

A VUS test result may:

• not be considered in health management strategies

• be reclassified (over time) in relationship to cancer, to benign (not clinically important) or **(likely) pathogenic** (associated with increased risks for cancer)

Interpretation of the molecular genetic tests: *Benign test meaning*

= An expected genetic variant was found (NOT known to be associated with an inherited cancer susceptibility syndrome, AND commonly seen in general population without cancer)

Communicating test results: *Negative test meaning*

= An expected (known to be associated with an inherited cancer susceptibility syndrome) genetic variant was NOT found.

A negative test result may be:

true negative: there is no susceptibility for the inherited cancer susceptibility in the person tested – cancer risk is the same as in the general population

uninformatively negative: there is a strong family history for cancer, but no genetically test-proven variant associated with a hereditary cancer syndrome was found

Even if the test is negative, **appropriate cancer screening and surveillance** based on personal and family history and on other risk factors **is warranted**.

Genetic counselling

- Interactive and customized, using a broad approach to emphasize context

  should always start with an assessment of the individual's concerns and reasons for seeking counseling and to guarantee that her/his personal needs and priorities will be addressed in the counseling process
- Positive, supportive interaction with the counseling team

  important determinant of the individual's satisfaction with the counseling process and adherence to recommendations
- Education of individuals about the genetic, biological, and environmental factors related to their cancer diagnosis and/or risk for disease

  information should be objective, based on current clinical evidence and delivered in a manner adequate to the individual's age, knowledge, cultural level, social environment, psychological frame and goals

  Exaggerated, as well as attenuated risk perception can interfere with the adoption of appropriate health behaviors

Genetic counselling: The counselor's limits

Ideal:

Clinical genetics health professionals working in teams according to well-established protocols, similar across different European (world) countries.

Reality:

Dedicated funding – low to none

Awareness – limited

Access to educational, counselling, research and follow up resources – currently available only at major medical centers

Number of health professionals involved in providing clinical genetic services – small

Time to accessing appropriate professional opinion and receiving a result from the laboratory – combined delay of 4-5 months is norm

Access of family members to accurate and understandable information – not generally available/not desired (structural, educational, cultural, emotional, financial issues)

Genetic counselling: The counselee's limits

Great interest claimed[1-3], but high rates of foregoing genetic counseling[4]:

- estimated cancer risk and its perception[2,5]
- expected benefit or limitations of genetic testing[6-8]
- general psychological distress[7,9]
- cancer-specific distress[7,9]
- lack of trust in one's emotional reactions when faced with negative events[11-13]
- expected level of family support[11-13]
- communications within the family[11-13]

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Genetic counseling: Stages

Pre-test counseling

Essential, especially if genetic testing for a pathogenic or likely pathogenic variant associated with a hereditary cancer syndrome is concerned

Based on the principle of the informed consent

Discussion (at least) on:

Test rationale and impact on medical management
Cancer risks associated with the pathogenic or likely pathogenic variant in question
Significance of possible test results and likelihood of a positive test result
Risk variability (inheritance patterns, penetrance, variable expressivity, potential for genetic heterogeneity)
Technical aspects and accuracy of the test
Economic considerations
Risks of genetic discrimination, psychosocial aspects, confidentiality issues
Potential significance of the test results for family members

Genetic counseling: Stages

Post-test counseling

Disclosure of results
Discussion of the significance of the results
Assessment of the impact of the results on the emotional state of the individual
Discussion on impact of the results on the medical management
Enumeration and explanations on follow-up modalities and timing
Possible inherited cancer risk to relatives
Importance of informing family members about test results
Awareness of available resources (disease-specific support groups, advocacy groups, research studies)
Provisions for supportive interventions against distress

Passive/active follow-up and/or Treatment. An example: HBOC

Breast/ovarian cancer risk: Risk models

The Modena model

• 1628 family histories collected from 1994 by the Modena Study Group for Familial Breast and Ovarian Cancer

• BC risk estimate assessed according to the Gail model, Claus tables and a slightly modified BRCAPro model, adapted to the Italian population

	High risk	(30-50% lifetime risk)	Pedigree classification	
I) at least 3 relatives diagnosed with BC (or OC) in 2 different generations	II) one BC/OC case is a first-degree relative of the other 2 (of the other I if the first criterion is not fulfilled)°	III) at least one case has been diagnosed at the age ≤ 40 or with bilateral BC		
•	•	•	Hereditary	HBC/*HBOC
•	•		Suspected Hereditary	SHBC/SHBOC
			Suspected Hereditary	SHBC/SHBOC
BC diagnosed at age ≤ 35, regardless of family history			Early Onset	EOBC
BC and OC in the same woman, regardless of family history			Breast Ovarian Cancer	BOC

	Intermediate risk	(18-29% lifetime risk)		
•			Familial	FBC/FBOC
	•	•	Strongly Suspected Familial	**SFBC+ **SFBOC+
Male BC, regardless of family history			Male Breast Cancer	MBC

	Slightly increased risk	(6-17% lifetime risk)		
	•		Suspected Familial	**SFBC/**SFBOC
		•	Suspected Familial	**SFBC/**SFBOC
BC/OC without any of the described criteria		•	Sporadic Breast Cancer	SpBC/SpOC

° male relatives excluded when calculating the degree of relationship

*If at least two of the malignancies are OC, the pedigree must be classified as HBOC even if the third criterion is not fulfilled.

**At least two cancer cases are required.

HBC – hereditary breast cancer;
HBOC – hereditary breast/ovarian cancer;
SHBC – suspected hereditary breast cancer;
SHBOC – suspected hereditary breast/ovarian cancer;
EOBC – early onset breast cancer;
BOC - breast ovarian cancer;
FBC – familial breast cancer;
FBOC – familial breast/ovarian cancer;
SFBC+ - strongly suspected familial breast cancer;
SFBOC+ - strongly suspected familial breast/ovarian cancer;
MBC – male breast cancer;
SFBC – weakly suspected familial breast cancer;
SFBOC – weakly suspected familial breast/ovarian cancer;
SpBc – sporadic breast cancer;
SpOC – sporadic ovarian cancer

Cortesi L, et al. *BMC Cancer* 2006, 6:210

Breast/ovarian cancer risk: "The usual suspects"

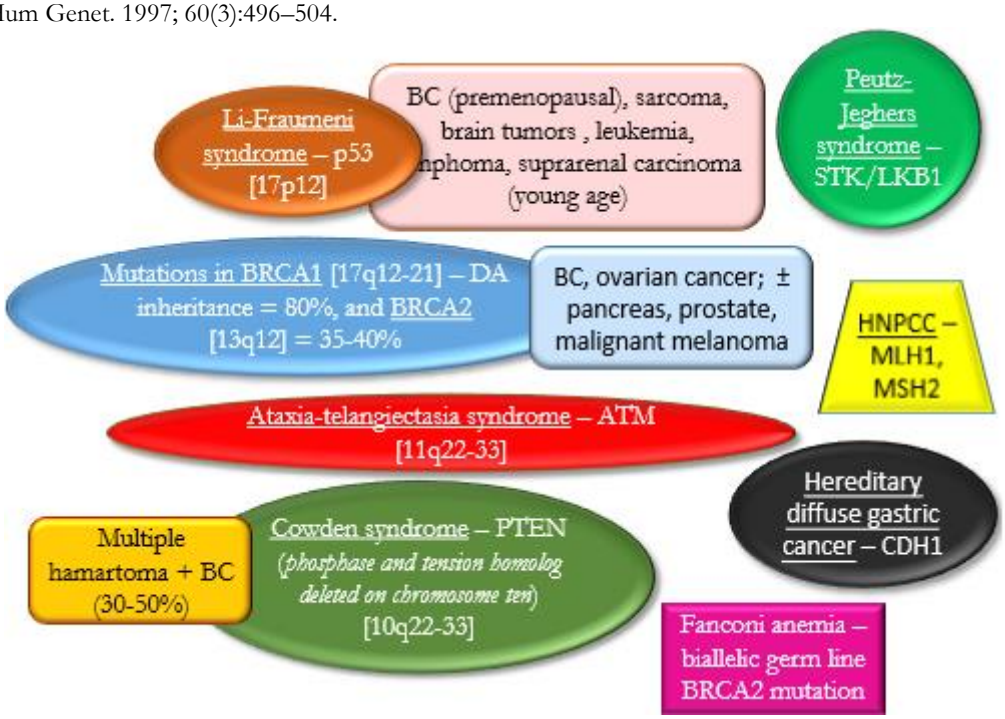
• Breast cancer (BC) predisposition is in most cases a complex trait, attributable to variants in multiple genes;

• 5-10% of all BCs associated with single gene mutations (Mendelian inheritance);

• Hereditary breast-ovarian cancer syndrome (HBOC) relatively common among cancer predisposition syndromes

- 1 : 345 general US population (0.3%)
- 1 : 44 Ashkenazi Jews (2%)
- much higher in patients with breast cancer even higher in patients with suspicious family history.

Devilee P. WHO Classification of Tumours of the Breast., 2012. Foulkes WD. N Engl J Med. 2008;359(20):2143–2153. Howlett NG, et al. Science. 2002:297(5581):606–609. King MC. Am J Hum Genet. 1997;60(5):1013–1020. Pharoah PD, et al. Nat Genet. 2002;31(1):33–36. Struewing JP, et al. N Engl J Med. 1997;336(20):1401–1408. Szabo CI, Whittemore AS, et al. Am J Hum Genet. 1997; 60(3):496–504.



Breast/ovarian cancer: Genetic testing principles

• HBOC-associated genes are predominantly classical tumor suppressors ("diffuse" mutations at numerous amino acid positions, not "hot spots")

• Comprehensive sequencing of the entire coding region of the gene of interest – recommended to ensure sufficient clinical sensitivity of testing for germ line breast cancer predisposition

- patients from populations with a known founder effect (e.g. Ashkenazi, Icelandic, Swedish, Hungarian, Dutch) may be candidates for genotyping of founder-specific positions
- clinical presentation may be highly suggestive (e.g., Cowden syndrome) – testing can focus on the single most likely causative gene (e.g., PTEN)
  - syndromes that include breast cancer usually have overlapping clinical presentations

• pathologic features of the tumor may suggest its genetic basis (e.g., lobular – CDH1; medullary or triple-negative – BRCA1; ER-positive – BRCA2)

NCCN Clinical Practice Guidelines in Oncology https://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf. Corso G, et al. Fam Cancer. 2016;15(2):215-219. Atchley DP, et al. J Clin Oncol. 2008;26(26):4282–4288. Mavaddat N, et al. Cancer Epidemiol Biomarkers Prev. 2012;21(1):134–147.

Breast/ovarian cancer: NCCN Genetic testing criteria

Testing clinically indicated if:

• *Any blood relative with a known pathogenic/likely pathogenic variant* in a cancer susceptibility gene

• *Individuals meeting the criteria below but with previous limited testing* (e.g., single gene and/or absent deletion duplication analysis) interested in pursuing multi-gene testing

• *Personal history of cancer*

1. Breast cancer with at least one of the following:

Diagnosed at age ≤45 y
Diagnosed at age 46–50 y with:
Unknown or limited family history; or
A second breast cancer diagnosed at any age; or
≥1 close blood relative with breast, ovarian, pancreatic, or high-grade (Gleason score ≥7) or intraductal prostate cancer at any age
Diagnosed at age ≤60 y with triple-negative breast cancer;
Diagnosed at any age with:
Ashkenazi Jewish ancestry; or
≥1 close blood relative with breast cancer at age ≤50 y or ovarian, pancreatic, or metastatic or intraductal prostate cancer at any age; or
≥3 total diagnoses of breast cancer in patient and/or close blood relatives;
Diagnosed at any age with male breast cancer

2. Epithelial ovarian cancer (including fallopian tube cancer or peritoneal cancer) at any age
3. Exocrine pancreatic cancer at any age
4. Metastatic or intraductal prostate cancer at any age
5. High-grade (Gleason score ≥7) prostate cancer with:

Ashkenazi Jewish ancestry; or
≥1 close relative with breast cancer at age ≤50 y or ovarian, pancreatic, or metastatic or intraductal prostate cancer at any age; or
≥2 close relatives with breast or prostate cancer (any grade) at any age.

6. A mutation identified on tumor genomic testing that has clinical implications if also identified in the germline
7. To aid in systemic therapy decision-making, such as for HER2-negative metastatic breast cancer

- *Family history of cancer*
  1. An affected or unaffected individual with a first- or second-degree blood relative meeting any of the criteria listed above (except individuals who meet criteria only for systemic therapy decision-making)
  2. An affected or unaffected individual who otherwise does not meet the criteria above but has a probability >5% of a *BRCA1/2* pathogenic variant based on prior probability models (e.g., Tyrer-Cuzick, BRCAPro, PennII)

Testing may be considered (with appropriate pre-test education and access to post-test management) **if:**

1. Bilateral breast cancer first diagnosed between the ages of 50 and 65 y
2. An unaffected Ashkenazi Jewish individual
3. An affected or unaffected individual who otherwise does not meet the criteria above but has a probability >5% of a *BRCA1/2* pathogenic variant based on prior probability models (e.g., Tyrer-Cuzick, BRCAPro, PennII)

There is a low probability (< 2.5%) that testing will have findings of documented clinical utility in:

1. Women diagnosed with breast cancer at age > 65 y, with no close relative with breast, ovarian, pancreatic, or prostate cancer;

2. Men diagnosed with localized prostate cancer with Gleason Score <7 and no close relative with breast, ovarian, pancreatic, or prostate cancer.

NCCN Guidelines v1.2020: Hereditary Cancer Testing Criteria,
https://www.nccn.org/professionals/physician_gls/pdf/genetics_bop.pdf

Breast/ovarian cancer: Genetic counselling/testing criteria

NCCN/ACMG/NSGC guidelines	Other criteria

• Female BC diagnosed ≤ 50 years
• TNBC diagnosed ≤ 60 years
• ≥ 2 primary BCs
• Invasive ovarian/fallopian tube cancer or primary peritoneal cancer
• Male BC
• Any HBOC-associated cancer (regardless of age) or Ashkenazy ancestry
• BC and either a relative with BC diagnosed ≤ 50 years or BC, or 2 relatives with BC, prostate or panc reatic cancer at any age
• Metastatic, regional, or high-/very high risk localized prostate cancer
BRCA pathogenic variant identified from genomic analysis of any tumor
	• Pathogenic variant in BRCA1/2 in a relative
	• ≥ 2 individuals with BC on the same side of the family, at least one diagnosed ≤ 50 years
	• First-/second degree relative with either: BC ≤ 45 years, ovarian cancer, male BC, pancreatic cancer, metastatic prostate cancer, or ≥ 2 individuals with BC on the same side of the family, at least one diagnosed ≤ 50 years
	• Family history of ≥ 3 cancers linked to hereditary cancer syndromes
	• Familial risk assessment tools (mathematic models):
	  • Ontario Family History Assessment Tool
	  • International Breast Cancer Intervention Study (IBIS)
	  • BRCAPRO
	  • Claus
	  • Tyrer-Cuzick
	  • NCI Breast Cancer Risk Assessment Tool (BCRAT)
	NOT the Gail model anymore

NCCN, National Comprehensive Cancer Network; ACMG, American College of Medical Genetics and Genomics; NSGC, National Society of Genetic Counsellors; TNBC, triple-negative breast cancer

KEY CRITERIA:

Female BC diagnosed ≤ 50 years
TNBC diagnosed ≤ 60 years
Personal/family history of ovarian cancer or male breast cancer

Other considerations for genetic testing recommendations

- **Impact of test results**
  - Management/treatment
  - Financial aspects
  - Blood relatives
- **Other potentially involved genes**
  - Consider multi-gene panel testing (NGS)

	Breast	Ovarian
e.g.,	ATM	BARD1
	BARD1	BRIP1
	CHEK2	MRE11
	TP53	MSH2
	PTEN	MSH6
	STK11	RAD51C
	CDH1	RAD51D
		TP53

Rebbeck TR et al., Cancer Res. 2011

- **Somatic pathogenic variants and MSI/MMR**
  - +/– associated with Lynch syndrome → confirmatory germline testing warranted
  - not all germline variants detectable in the tumor → history assessment necessary
  - somatic variants confirmed in the germline in patients not meeting testing criteria → panel testing recommended

Proactive management options

Breast screening

Appropriate imaging modalities and surveillance intervals unclear

guidelines (ACS): annual MRI + screening mammogram + clinical breast examination for women aged ≥ 25 years with a genetic predisposition

BRCA1/2 pathogenic/likely pathogenic variant carriers < 30 years of age: MRI preferred over mammography (potential radiation exposure risk and less sensitivity)

Ovarian screening

• transvaginal ultrasound (TVUS) + CA-125 starting at 30-35 years of age for high-risk women seems superior to none, or either modality alone (but no significant mortality reduction observed)

Risk-reducing surgery

• mastectomy and/or salpingo-oophorectomy (40y for *BRCA1*, 40-45y for *BRCA2* carriers) discussed during post-test counseling

Other screening recommendations

• Men testing positive for a *BRCA1/2* pathogenic/likely pathogenic variant

Breast cancer: annual clinical breast examination and monthly breast self-examination starting at 35 years of age; regularly scheduled mammography not recommended

Prostate cancer: PSA + DRE starting at 45 years of age recommended for *BRCA2* carriers and considered for *BRCA1* carriers

• Men and women testing positive for a *BRCA1/2* pathogenic/likely pathogenic variant

Melanoma: yearly full body skin and eye exam

Pancreatic cancer: investigational screening protocols should be considered

• endoscopic ultrasound (EUS) and endoscopic retrograde cholangiopancreatography (ERCP) identified as potential screening tools, but no clear schedule recommendations available

Breast/Ovarian Cancer Risk. Q & A

Risk Assessment, Genetic Counseling, and Genetic Testing for BRCA-Related Cancer in Women. Evidence Report and Systematic Review

Effectiveness of Risk Assessment, Genetic Counseling, and Genetic Testing in Reducing Incidence and Mortality of BRCA1/2-Related Cancer

• ***Key Question 1.*** In women with unknown BRCA1/2 mutation status, does risk assessment, genetic counseling, and genetic testing result in reduced incidence of BRCA1/2-related cancer and cause-specific and all-cause mortality?

• *Data & Results*: No studies identified.

Evidence Report

Accuracy of Risk Assessment and Pre-Test Genetic Counseling

Key Question 2a. What is the accuracy of familial risk assessment for BRCA1/2-related cancer when performed by a non-specialist in genetics in a clinical setting?

Data: 14 discriminatory accuracy studies (n = 43,813) of 8 risk assessment tools*)

Results: moderate/high diagnostic accuracy in predicting BRCA1/2 mutations in individuals (AUC 0.68-0.96)

Key Question 2b. What are the benefits of pretest genetic counseling in determining eligibility for genetic testing for BRCA1/2-related cancer?

Data & Results: 28 studies (30 articles; n = 8060) on:

• *Understanding of cancer risk/intention for genetic testing*: 14/1 studies ⊕; 1/4 studies ⊖; 1/0 studies ?

• *Agreement with genetic counselor's appraisal* – 1 study ⊕ (1 year vs. immediately after = 49% vs 35%)

• *Measures taken after genetic counseling* – 1 study ⊕ (but only in high risk); 8 studies ⊖; 8 studies ?

• *Anxiety/depression associated with genetic counseling* – 0/0 studies ⊕; 5/1 studies ⊖; 8/6 studies ?

*) Ontario Family History Assessment Tool (FHAT), 7-question Family History Screening (FHS-7), Manchester Scoring System (MSS), PAT, Referral Screening Tool (RST), International Breast Cancer Intervention Study (IBIS) risk model, and brief versions of BRCAPRO

⊕ increases; ⊖ decreases; ? mixed results/no associations

Nelson HD et al. *JAMA*. 2019; 322(7):666-685

***BRCA1/2* Mutation Testing and Post-Test Genetic Counseling**

Key Question 2c. What are optimal testing approaches to determine the presence of pathogenic *BRCA1/2* mutations in women at increased risk for *BRCA1/2*-related cancer?

Data: 1 good-quality RCT - Ashkenazi Jewish women (n=691) and men (n=343) randomized to population-based *BRCA1/2* mutation testing vs family history-based testing; 3 years follow-up

Results:

overall *BRCA1/2* mutations prevalence = 2.45% (13 carriers identified by population testing, 9 by family history)

210 of the 438 family-history-negative participants → complete testing → additional 5 carriers

health outcomes related to increased detection (cancer incidence, mortality, harms): not studied

short-term measures of anxiety, health anxiety, depression, distress, uncertainty, and QoL: similar

Key Question 2d. What are optimal posttest counseling approaches to interpret results and determine eligibility for interventions to reduce risk of *BRCA1/2*-related cancer?

Results: No studies identified.

RCT, randomized clinical trial Nelson HD et al. *JAMA*. 2019; 322(7):666-685

Effectiveness of Interventions to Reduce BRCA1/2-Related Cancer and Mortality in BRCA1/2 Mutation Carriers

Key Question 4. Do interventions reduce incidence of *BRCA*-related cancer and mortality in women at increased risk?

Data & Results:

No effectiveness trials of intensive screening for BC or OC in BRCA carriers were published.

2 studies for BC (1364 carriers): sensitivity 63-69% (MRI), 25-62% (Mx), 66-70% (both); specificity ≥ 91% (either)

1 study for OC (459 carriers): sensitivity 43% (TVUS), 71% (CA-125), 71% (both); specificity 99% (either)

No trials of risk-reducing medications or surgery reported results specifically for BRCA carriers.

meta-analysis of 8 placebo-controlled RCTs (n = 54 651) of tamoxifen, raloxifene, anastrozole and exemestane: lower risk of invasive BC after 3-5 years of use

1 trial of tamoxifen vs raloxifene (n = 19 747): tamoxifen better (RR 1.24; significant for ER-positive).

6 observational studies (n = 2546) of mastectomy, 2+7 of (salpingo-) oophorectomy (n = 2379 + n = 6807)

mastectomy: 90-100% reduction in BC incidence (high-risk women and BRCA carriers), and 81-100% reduction of BC-specific mortality (1 study, n = 639); (salpingo-)oophorectomy: no associations with BC risk

2 studies of oophorectomy (n = 2108): 69-100% reduction in OC risk, but not cancer-specific mortality

BC - breast cancer;
OC - ovarian cancer;
MRI - magnetic resonance imaging;
Mx - mammography;
TVUS - trans-vaginal ultrasound;
RCT - randomized clinical trial. Nelson HD et al. *JAMA*. 2019; 322(7):666-685

Harms of Interventions to Reduce BRCA1/2-Related Cancer and Mortality in BRCA1/2 Mutation Carriers

Key Question 5. What are adverse effects of interventions to reduce risk for *BRCA1/2*-related cancer?

Data & Results

• No trials on harms of intensive screening for BC or OC in BRCA carriers were published.

- *BC screening*: 3 studies (n = 2631): false-positive/negative results, recall rates, and diagnostic procedures higher with MRI than Mx; 3 studies (n = 513): no discomfort, pain, anxiety, depression after MRI/ Mx/ CBE; BC worry decreased over time
- *OC screening*: false-positive rate 3.4% (55/1595) for TVUS and diagnostic surgery rate 55% (6/11) (benign if TVUS+CA-125)

- No studies evaluated the adverse effects of risk-reducing medications or surgery specifically in BRCA carriers.
  - AEs reported in 8 placebo-controlled RCTs of tamoxifen, raloxifene, anastrozole and exemestane, and 1 RCT of tamoxifen vs raloxifene: tamoxifen (RR 1.93) and raloxifene (RR 1.56) – increased thromboembolic events; tamoxifen – increased endometrial cancer (RR 2.25) and cataracts; all medications – some undesirable AEs (e.g., vasomotor, musculoskeletal);
  - 12 observational studies (n = 2684) for mastectomy, and 5 studies (n = 530) for (salpingo-) oophorectomy: surgical complications ≥ 50%, and 4% (7/159); worse body image/ psychological symptoms, and vasomotor symptoms/sexual functioning/fatigue;

BC - breast cancer;
OC - ovarian cancer;
MRI - magnetic resonance imaging;
Mx - mammography;
TVUS - trans-vaginal ultrasound;
RCT - randomized clinical trial. Nelson HD et al. *JAMA*. 2019; 322(7):666-685

Take home message

✓ **Molecular techniques are complementary to the immunohistochemical (IHC) technique**, assisting IHC diagnosis when its results are nonspecific.

✓ **Each molecular technique has specific indications and applications**, according to the investigated molecular abnormality.

✓ **The interpretation of genetic tests can provide the following possible results**: positive, negative, true negative, uninformative negative, variant of uncertain significance, or benign.

✓ Understanding of these molecular techniques and their applications provides a **better elucidation of their diagnostic use and clinical-pathological characteristics** of the neoplastic tissues.

✓ **The process of familial risk assessment** in primary care, referral and evaluation by genetic counselors, genetic testing, and use of intensive screening and risk-reducing medications and surgical procedures (where indicated) **is complex**.

✓ Each step of the pathway requires careful interpretation of information, consideration of future risks, and shared decision-making.

✓ **Results of genetic tests are indicative of risk, not predictive of disease occurrence**, and should be communicated as such; data available on significance of genetic alterations are continuously changing, and follow-up with patients is paramount.

✓ **Services must be well integrated and highly individualized** to optimize benefits and minimize harms for patients as well as their families.

✓ **Several evidence/knowledge gaps relevant to prevention remain**, and additional studies are necessary[1].

[1] Nelson HD et al. *JAMA*. 2019; 322(7):666-685

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II.6. The monitoring of people at high risk for cancer: screening, preventive measures. How to ensure the quality of monitoring

Learning objectives

At the conclusion of this presentation, participants will be able to identify, differentiate, summarize, evaluate, and apply the following aspects related to the most common hereditary cancers:

- Hereditary cancer syndromes
- Identification of different risk categories for colorectal cancer (CRC)
- Histological classification of colorectal polyps, and of tumors of the colon and rectum
- Prevention and screening algorithms for CRC
- High risk population for breast cancer
- Histological and molecular classification of breast carcinoma
- Prevention and screening algorithms for breast cancer
- Quality of monitoring principles

Introduction

✓ Due to the accessibility to the existing data, all countries could manage cancer control an all four directions:

- prevention
- early detection
- diagnosis and treatment strategies
- palliative care

✓ Most cancers occur by chance, as a result of lifestyle choices or environmental conditions.

✓ The cancer risk in hereditary cancer families (inherited gene mutation) is much higher than in the general population.

✓ **Sporadic Cancer** – without a family history of cancer or an inherited gene change;

✓ **Familial Cancer** – caused by a combination of genetic and environmental risk factors;

✓ **Hereditary Cancer** – occurs when an altered gene is passed down from parent to child.

Hereditary Cancer Syndromes

Correspondence between hereditary cancer syndromes, gene mutations, and tumor types

HEREDITARY CANCER SYNDROME	GENE MUTATIONS	TUMOR/CANCER TYPES	CANCER ASSOCIATIONS
Hereditary Breast & Ovarian Cancer Syndrome	BRCA1 BRCA2	Breast cancer Ovarian cancer	Higher incidence of pancreatic cancer, melanoma Higher chance for breast and prostate cancer (men with BRCA mutations)
Cowden Syndrome	PTEN	Hamartomas (skin and mucous membranes, intestinal tract, brain)	Increased risk for breast, uterus and thyroid cancer
Hereditary Non-polyposis Colorectal Cancer Syndrome (Lynch Syndrome)	DNA mismatch repair genes (MLH1, MSH2, MSH6 or PMS2)	Colorectal cancer Endometrial cancer Other tumors	Muir Torre Syndrome (increased risk for skin tumors) Turcot Syndrome (associated with brain tumors)
Hereditary Leukemia and Hematologic Malignancies Syndromes	Germline mutations, including: ANKRD26, CEBPA, DDX41, ELANE, ETV6, GATA2, HAX1, RUNX1, SAMD9, SAMD9L, SRP72, ETV6,	Leukemia Myelodysplastic syndrome Acute myeloid leukemia Aplastic anemia	Leukemia predisposition syndromes include: Fanconi anemia Diamond-Blackfan anemia Shwachman Diamond syndrome

	PAX5, TP53, IKZF1 (Godley, Shimamura, 2017)		Dyskeratosis congenita/telomere biology disorders
Familial Adenomatous Polyposis (FAP)	APC (autosomal dominant pattern) MUTYH (autosomal recessive pattern)	Multiple adenomatous colorectal polyps, with a high lifetime risk of colorectal cancer (CRC)	Attenuated FAP (AFAP) Gardner's Syndrome Turcot Syndrome
Li-Fraumeni Syndrome	TP53	Soft tissue sarcomas Breast cancer Leukemia Lung cancer Brain tumors Adrenal gland cancer	Rare early childhood cancers: Embryonal rhabdomyosarcoma Adrenal cortical cancer, Choroid plexus cancers Other types of cancer: Colon/GI tract cancer, Uterine, ovarian cancer Prostate, testicular cancer, Thyroid cancer, Renal cancer, Lung cancer, Leukemia
Von Hippel-Lindau Disease	Von Hippel-Lindau disease (VHL)	Hemangioblastomas Angiomas	Higher risk for renal cancer
Multiple Endocrine Neoplasias (MEN)	MEN1, RET, CDNK1B	MEN1/Wermer disease: Hyperparathyroidism, Pancreas tumors Pituitary gland tumors MEN2A: Medullary thyroid carcinoma Pheochromocytoma, Hyperparathyroidism MEN2B: Neuromas Physical characteristics similar to Marfan syndrome Medullary thyroid carcinoma MEN4: Parathyroid and anterior pituitary tumors	Adrenal cortical tumors, carcinoid tumors and infrequent pheochromocytomas, and some parts of the digestive tract (with MEN1) Adrenal, renal, and reproductive organ tumors (with MEN4)

Prevention and screening

✓ Cancer prevention involves several stages, including:

✓ **Primary prevention** - reducing the incidence of cancer by eliminating the main risk factors (smoking, nutrition, poor physical activity)

✓ **Secondary prevention** - reducing cancer mortality, by early detection, when the therapeutic chances are increased

✓ The best method of early detection is the **screening** of the entire population.

✓ In 2003, the Council of the European Union (CEU) adopted a set of Recommendations for cancer screening, with a view to **breast, cervical,** and **colorectal** cancer screening programs, responsible for most cancer deaths worldwide, publishing guidelines for quality assurance in screening and diagnosis for these three main types of cancer.

✓ **Tertiary prevention** – clinical activities that prevent further deterioration or reduce complications after a declared disease.

High risk population for colorectal cancer (MD Anderson Cancer Center, 2019)

✓ Personal history of precancerous colon polyps (adenomas)

✓ Family history of colorectal cancer or precancerous polyps (adenomas)

✓ Personal history of Familial Adenomatous Polyposis or suspected Familial Adenomatous Polyposis without yet having undergone genetic testing

✓ Personal history of Hereditary Nonpolyposis Colorectal Cancer or family history of Hereditary Nonpolyposis Colorectal Cancer

✓ Inflammatory bowel disease (chronic ulcerative colitis or Crohn's disease)

Histological classification of the two major classes of colorectal polyps and WHO histological classification of colonic and rectal tumors

WHO histological classification of tumours of the colon and rectum[2]

Epithelial tumours		Non-epithelial tumours	
Adenoma	8140/0	Lipoma	8850/0
Tubular	8211/0	Leiomyoma	8890/0
Villous	8261/0	Gastrointestinal stromal tumour	8936/1
Tubulovillous	8263/0	Leiomyosarcoma	8890/3
Serrated	08213/0	Angiosarcoma	9120/3
		Kaposi sarcoma	9140/3
		Malignant melanoma	8720/3
Intraepithelial neoplasia[3] (dysplasia) associated with chronic inflammatory diseases		Others	
Low-grade glandular intraepithelial neoplasia			
High-grade glandular intraepithelial neoplasia			
Carcinoma		Malignant lymphomas	
Adenocarcinoma	8140/3	Marginal zone B-cell lymphoma or MALT Type	9699/3
Mucinous adenocarcinoma	8480/3	Mantle cell lymphoma	9673/3
Signet-ring cell carcinoma	8490/3	Diffuse large B-cell lymphoma	9680/3
Small cell carcinoma	8041/3	Burkitt lymphoma	9687/3
Squamous cell carcinoma	8070/3	Burkitt-like/atypical Burkitt-lymphoma	9687/3
Adenosquamous carcinoma	8560/3	Others	
Medullary carcinoma	8510/3		
Undifferentiated carcinoma	8020/3	**Secondary tumours**	
Carcinoid (well differentiated endocrine neoplasm)	8240/3	**Polyps**	
EC-cell, serotonin-producing neoplasm	8241/3	Hyperplastic (metaplastic)	
L-cell, glucagon-like peptide and PP/PYY producing tumour		Peutz-Jeghers	
Others		Juvenile	
Mixed carcinoid-adenocarcinoma	8244/3		
Others			

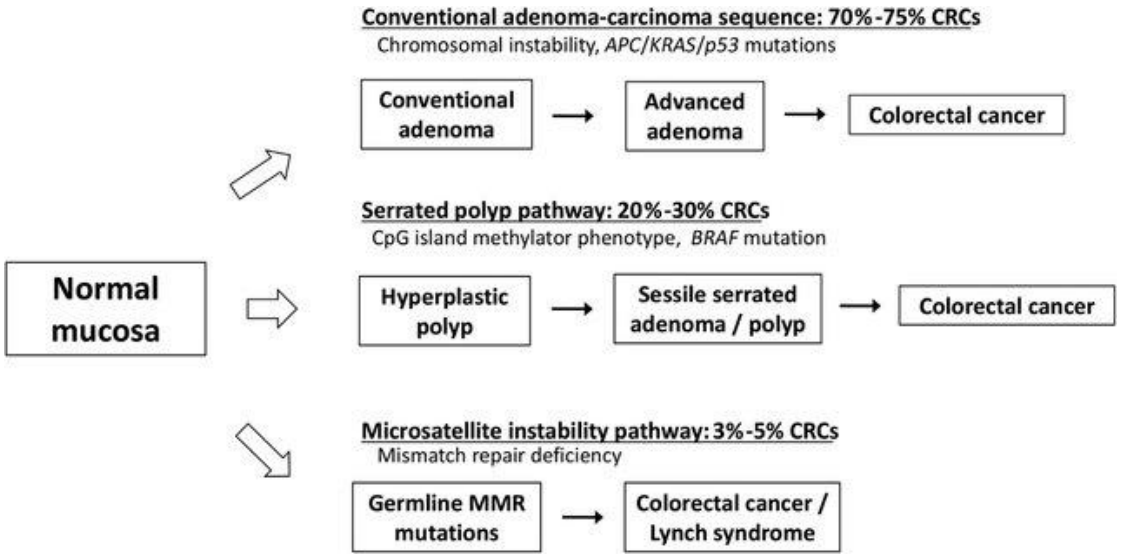
World Health Organisation

I. CONVENTIONAL ADENOMAS	II. SERRATED LESIONS
a. Dysplasia grade i. High grade ii. Low grade b. Villousity i. Tubular ii. Tubulovillous iii. Villous	a. Hyperplastic polyps (not considered precancerous) b. Sessile serrated polyp i. Without cytologic dysplasia ii. With cytologic dysplasia c. Traditional serrated adenoma

[2] This classification is modified from the previous WHO histological classification of tumours {845} taking into account changes in our understanding of these lesions. In the case of endocrine neoplasms, it is based on the recent WHO classification {1784} but has been simplified to be of more practical utility in morphological classification.

[3] Morphology code of the International Classification of Diseases for Oncology (ICD-0) {542} and the Systematised Nomenclature of Medicine (http://snomed.org). Behaviour is coded/0 for benign tumours, /3 for malignant tumours, /2 for *in situ* carcinomas and grade III intraepithelial neoplasia, and /1 for unspecified, borderline or uncertain behaviour. Intraepithelial neoplasia does not have a generic code in ICD-0. ICD-0 codes are available only for lesions categorized as glandular intraepithelial neoplasia grade III (8148/2), and adenocarcinoma *in situ* (8140/2).

Main molecular pathways in CRC pathogenesis. CRC: colorectal cancer; MMR: mismatch repair. (Li, 2018)



Primary prevention in CRC

List of the main factors and their estimated risk of developing CRC

Factors	Particularities	Estimated risk
Macronutrients	Fats Meat Fibers, vegetables, and fruits Milk and other dairy products	Contradictory results Contradictory results (red and processed meat can increase the risk of CRC development) Fiber intake inversely associated with the risk of CRC. Protective effect disappears when other diet-related risk factors are present. Fruits and vegetables are associated with a non-significant decreasing of the CRC risk/may interfere with the cancer localization. Protector effect for distal colon neoplasias
Micronutrients	Folate acid Calcium Vitamin D	No evidence for increasing the risk Protector effect of dietary calcium consumption High doses cut the risk of CRC
Antioxidants	Beta-carotenes Vitamin E Selenium Their associations	Do not modify the risk of CRC Do not have a beneficial effect on CRC recurrence prevention
Other factors	Lifestyle Economic development	Associated with a higher incidence of CRC
Physical activity, obesity, energy balance	Regular physical exercise (intensity, frequency, duration) Occupational and recreational activity BMI Abdominal obesity (waist-hip index, waist perimeter)	Decrease the risk of CRC with 40% Significant reduction in the risk of CRC >30 – increase the risk of CRC, higher for men Increase the risk of CRC in both genders
Alcohol intake	> 45g/day	Associated with the risk of developing CRC
Smoking	Difference between active, occasional, former smokers	Associated with developing CRC
Age	After 50 yrs old	Significantly increases the risk of CRC

Acetylsalicylic acid and non-steroidal anti-inflammatory drugs	ASA ≥ 300 mg/day	Significantly lowers the recurrence of adenomas Lowers the risk of CRC
Statins		Controversed/no beneficial effect on CRC prevention
Hormone treatment in postmenopausal women		Non-significant protector effect

CRC SCREENING METHODS (Li, 2018)

The most important screening tests used in the detection of CRC

ENDOSCOPIC METHODS	Colonoscopy Flexible sigmoidoscopy	Colonoscopy represents the most sensitive method for CRC screening and the reference standard for assessing the performance of other CRC screening tests. Flexible sigmoidoscopy examines the lower half of the colon.
STOOL-BASED TESTS	gFOBT (guaiac-based fecal occult blood test) FIT (fecal immunochemical test) FIT-DNA	gFOBT can be falsely positive due to the presence of blood from red meat or certain food. FIT uses antibody technology to detect intact human hemoglobin in stool, therefore, it does not require dietary restrictions. This test combines FIT with testing for DNA markers that are shed into the stool. A positive result should be followed by colonoscopy.
RADIOGRAPHIC TEST	CT Colonography	CT colonography is a radiologic method of CRC screening. If positive, follow-up colonoscopy should be performed.
BLOOD-BASED SCREENING TESTS	Liquid biopsy	Liquid biopsy consists of the analysis of circulating tumor cells (CTCs), cell-free tumor DNA (ctDNA) and/or protein markers detectable in the blood.

Screening approaches for colorectal cancer

- ✓ OPPORTUNISTIC SCREENING
- ✓ PROGRAMMATIC SCREENING
- ✓ Multiple options
- ✓ Sequential
- ✓ Risk-stratified

Approaches to offering screening in the opportunistic setting (Rex et al., 2017)

APPROACH	DESCRIPTION
Sequential testing	A preferred test is offered first. If the patients decline another option(s) is offered.
Risk stratified approach	Colonoscopy is offered to patients predicted to have a high prevalence of advanced pre-cancerous lesions; other tests are offered to patients predicted at low risk.
Multiple options	The relative benefits, risks, and costs of 2 or more options are presented

CRC screening for average-risk individuals (Li, 2018)

Screening method	Frequency	Efficacy	Main issues for informed decisions
Endoscopic methods			
Colonoscopy	Every 10 years	Reduction in mortality in a prospective cohort study.	Most sensitive. May require sedation. Can detect precancerous lesions. Requires full bowel preparation. Only part of colon examined. Can detect precancerous lesions. Require limited bowel preparation.
Sigmoidoscopy	Every 5 years	Reduction in mortality in RCTs	Performed at home but should be repeated annually. Limited ability in detecting precancerous lesions. Needs follow-up colonoscopy if result is positive.
Stool-based tests			
gFOBT	Every year[b]	Reduction in mortality in RCTs	Performed at home but should be repeated annually. Limited ability in detecting precancerous lesions. Needs follow-up colonoscopy if result is positive.
FIT	Every year[c]	Higher sensitivity and specificity in detecting CRC than gFOBT, but RCTs lacking.	Performed at home but should be repeated annually. Limited ability in detecting precancerous lesions. Needs follow-up colonoscopy if result is positive.
FIT-DNA	Every 1-3 years?	More sensitive but less specific than FIT only. Effect on mortality unknown.	Performed at home. More expensive than gFOBT and FIT. Limited ability in detecting precancerous lesions. Needs follow-up colonoscopy if result is positive.
Radiologic method			
CT colonography	Every 5 years	Effect on mortality unknown	Needs bowel preparation. Lower risk than colonoscopy but less sensitive. Needs follow-up coloscopy if polyp(s) detected.
Biomarker			
Septin9 DNA	Unknown	Effect on mortality unknown	First FDA approved serum test for CRC screening. Less sensitive and less specific than colonoscopy. May be more convenient than other screening tests.

CRC: colorectal cancer; RCT: randomized controlled trial;
gFOBT: guaiac-based fecal occult blood test;
FIT: fecal immunochemical test; CT: computed tomography.

[a] Most recommendations in this table are based on the current U.S. Preventive Service Task Force guidelines and U.S. Multi-Society Task Force recommendations.[23, 46] Guidelines may vary in different counties.

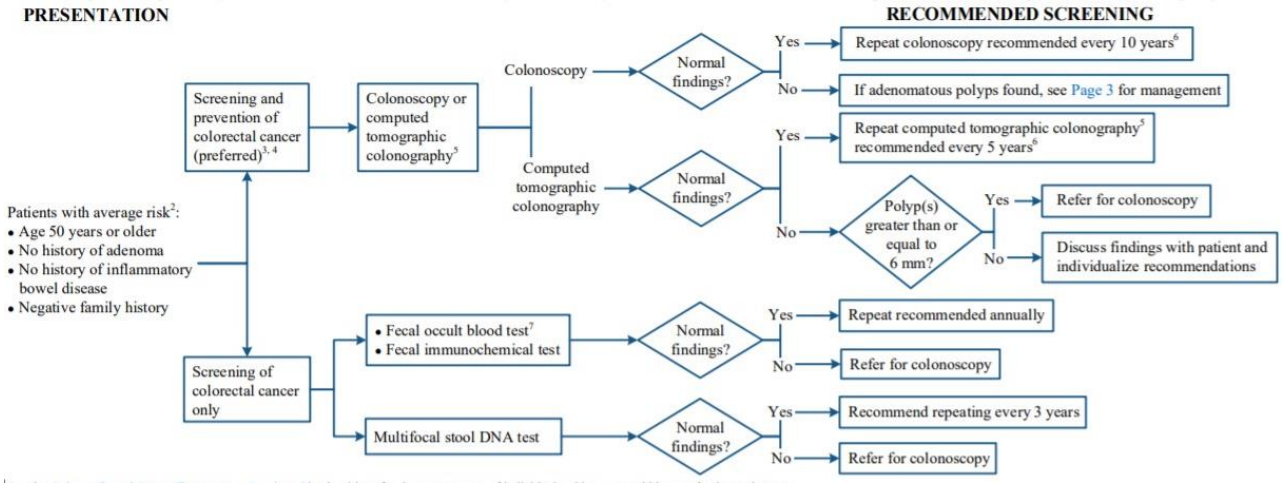
[b,c] The consensus from the International Agency for Research on Cancer (IARC) Handbook Working Group recommends screening every 2 years with gFOBT without rehydration and every 1-2 years with higher sensitivity guaiac tests (with rehydration). IARC also recommends screening with FIT every 2 years.

MSTF recommendations for persons with high-risk family histories not associated with polyp syndromes (Rex et al., 2017)

Family history	Recommended screening
Lynch syndrome Family colon cancer syndrome X	See reference 34 Colonoscopy every 3-5 years beginning 10 years before the age at diagnosis of the youngest affected relative
Colorectal cancer or an advanced adenoma in two first-degree relatives diagnosed at any age OR colorectal cancer or an advanced adenoma in a single first-degree relative at age < 60 years	Colonoscopy every 5 years beginning 10 years before the age at diagnosis of the youngest affect interval or age 40, whichever is earlier; for those with a single first-degree relative with colorectal cancer in whom no significant neoplasia appears by age 60 years, physicians can offer expanding the interval between colonoscopies
Colorectal cancer or an advanced adenoma in a single first-degree relative at age ≥ 60 years	Begin screening at age 40 years; tests and intervals are as per the average-risk screening recommendations (Table 4)

Colorectal Cancer Screening – Average Risk Population (MD Anderson Algorithm)

Note: Screening for adults age 76 to 85 years old should be evaluated on an individual basis by their health care provider to assess the risks and benefits of screening. Colorectal cancer screening is not recommended over age 85 years.



[1] See the Colon or Rectal Cancer Treatment or Survivorship algorithms for the management of individuals with a personal history of colorectal cancer

[2] African Americans have a higher risk of large polyps and tumours from ages 50-65 years; thus it is important to start screening this population earlier. Limited evidence supports initiating screening in African Americans at age 45 years old. Follow-up frequency would be based on colonoscopy findings.

[3] While there is good evidence to support fecal occult blood test, tests that both screen for and prevent colon cancer and the preferred screening modality. Annual fecal occult blood tests should not be performed if colonoscopy or CT colonography is used as the screening measure in an average-risk patient.

[4] Flexible sigmoidoscopy is an alternate option but is not the preferred endoscopic modality as the entire colon is not visualised.

[5] Preauthorisiation with patient's insurance carrier is always advised.

[6] Discontinuation of screening should be considered when persons up to date with screening, who have prior negative screening (particularly colonoscopy), reach age 75 or have < 10 years of life expectancy

[7] High sensitivity fecal occult blood test (guaiac-based or immunochemical)

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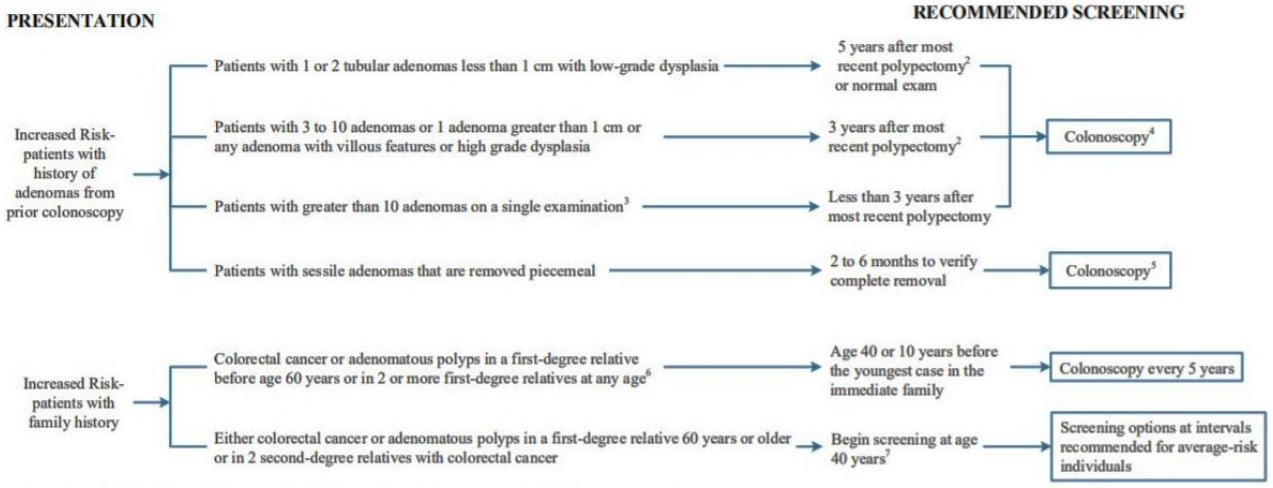


[1] See the Colon or Rectal Cancer Treatment or Survivorship algorithms for the management of individuals with a personal history of colorectal cancer

[2] Precise timing based on clinical factors, patient and physician preference

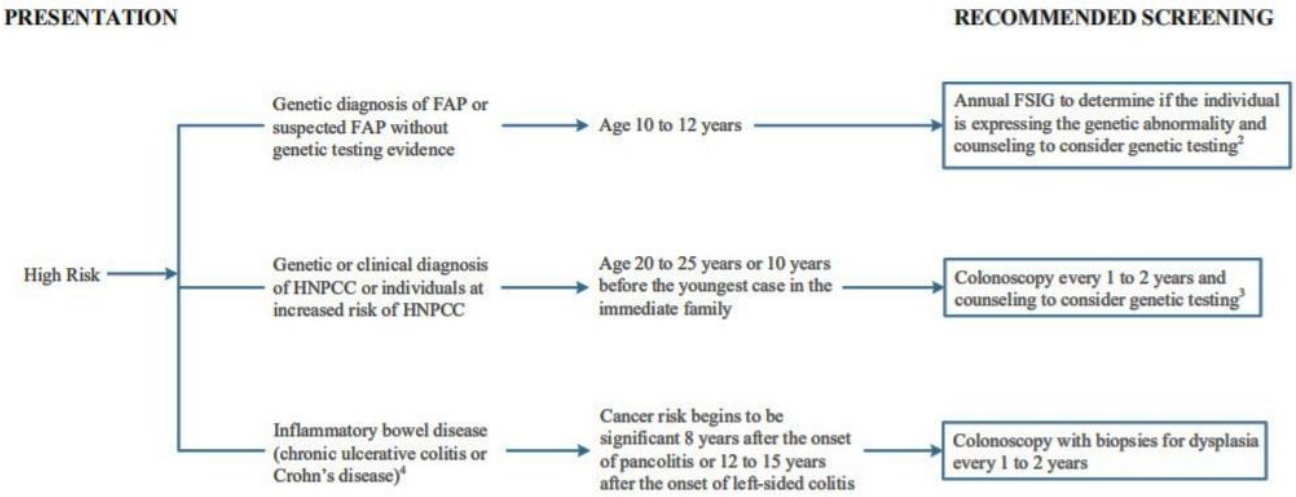
[3] Genetic evaluation for familial cancer syndromes is recommended

[4] Subsequent follow-up is based on the number and size of polyps at the time of colonoscopy as well as the degree of dysplasia. If the follow-up colonoscopy is negative for adenomatous polyps, follow-up in 5 years is recommended.

[5] Surveillance individualised based on Endoscopist's judgement.

[6] Consider Familial Syndrome

[7] Screening should begin at an earlier age, but individuals may be screened with any recommended form of testing



FAP = familial adenomatous polyposis
FSIG = flexible sigmoidoscopy
HNPCC = hereditary nonpolyposis colorectal cancer

[1] See the Colon or Rectal Cancer Treatment or Survivorship algorithms for the management of individuals with a personal history of colorectal cancer

[2] If the genetic test is positive, colectomy should be considered

[3] Genetic testing for HNPCC should be offered to first-degree relatives of persons with a known inherited MMR gene mutation. It should also be offered when the family mutation is not known, but 1 of the first 3 of the modified Bethesda Criteria is present.

[4] These patients are best referred to a center with experience in the surveillance and management of inflammatory bowel disease.

High-risk population for breast cancer (MD Anderson Cancer Center, 2019)

✓ History of radiation treatment to the chest

✓ Genetic mutation, including an abnormality in the BRCA 1 or BRCA 2 genes, CDH1, Bannayan-Riley-Ruvalcaba Syndrome

✓ History of lobular carcinoma in situ (LCIS)

✓ History of Atypical Ductal Hyperplasia (ADH)/Atypical Lobular Hyperplasia (ALH)

✓ Five-year risk of breast cancer 1.7% or greater at age 35 or older, as defined by a Gail Model calculation.

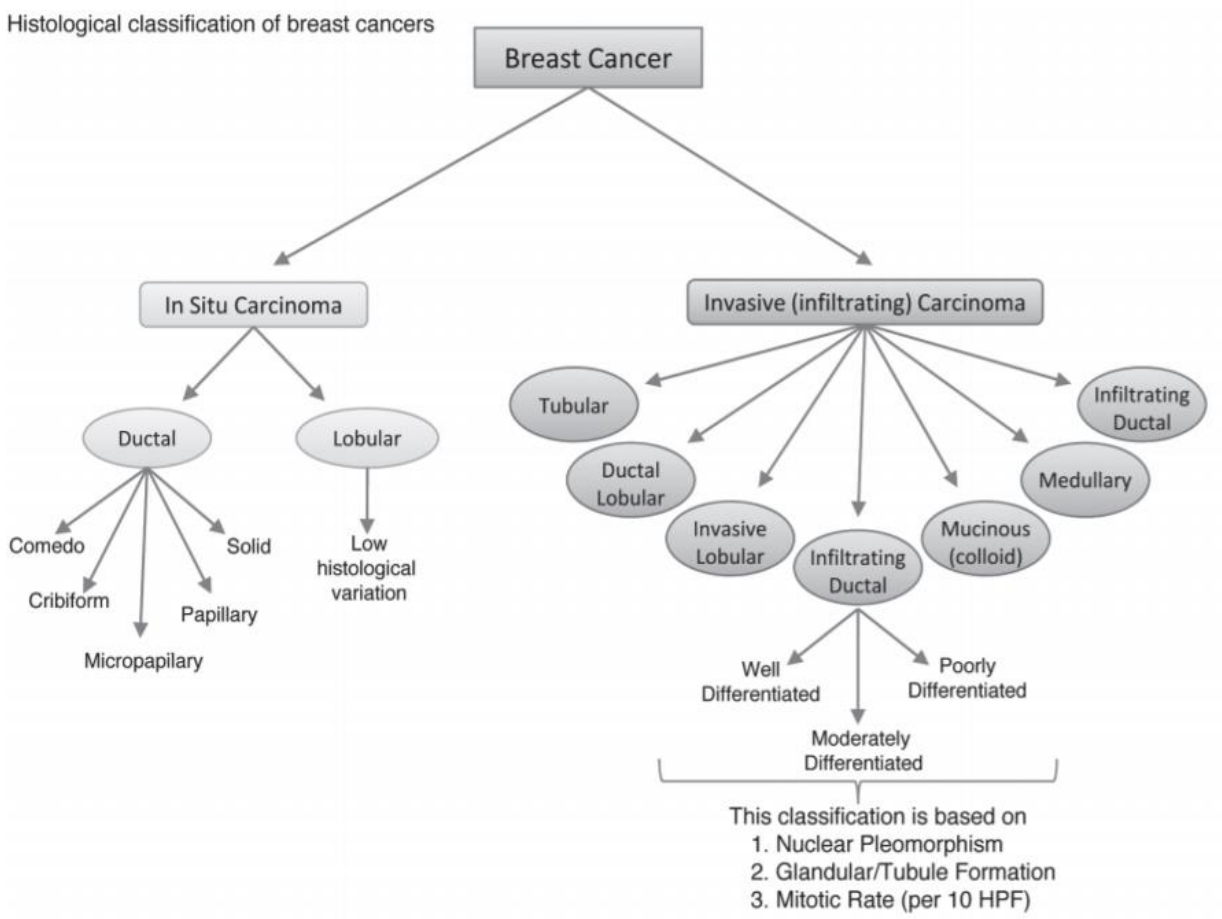
✓ A life-time risk of breast cancer 20% or greater, as defined by models dependent on family history.

Classification of precursors lesions and breast carcinoma (Sinn, 2013)

Type	Classification
Precursor lesions	
Ductal carcinoma *in situ*	8500/2
Lobular neoplasia	
Lobular carcinoma *in situ*	
Classic lobular carcinoma *in situ*	8500/2
Pleomorphic lobular carcinoma *in situ*	8519/2*
Atypical lobular hyperplasia	
Intraductal proliferative lesions	
Usual ductal hyperplasia	
Columnar cell lesions including flat epithelial atypia	
Atypical ductal hyperplasia	
Papillary lesions	
Intraductal papilloma	8503/0
Intraductal papilloma with atypical hyperplasia	8503/0
Intraductal papilloma with ductal carcinoma *in situ*	8503/2*
Intraductal papilloma with lobular carcinoma	8520/2
Intraductal papillary papilloma	8503/2
Encapsulated papillary carcinoma	8504/2
Encapsulated papillary carcinoma with invasion	8504/3
Solid papillary carcinoma	
In situ	8509/2
Invasive	8509/3
Invasive carcinoma of no special type (NST)	8500/3
Pleomorphic carcinoma	8522/3
Carcinoma with osteoclast-like stromal giant cells	8035/3
Carcinoma with choriocarcinomatous features	
Carcinoma with melanotic features	
Invasive lobular carcinoma	8520/3
Classic lobular carcinoma	
Solid lobular carcinoma	
Alveolar lobular carcinoma	
Pleomorphic lobular carcinoma	
Tubulolobular carcinoma	
Mixed lobular carcinoma	
Tubular carcinoma	8211/3
Cribriform carcinoma	8201/3
Mucinous carcinoma	8480/3
Carcinoma with medullary features	
Medullary carcinoma	8510/3
Atypical medullary carcinoma	8513/3
Invasive carcinoma NST with medullary features	8500/3
Carcinoma with apocrine differentiation	
Carcinoma with signet-ring-cell differentiation	
Invasive micropapillary carcinoma	8507/3
Metaplastic carcinoma of no special type	8575/3
Low-grade adenosquamous carcinoma	8570/3
Fibromatosis-like metaplastic carcinoma	8572/3
Squamous cell carcinoma	8070/3
Spindle cell carcinoma	8032/3
Metaplastic carcinoma with mesenchymal differentiation	
Chondroid differentiation	8571/3
Osseous differentiation	8571/3
Other types of mesenchymal differentiation	8575/3
Mixed metaplastic carcinoma	8575/3
Myoepithelial carcinoma	8982/3
Epithelial-myoepithelial tumors	
Adenomyoepithelioma with carcinoma	8983/3
Adenoid cystic carcinoma	8200/3

Rare types	
Carcinoma with neuroendocrine features	
Neuroendocrine tumour, well-differentiated	8246/3
Neuroendocrine carcinoma poorly differentiated (small cell carcinoma)	8041/3
Carcinoma with neuroendocrine differentiation	8574/3
Secretory carcinoma	8502/3
Invasive papillary carcinoma	8503/3
Acinic cell carcinoma	8550/3
Mucoepidermoid carcinoma	8430/3
Polymorphous carcinoma	8525/3
Oncocytic carcinoma	8290/3
Lipid-rich carcinoma	8314/3
Glycogen-rich clear cell carcinoma	8315/3
Sebaceous carcinoma	8410/3

Histological classification of breast carcinoma used by clinicians, based on architectural features and growth patterns. HPF: high power field. (Malhotra, 2010)



Molecular classification of breast carcinoma (Makki, 2015)

Immunohistochemical pattern for the molecular subtypes of breast carcinoma

Molecular subtype	Luminal A	Luminal B	HER2/NEU	Basal-like
ER, PR	ER and/or PR+	ER and/or PR+	ER-, PR-	ER-, PR-
HER 2	HER2-	HER2+/ HER2-	HER2+	HER2-

ER, estrogen receptor;
PR, progesterone receptor;
HER2/HER2/neu, human epidermal growth factor receptor 2

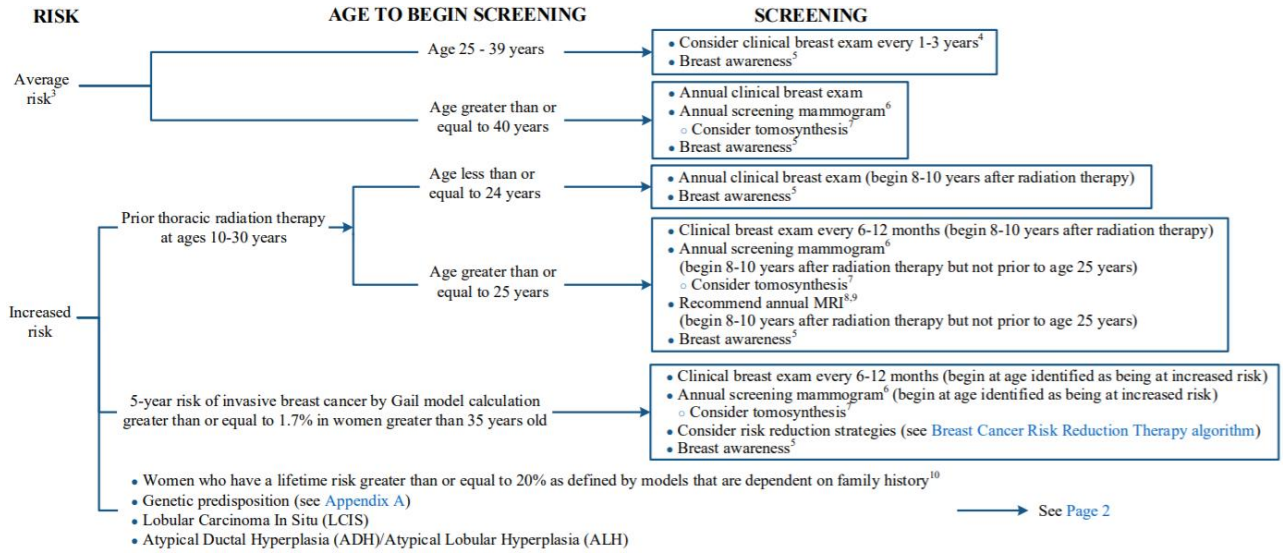
Primary prevention of breast cancer (Sauter, 2018)

The main risk factors and their involvement in breast carcinogenesis

Factors	Particularities	Estimated risk
Dietary modification	Obesity, specific foods, independent of weight gain or loss	associated with a higher risk of premenopausal estrogen receptor negative and triple negative breast cancers less certain influence breast cancer risk
Physical activity	BMI <25 Premenopausal women Estrogen and progesterone receptor-negative breast Cancer	12% reduction in risk among those who were physically active stronger associations with physical activity and breast cancer risk
Tobacco and alcohol	Smoking with concomitant alcohol use	incidence is 24% higher among smokers than non-smokers a stronger association between smoking and breast cancer risk among women who started smoking before the birth of their first child
Exogenous use of estrogens and progestins	the use of combined estrogen and progesterone formulations after menopause birth control pill (BCP) use IUDs with progestins releasing	increased risk of breast cancer increase risk during active use and higher hormone dose increased risk of breast cancer
Ionizing radiation	radiation exposure from multiple chest X-rays computerized tomography (CT) scans of the chest or heart therapeutic radiation	increased mortality from breast cancer with increasing radiation dose, with the increased risk appearing 15 yrs after radiation exposure, the risk remaining elevated up to 50 yrs later increased breast cancer risk
Pregnancy and nursing	Immediately following childbirth Nursing, length of nursing	increased risk of breast cancer for women of all age groups no association between lactation and breast cancer risk among women of normal risk

Breast Cancer Screening

Note: This algorithm is not intended for women with a personal history of breast cancer. Breast cancer screening may continue as long as a woman has a 10-year life expectancy and no co-morbidities that would limit the diagnostic evaluation or treatment of any identified problem. Women should be counseled about the benefits, risks and limitations of screening mammography.



[1] For transgender patients, recommend performing a breast cancer risk assessment and making individualized screening recommendations

[2] See the Breast Cancer Treatment or Survivorship algorithms for the management of women with a personal history of breast cancer

[3] Women who do not meet one of the increased risk categories

[4] Effectiveness of clinical breast exams has not been assessed in women 20-39 years of age

[5] Women should be familiar with their breasts and promptly report changes to their healthcare provider

[6] Augmented breasts need additional views for complete assessment

[7] Tomosynthesis improves cancer detection and decreases recall rates

[8] Risk of breast cancer begins to increase 8-10 years after thoracic exposure. The optimal age to begin MRI screening in this high risk population is not currently known.

[9] Current practice at MD Anderson is to alternate the mammogram and breast MRI every 6 months. While there is no data to suggest that this is the optimal approach, it is done with the expectation that interval cancers may be identified earlier. Other screening regimens, such as breast MRI performed at the time of the annual mammogram, are also acceptable.

[10] Risk models that are largely dependent on family history include Tyrer-Cuzick and Claus

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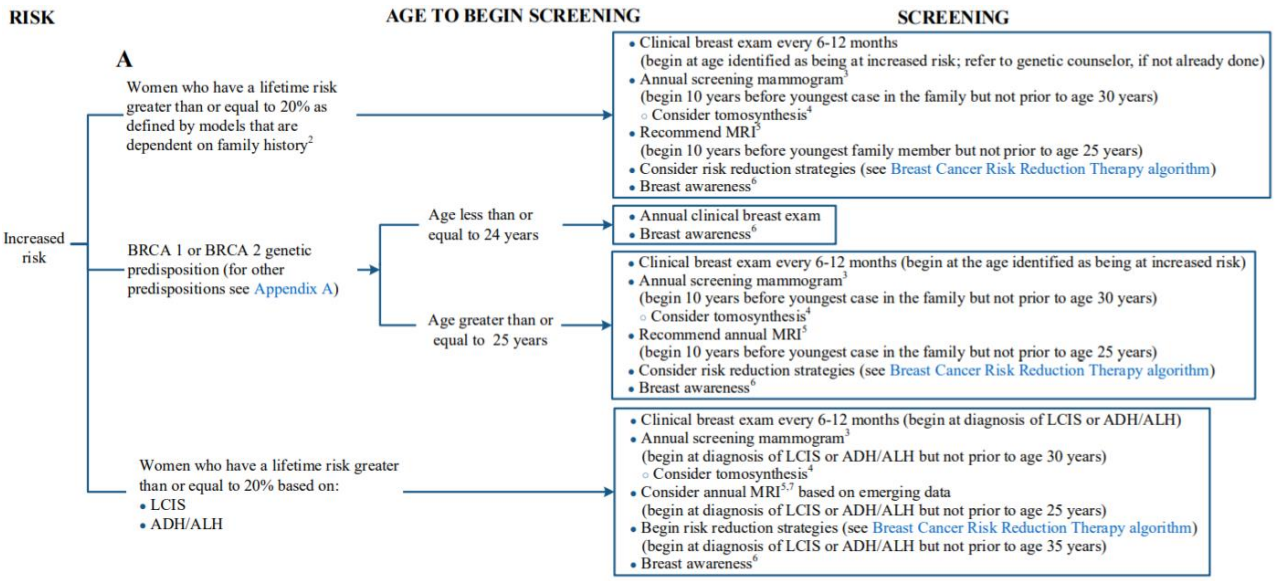

[1] See the Breast Cancer Treatment or Survivorship algorithms for the management of women with a personal history of breast cancer

[2] Risk models that are largely dependent on family history include Tyrer-Cuzick and Claus

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[6] Women should be familiar with their breast and promptly report changes to their healthcare provider

[7] Patient should be educated that insurance may not cover the MRI

Breast Management based on Genetic Test Results

ATM	Increased risk of breast cancer • Screening: annual mammogram with consideration of tomosynthesis and consider breast MRI with contrast starting at age 40 years[3,4] • RRM: evidence insufficient, manage based on family history
BARD1	Potential increase in breast cancer risk, with insufficient evidence for management recommendations
BRIP1	Unknown or insufficient evidence
CDH1	Increased risk of lobular breast cancer • Screening: annual mammogram with consideration of tomosynthesis and consider breast MRI with contrast starting at age 30 years[3,4] • RRM: evidence insufficient, manage based on family history
CHEK2	Increased risk of breast cancer • Screening: annual mammogram with consideration of tomosynthesis and consider breast MRI with contrast starting at age 40 years[3,4] • RRM: evidence insufficient, manage based on family history
MSH2, MLH1, MSH6, PMS2, EPCAM	Unknown or insufficient evidence for breast cancer risk[4] • Manage based on family history, as per Box A on Page 2
NBN	Increased risk of breast cancer • Screening: annual mammogram with consideration of tomosynthesis and consider breast MRI with contrast starting at age 40 years[3,4] • RRM: evidence insufficient, manage based on family history
NF1	Increased risk of breast cancer • Screening: annual mammogram with consideration of tomosynthesis starting at age 30 years and consider breast MRI with contrast starting at age 30-50 years[3,4] • RRM: evidence insufficient, manage based on family history

RRM = risk-reducing mastectomy

[1] The following genes and others are found on some of the panels, but there is insufficient evidence to make any recommendations for breast MRI, or RRM: BARD1, FANCC, MRE11A, MUTYH heterozygotes, RECQL4, RAD50, RINT1, SLX4, SMARCA4, or XRCC2

[2] See Genetic Counselling algorithm

[3] May be modified based on family history (typically beginning screening 5-10 years earlier than the youngest diagnosis in the family but no later than stated in the table) or specific gene pathogenic/likely pathogenic variant

[4] For women with pathogenic/likely pathogenic variants who are treated for breast cancer and have not had bilateral mastectomy, screening should continue as described

PALB2	Increased risk of breast cancer • Screening: annual mammogram with consideration of tomosynthesis and consider breast MRI with contrast starting at age 30 years[1,2] • RRM: evidence insufficient, manage based on family history
PTEN	Increased risk of breast cancer • See NCCN Guidelines for Genetic/Familial High-Risk Assessment: Cowden Syndrome Management
RAD51C	Unknown or insufficient evidence for breast cancer risk
RAD51D	Unknown or insufficient evidence for breast cancer risk
STK11	Increased risk of breast cancer • Screening: see NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal • RRM: evidence insufficient, manage based on family history
TP53	Increased risk of breast cancer • See: Li-Fraumeni Syndrome Screening algorithm

RRM = risk-reducing mastectomy

[1] May be modified based on family history (typically beginning screening 5-10 years earlier than the youngest

diagnosis in the family but not later than stated in the table) or specific gene pathogenic/likely pathogenic variant

[2] For women with pathogenic/likely pathogenic variants who are treated for breast cancer and have not had bilateral mastectomy, screening should continue as described

Quality of monitoring – Cancer standards

- Multidisciplinary Commission - Multidisciplinary Cancer Case Conferences/Meetings
- Cancer programs – annual review of standards
- Appropriate facilities and equipment for the care of cancer patients
- Genetic counseling and risk assessment
- Implementation of a personalized oncogenetic monitoring plan
- Paliative and rehabilitation care services, oncology nutrition services
- Patient care expectations
- Patients compliance – long-term follow-up
- High-quality of data surveillance and systems
- Educational screening and prevention events
- Commission on cancer special studies

Quality control (European Guide for Quality National Cancer Control Programs)

✓ Specific quality assurance mechanisms vary enormously by **screening procedure**.

✓ Programme planners are encouraged to carefully heed comprehensive European guidelines – few fundaments upon which any quality control programme should be based:

1. **Institutionalisation of quality**, with careful attention from policymakers and programme managers as well as clear lines of responsibility and strict accountability mechanisms;

2. **Systematic implementation** of all of the following: **clinical guidelines**, **screening protocols**, **accreditation of professionals and facilities**, **monitoring and auditing schemes**, and **close linkage with a central cancer registry;**

3. **Internal Quality Control** procedures and rigorous **External Quality Assessment Schemes** in screening centres and laboratories - to ensure that: wait times are limited, screening equipment is up-to-date, storage facilities for samples are adequate, staff is well trained;

4. **Close monitoring** by public health specialists and health system managers, to ensure equitable and accessible population coverage as well as health system capacity to quickly and efficiently handle patient follow-up and treatment in case of an abnormal test result.

Take home message

✓ Cancers can be **sporadic**, **familial**, and hereditary.

✓ There are hereditary cancer syndromes whose gene mutations predispose to certain types of malignancies, which associate an increased risk for other tumors.

✓ **Breast and colorectal cancers**, together with cervical cancer are responsible for **most of the cancer deaths globally**.

✓ **Cancer prevention** involves **primary**, **secondary**, and **tertiary prevention**.

✓ **Primary prevention** considers reducing the incidence of cancer by **eliminating the main risk factors** (smoking, nutrition, reduced physical activity).

✓ **Secondary prevention** aims at reducing mortality through **early detection**, the best method being **the screening** of the entire population.

✓ **Tertiary prevention** includes the **specific clinical activities** that prevent further deterioration or reduce complications after a declared disease.

✓ Specific quality control mechanisms vary depending on the screening method.

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II.7.1. Good clinical practice in the management of the hereditary risk of breast and ovarian cancer

Learning objectives

- know which is the role of initial counselling and follow-up of BRCA mutation carriers
- know which are the breast and ovarian cancer risk-reduction measures
- know which is the role of screening for the detection of breast cancer
- know which is the role of risk-reducing agents as a prevention of breast cancer
- know which is the role of risk-reducing surgery as a prevention of breast and ovarian cancer

Introduction

• The presentation will focus on cancer prevention and screening among individuals known to harbour a pathogenic BRCA1/2 mutation.

• The presence of a BRCA1 or BRCA2 mutation accounts for the majority of hereditary breast and ovarian cancer syndromes.

• Hereditary breast and ovarian cancer (HBOC) syndrome represents 5-10% of all breast cancer.

• The risk of ovarian cancer with pathogenic mutations in BRCA1 is 63% by the age of 70 years, and that with pathogenic mutations in BRCA2 is 27% by the age of 70 years.

• A treatment strategy combining a plan for existing breast cancer and for reduction of future breast and ovarian cancer risk is required.

Initial counselling and follow-up of BRCA mutation carriers

• Following a diagnosis of the presence of a BRCA1/2 mutation follow-up counselling outlining options for screening for early detection, risk-reducing measures and issues pertaining to fertility in women who have not completed their family is fundamental.

• The difference between the goals of screening and those of risk-reducing measures (including surgery, chemoprevention and lifestyle measures) must be clarified to the patient.

• Individuals older than 25 years in a family known to harbour a BRCA1/2 should be encouraged to undergo testing and, if positive, to consider risk-reducing measures.

Surveillance	Surgical therapy	Chemoprevention
• Annual mammography • Breast magnetic resonance imaging (MRI) • CA-125 performed once every 6 months remains the alternative ovarian screening test	• Risk-reducing mastectomy (RRM) is expected to be approximately 90% preventive, considering onset in other tissues. • Options for simultaneous reconstruction are also considered. • When considering risk reduction salpingo-oophorectomy (RRSO), counseling is provided regarding postoperative menopause. Because the possibility of peritoneal cancer remains, the preventive effect is approximately 80%.	• Prophylactic endocrine therapy significantly reduces the risk of breast cancer in HBOC patients. • For risk reduction with endocrine therapy is recommended oral administration of: - 20 mg/day **tamoxifen** for 5 years or - 60 mg/day **raloxifene** for 5 years.

Breast cancer risk reduction measures include

Lifestyle modifications:

1. Numerous observational studies have suggested that breastfeeding may reduce the risk of breast cancer among BRCA1/2 carriers;
2. Regular exercise, maintaining healthy body weight and limiting alcohol consumption should also be encouraged;
3. Hormone replacement therapy (HRT) should be avoided.

Screening

• Clinical breast examination every 6–12 months is recommended from the age of 25 or 10 years before the youngest breast cancer diagnosis in the family;

• Breast magnetic resonance imaging (MRI) is well established as the most sensitive screening tool for the high-risk population.

• Annual screening MRI should be commenced from the age of 25 with the addition of annual mammography from the age of 30;

• Retrospective data suggest an association between increased breast cancer risk and exposure to diagnostic radiation before the age of 30;

• Ultrasound may be considered as an adjunct to mammography at all ages and as an alternative when MRI is not available.

Risk-reducing agents

• There is limited data available regarding the use of selective estrogen receptor modulators (tamoxifen, raloxifene) and aromatase inhibitors as primary prevention among BRCA1/2 mutations;

• Several observational studies have suggested that tamoxifen use reduces the risk of contralateral breast cancer among BRCA1/2-associated breast cancer patients;

• There is no evidence to suggest that, with respect to hormonal therapy, patients with BRCA1/2-associated breast cancer should be treated any differently to those with non-BRCA-associated breast cancer.

Risk-reducing surgery

• Bilateral risk-reducing mastectomy (RRM) is the most effective method for reducing breast cancer risk among BRCA1/2 mutation carriers reducing the risk of breast cancer by approximately 90%;

• The studies have been either retrospective or prospective in nature, many with over 10 years of long-term follow-up.

What is the effect of risk-reducing mastectomy (RRM)?

• The effect of RRM has not shown significant differences in mortality compared to other options in some studies.

• One study reported that prophylactic RRM reduced the development of new primary breast cancer by > 90% and improved prognosis.

• Patients who received the most survival benefit from RRM were:

- those who developed breast cancer prior to 40 years of age,
- those with non-triple negative breast cancer,
- those with histological grades of 1 or 2 even in triple-negative breast cancer,
- those who had not received adjuvant chemotherapy.

• Major prerequisites for indicating RRM in HBOC patients are the patient's wish and the perceived value of the procedure.

What is the effect of risk-reduction salpingo-oophorectomy?

RRSO

- reduces the risk of developing ovarian cancer and fallopian tube cancer
- leads to improved prognosis.
- reduces the risk of developing ovarian cancer, fallopian tube cancer, and peritoneal cancer by approximately 80%
- because the peritoneum cannot be resected, the onset risk rate remains

Even after removal of the breast before the onset of cancer, occult cancer may be detected **the extent to which the tissue from RRM should be examined**.

Issue !

Does RRSO reduce the occurrence of breast cancer?

• Prophylactic RRSO reduced the risk of breast cancer in BRCA1 and BRCA2 mutation carriers;
• It is effective in the risk reduction of breast cancer occurrence in those without breast cancer.

Surgery in HBOC

Breast cancer treatment of HBOC carriers

• The extent of the lesion and cancer stage will determine the choice of mastectomy or breast-conserving surgery;
• A relative contraindication for breast-conserving surgery in patients with premenopausal breast cancer with BRCA1/2 mutations includes the high probability of ipsilateral breast tumor recurrence (IBTR) after breast-conserving surgery.
• Recurrence was observed distant from the primary tumor in many reports, indicating that IBTR due to new primary cancer accounted for most of the recurrence rate.
• Chemotherapy and oophorectomy is associated with reduction of IBTR risk.
• There was no diference in prognosis between the BRCA1/2 mutation group and the non-carrier control group in patients who underwent breast-conserving surgery.
• And no diference in prognosis between surgical procedures.

Radiation therapy after lumpectomy in HBOC patients.

• It is necessary to consider whether tumor cells in HBOC have the same sensitivity to radiation as tumor cells in sporadic breast cancer.
• BRCA1/2 mutation-associated breast cancer cells have high sensitivity to irradiation.
• For Li-Fraumeni syndrome patients with TP53 mutations, radiation therapy should be avoided due to the high rate of cancer development from non-cancer sites.

Systemic therapy for HBOC

• Poly ADP-ribose polymerase (PARP) inhibition causes synthetic lethality with defective DNA repair via inhibition of homologous recombination.
• PARP inhibitors are effective at well-tolerated doses and have antitumor activity against BRCA1- and BRCA2-associated cancers.
• There is a high chemotherapy sensitivity in patients with BRCA1/2 mutations.
• In case of invasive cancer, it may be recommended that all patients should receive chemotherapy.

Ovarian cancer treatment of HBOC carriers

Is similar to that of sporadic ovarian cancer, and involves standard surgery and postoperative chemotherapy.

Future outlook and challenges in HBOC diagnosis

• The demand for genetic counseling and testing is increasing, necessitating the need for increased genetic counseling and risk reduction.

• In order to better diagnose and treat HBOC, it is important to work toward increasing awareness among patients, healthcare providers, and society, and developing medical systems facilitating optimal diagnosis and treatment

Risk reducing surgery

• A variety of techniques exist: ranging from total mastectomy, through to skin-sparing mastectomy (**SSM**) and nipple-sparing mastectomy (**NSM**), which aim to improve cosmetic results

• Immediate breast reconstruction surgery should be offered

• SSM and NSM have similar safety outcomes as total mastectomy, after breast cancer diagnosis

• The possibility of an occult breast cancer being diagnosed at the time of surgery is <5% and thus routine sentinel lymph node biopsy is not indicated;

• Studies have found that women that chose risk-reducing surgery (RRS) were more likely to perceive their risk of breast cancer more highly than women who did not opt for surgery.

• Contralateral risk-reducing mastectomy (CRRM) among patients with a previous breast cancer diagnosis can be considered.

• Several retrospective and prospective studies with long-term follow-up have all demonstrated a significant reduction in contralateral breast cancer events, and two studies demonstrated a significant reduction in the risk of breast cancer-related death.

• Risk-reducing salpingo-oophorectomy (**RRSO**) has repeatedly been reported in several retrospective and prospective studies to reduce the risk of breast cancer among BRCA1/2 mutation carriers when carried out in premenopausal women.

• **Prophylactic surgery at 40 years of age for B1/B2 can be postponed to 45 years for B2. PALB2 as B1/B2**

Reproductive considerations in BRCA mutation carriers

• BRCA1/2 carriers can be reassured that there is no convincing evidence that mutation carriers have reduced ovarian reserve or fertility.

• For women who wish to undergo Risk-reducing salpingo-oophorectomy (**RRSO)** and have not yet completed childbearing, fertility preservation options such as oocyte and embryo cryopreservation should be discussed.

• Women harbouring a BRCA1/2 mutation who have been diagnosed with a malignancy should be counselled about options for fertility preservation before the commencement of oncology treatment.

Take Home Message

✓ The role of initial counselling and follow-up of BRCA mutation carriers is to outline the options of screening for early detection, risk-reducing measures and issues pertaining to fertility in women who have not completed their family.

✓ The methods used for screening include: clinical breast examination, breast magnetic resonance imaging, mammography and ultrasound.

✓ Selective estrogen receptor modulators (tamoxifen, raloxifene) and aromatase inhibitors can be used as risk-reduction agents (primary prevention) for BRCA1/2 mutations carriers.

✓ Risk-reduction surgery include risk-reducing mastectomy and risk-reduction salpingo-oophorectomy.

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II.7.2. Good clinical practice in the management of the hereditary risk of breast cancer

Learning objectives

✓ At the conclusion of this presentation, participants will be able to identify, differentiate, summarize, evaluate, and apply the following aspects related to the management of the hereditary risk of breast cancer:

- Breast cancer screening
- Diagnostic strategy for breast cancer
- Breast cancer staging

Introduction

✓ It is considered as the most important risk factors for breast cancer (BC): genetic predisposition, estrogens exposure, low parity, ionising radiation, high breast density, a background of atypical hyperplasia.

✓ Genetic predisposition to breast cancer may be related to mutation in a particular gene or group of genes, including BRCA1/2, which increase the lifetime risk of breast cancer development.

✓ The genetic screening improves the surveillance of people for breast as well as other cancers, providing a better prophylaxis and risk-reducing interventions.

✓ Among numerous guidelines for BC screening in individuals with high risk of breast cancer (family history/known BRCA1/2 mutation), many of them indicate a multimodal screening approach.

Breast cancer screening

ESMO Recommendations for breast cancer screening.

Recommended age	Recommended period	Recommended method
50–69 years	annual or every 2 years	mammography
40–49 years 70–74 years	annual or every 2 years	mammography- less established benefit
strong familial history of breast cancer, with or without proven BRCA mutations	annual	MRI and mammography (concomitant or alternating)

MRI – magnetic resonance imaging

Diagnosis of breast cancer

• **Clinical examination** - bimanual palpation of the breasts and regional lymph nodes, distant metastases assessment;

• **Imaging** – bilateral mammography and ultrasound of the breast and regional lymph nodes;

• **Pathological assessment** – diagnostic confirmation and tumor evaluation (histopathological and immunohistochemical).

Diagnosis strategy for early breast cancer

ESMO Diagnosis Guidelines for early breast cancer

Evaluation of general health status	History Menopausal status Physical examination Blood count Liver, renal and cardiac function tests
Evaluation of primary tumor	Physical examination Mammography Breast ultrasound (US) Breast MRI in selected cases Core biopsy with histopathological and immunohistochemical assessment of the tumor (histology, grade, ER, PR, HER2 and Ki67)
Evaluation of regional lymph nodes	Physical examination Ultrasound Ultrasound-guided biopsy if suspicious
Evaluation of metastatic disease	Physical examination Other tests are not routinely recommended, unless aggressive tumor or suggestive symptoms

ER, estrogen receptor;
PR, progesterone receptor;
HER2, human epidermal growth factor receptor 2;
Ki67, nuclear protein, marker for cellular proliferation;
MRI, magnetic resonance imaging;
US, ultrasound

ESMO Recommendations for breast cancer diagnosis

• **Breast imaging** - bilateral mammogram and ultrasound of breasts and axillae in all cases; MRI - in uncertain cases following standard imaging and in special clinical situations.

• **Pathological evaluation** – histopathological examination of the primary tumor and cytological/histopathological examination of the axillary nodes (if involvement is suspected).

• **Pathological report** - histological type, grade, IHC evaluation of ER, PR, HER2, Ki67 (invasive cancer). Tumors should be also grouped into molecular subtypes.

• **TIL (Tumour-infiltrating lymphocyte) scoring** - prognostic value

• **Genetic counselling** and **testing for BRCA1 and BRCA2 mutations** - breast cancer patients from high-risk groups.

Pathological diagnosis

• World Health Organization (WHO) and American Joint Committee on Cancer (AJCC) classification;

• TNM (tumor, node, metastasis) staging system - anatomical and prognostic information: tumor grade, estrogen receptor (ER), progesterone receptor (PR) status, human epidermal growth factor receptor 2 (HER2);

• Pathological diagnosis - core needle biopsy (ultrasonography/stereotactic guidance) or at least a fine-needle aspiration (FNA) proving carcinoma before any treatment;

• In case of preoperative systemic therapy, the core needle biopsy (2-3 cores) is mandatory for the diagnosis of invasive carcinoma and IHC evaluation of biomarkers;

• For multifocal/multicentric tumors - all lesions should be biopsied ;

• Only in repeated negative core biopsies - an excisional biopsy should not be carried out;

• The pathological report - presence/absence of ductal carcinoma in situ (DCIS), the histological tumor type/grade, immunohistochemical (IHC) evaluation of ER, PR, HER2 expression/gene amplification, Ki67 proliferation marker;

• If ER, PR, HER2 are negative in the biopsy specimen – retesting in the surgical specimen – discrepancy - the surgical specimen results are definite;

• For prognostic assessment and treatment decision - tumors should be grouped into intrinsic molecular subtypes;

• Tumour-infiltrating lymphocyte (TIL) score - prognostic value in triple-negative breast cancer (TNBC) and HER2-positive breast cancer, as well as prediction of pathological complete response (pCR) to chemotherapy (ChT);

Genetic counselling and testing for germline BRCA1 and BRCA2 mutations

• strong family history of breast, ovarian, pancreatic and/or high grade/metastatic prostate cancer;

• diagnosis of breast cancer before the age of 50;

• diagnosis of triple-negative breast cancer before the age of 60;

• personal history of ovarian cancer or second breast cancer or male sex.

WHO Classification of breast cancer

Type	Classification
Precursor lesions	
Ductal carcinoma *in situ*	8500/2
Lobular neoplasia	
Lobular carcinoma *in situ*	
Classic lobular carcinoma *in situ*	8500/2
Pleomorphic lobular carcinoma *in situ*	8519/2*
Atypical lobular hyperplasia	
Intraductal proliferative lesions	
Usual ductal hyperplasia	
Columnar cell lesions including flat epithelial atypia	
Atypical ductal hyperplasia	
Papillary lesions	
Intraductal papilloma	8503/0
Intraductal papilloma with atypical hyperplasia	8503/0
Intraductal papilloma with ductal carcinoma *in situ*	8503/2*
Intraductal papilloma with lobular carcinoma	8520/2
Intraductal papillary papilloma	8503/2
Encapsulated papillary carcinoma	8504/2
Encapsulated papillary carcinoma with invasion	8504/3
Solid papillary carcinoma	
In situ	8509/2
Invasive	8509/3
Invasive carcinoma of no special type (NST)	8500/3
Pleomorphic carcinoma	8522/3
Carcinoma with osteoclast-like stromal giant cells	8035/3
Carcinoma with choriocarcinomatous features	
Carcinoma with melanotic features	
Invasive lobular carcinoma	8520/3
Classic lobular carcinoma	
Solid lobular carcinoma	
Alveolar lobular carcinoma	
Pleomorphic lobular carcinoma	
Tubulolobular carcinoma	
Mixed lobular carcinoma	
Tubular carcinoma	8211/3

Cribriform carcinoma	8201/3
Mucinous carcinoma	8480/3
Carcinoma with medullary features	
Medullary carcinoma	8510/3
Atypical medullary carcinoma	8513/3
Invasive carcinoma NST with medullary features	8500/3
Carcinoma with apocrine differentiation	
Carcinoma with signet-ring-cell differentiation	
Invasive micropapillary carcinoma	8507/3
Metaplastic carcinoma of no special type	8575/3
Low-grade adenosquamous carcinoma	8570/3
Fibromatosis-like metaplastic carcinoma	8572/3
Squamous cell carcinoma	8070/3
Spindle cell carcinoma	8032/3
Metaplastic carcinoma with mesenchymal differentiation	
Chondroid differentiation	8571/3
Osseous differentiation	8571/3
Other types of mesenchymal differentiation	8575/3
Mixed metaplastic carcinoma	8575/3
Myoepithelial carcinoma	8982/3
Epithelial-myoepithelial tumors	
Adenomyoepithelioma with carcinoma	8983/3
Adenoid cystic carcinoma	8200/3
Rare types	
Carcinoma with neuroendocrine features	
Neuroendocrine tumour, well-differentiated	8246/3
Neuroendocrine carcinoma poorly differentiated (small cell carcinoma)	8041/3
Carcinoma with neuroendocrine differentiation	8574/3
Secretory carcinoma	8502/3
Invasive papillary carcinoma	8503/3
Acinic cell carcinoma	8550/3
Mucoepidermoid carcinoma	8430/3
Polymorphous carcinoma	8525/3
Oncocytic carcinoma	8290/3
Lipid-rich carcinoma	8314/3
Glycogen-rich clear cell carcinoma	8315/3
Sebaceous carcinoma	8410/3

Molecular classification of breast cancer

Molecular Subtype	Characteristics	
Luminal A	**Luminal A-like** ER-positive HER2-negative Ki67 low PR high Low-risk molecular signature	
Luminal B	**Luminal B-like (HER2-negative)** ER-positive HER2-negative and either Ki67 high or PR low High-risk molecular signature	**Luminal B-like (HER2-positive)** ER-positive HER2-positive Any Ki67 Any PR
HER2	**HER2-positive (non-luminal)** HER2-positive ER, PR absent	
Basal-like	**Triple-negative** ER and PR absent HER2-negative	

Staging for breast cancer

pN Category	pN Criteria
pN2b	Metastases in clinically detected internal mammary lymph nodes with or without microscopic confirmation: with pathologically negative axillary nodes
pN3	Metastases in 10 or more axillary lymph nodes; *or* in infraclavicular (Level III axillary) lymph nodes; *or* positive ipsilateral internal mammary lymph nodes by imaging in the presence of one or more positive Level L II axillary lymph nodes; *or* in more than three axillary lymph nodes and micrometastases or maerometastases by sentinel lymph node biopsy in clinically negative ipsi lateral internal mammary lymph nodes; *or* in ipsilateral supraclavicular lymph nodes
pN3a	Metastases in 10 or more axillary lymph nodes (at least one tumor deposit larger than 2.0 mm); *or* metastases to the infraclavicular (Level III axillary lymph) nodes
pN3b	pN la or pN2a in the presence of cN2b (positive internal mammary nodes by imaging); *or* pN2a in the presence of pNlb
pN3c	Metastases in ipsilateral supraclavicular lymph nodes

Note*:* (sn) and (f) suffixes should be added to the N category to denote confirmation of metastasis by sentinel node biopsy or FNA/core needle biopsy respectively, with NO further resection of nodes.

Definition of Regional Lymph Nodes - Pathological (pN)

pN Category	pN Criteria
pNX	Regional lymph nodes cannot be assessed (e.g., not removed Tor pathological study or previously removed)
pN0	No regional lymph node metastasis identified or ITCs only
pN0(i+)	ITCs only (malignant cell clusters no larger than 0.2 mm| in regional lymph node(s)
pN0(mol+)	Positive molecular findings by reverse transcriptase polymerase chain reaction I RT-PCRl; no ITCs detected
pN1	Micrometastases; or metastases in 1—3 axillary lymph nodes: and/or clinically negative internal mammary nodes with micrometastases or macrornetastases by sentinel lymph node biopsy
pN1mi	Micrometastases I approximately 200 cells, larger than 0.2 mm. but none larger than 2.0 mm)
pN1a	Metastases in 1-3 axillary lymph nodes, at least one metastasis larger than 2.0 mm
pN1b	Metastases in ipsilateral internal mammary sentinel nodes, excluding ITCs
pN1c	pN I a and pN1b combined
pN2	Metastases in 4—9 axillary lymph nodes: or positive ipsilaieral internal mammary lymph nodes by imaging in the absence of axillary lymph node metastases
pN2a	Metastases in 4-9 axillary lymph nodes (at least one tumor deposit larger than 2.0 mm)

Definition of Primary Tumor (T) – Clinical and Pathological

T Category T Criteria

TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis (DCIS)*	Ductal carcinoma in *situ*
Tis (Paget)	Paget disease of the nipple NOT associated with
	(Paget) invasive carcinoma and/or carcinoma *in situ* (DCIS) in the underlying breast parenchyma. Carcinomas in the breast parenchyma associated with Paget disease are categorized based on the size and characteristics of the parenchymal disease, although the presence of Paget disease should still be noted.
T1	Tumor ≤ 20 mm in greatest dimension
T1mi	Tumor ≤ 1 mm in greatest dimension
T1a	Tumor > 1 mm but ≤ 5 mm in greatest dimension (round any measurement >1.0-1.9 mm to 2 mm).
T1b	Tumor > 5 mm but ≤ 10 mm in greatest dimension
T1c	Tumor > 10 mm but ≤ 20 mm in greatest dimension
T2	Tumor > 20 mm but ≤ 50 mm in greatest dimension

T3	Tumor > 50 mm in greatest dimension
T4	Tumor of any size with direct extension to the chest wall and/or to the skin (ulceration or macroscopic nodules); invasion of the dermis alone does not qualify as T4
T4a	Extension to the chest wall; invasion or adherence to pectoralis muscle in the absence of invasion of chest wall structures does not qualify as T4
T4b	Ulceration and/or ipsilateral macroscopic satellite nodules and/or edema (including *peau d'orange*) of the skin that does not meet the criteria for inflammatory carcinoma
T4c	Both T4a and T4b are present
T4d	Inflammatory carcinoma (see section "Rules for Classification")

*Note: Lobular carcinoma *in situ* (LCIS) in a benign entity and is removed from TNM staging in the *AJCC Cancer Staging Manual*, 8th Edition.

Definition of Distant Metastasis (M)

M Category	M Criteria
M0	No clinical or radiographic evidence of distant metastases*
cM0(i+)	No clinical or radiographic evidence of distant metastases in the presence of tumour cells or deposits no larger than0.2 mm detected microscopically or by molecular techniques in circulating blood, bone marrow, or other nonregional nodal tissue in a patient without symptoms or signs of metastases
cM1	Distant metastases detected by clinical and radiographic means
pM1	Any histologically proven metastases in distant organs; or if in non-regional nodes, metastases greater than 0.2 mm

*Note that imaging studies are not required to assign the cM0 category

Take home message

✓ As a breast cancer screening method, mammography is recommended in women between 50-69 years, annually or every 2 years.

✓ In strong familial history of breast cancer, with(out) proven BRCA mutations, mammography associated with MRI are recommended annually.

✓ Breast cancer diagnosis takes in consideration imaging, pathological evaluation and genetic testing for BRCA1 and BRCA 2 mutations.

✓ The pathological report should include the tumor molecular subtype.

✓ Gene expression profiles may be used, together with pathology assessment, for additional prognostic/predictive information and for evaluation of the need for adjuvant therapy.

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Learning objectives

• To be familiar with the Imaging protocol used for surveillance of patients with predisposition for hereditary risk of breast cancer (BRCA1 and BRCA2 mutation carriers, PALB2, PTEN, TP53, CDH1 mutations);

• To know the role of breast ultrasound, magnetic resonance imaging and mammography in the surveillance of patients with hereditary risk of breast cancer.

Introduction

Breast cancer is the most common cancer in all women at any age (29% of all female tumors) and is the first leading cause of cancer related death in women (Ferlay);

The most important risk is the hereditary factor: the individual risk increases directly proportional to the number of affected relatives and with the decrease of the age onset of the first carcinoma (Laloo).

There are different strategies to reduce the risk of developing breast cancer: clinical and imaging surveillance, lifestyle modifications, chemoprevention and prophylactic surgery.

Screening programs are available in European countries;

Ferlay J et. al. (2012), Lalloo F. & Evans DG (2012)

Imaging surveillance

• It is not a true risk-reduction measure but aims to identify the tumor in an early stage.

• Imaging methods used for surveillance of patients with hereditary risk for breast cancer are:

1. magnetic resonance imaging;
2. mammography;
3. ultrasound.

• Breast cancer surveillance protocol is age-dependent.

Imaging surveillance in patients with hereditary risk for breast cancer

ACOG guidelines

• 25–29: clinical breast examination every 6 to 12 months and breast imaging annually (optimally, MRI with contrast).

• ≥ 30: annual mammography and MRI, alternating every 6 months.

ESMO protocol

• 25-30 years: annual MRI;

> 30 years: annual MRI and, with a maximum delay of 2 months, mammography, alternating every 6 months with breast examination;

Mammography with only one incidence when an MRI is also carried out if the patient has no history of breast cancer.

> 65 years: stop the MRI; mammography annually

Imaging surveillance for breast cancer in patient without mutation

• Eisinger score > 5: same management for patient with breast cancer and 1st degree relatives women (MRI and mammography)

• Eisinger > 2 and < 6: mammography 5 years before the youngest breast cancer in family (not before 40 years of age) for patient with breast cancer and 1st degree relatives (woman) (can be until 2nd degree relatives if the relative is a man between two woman);

Breast MRI for patients with hereditary risk for breast cancer - Indication

• Breast magnetic resonance imaging (MRI) is well established as the most sensitive screening tool for the high-risk population.

• Annual screening MRI should be commenced from the age of 25 with the addition of annual mammography from the age of 30 [II, A].

Recommended annual MRI surveillance to women:

• 30–49 years who have not had genetic testing, but have a greater than 30% probability of being a BRCA carrier OR with a known BRCA1 or BRCA2 mutation;

➢ 20–49 years who have not had genetic testing but have a greater than 30% probability of being a TP53 carrier OR with a known TP53 mutation;

➢ 30–49 years with a personal history of breast cancer who remain at high risk of breast cancer, including those who have a BRCA1 or BRCA2 mutation.

➢ + 50–69 years with a known TP53 mutation.

Protocol

- dedicated breast coil;

- precontrast sequences: 3-plane localizer, axial T2-weighted with fat saturation and diffusion weighted imaging of each breast.

- gadolinium-based contrast agent intravenously administered, at 2 cc/sec followed by a 20-cc saline flush.

- Postcontrast: sagittal 3D gradient echo T1-weighted dynamic imaging with and without chemical fat saturation.

- Delayed high-resolution axial and sagittal T1- weighted fast spoiled gradient echo sequences with fat saturation.

Mammography for patients with hereditary risk for breast cancer – ESMO guidelines

• Annual screening by mammography from the age of 30 if MRI screening is not available [II, A].

• An association between increased breast cancer risk and exposure to diagnostic radiation before the age of 30.

• The decision to implement breast mammography under the age of 40 should take into consideration increased breast density at younger ages.

• There are no robust data supporting alternating 6-monthly radiology surveillance with MRI and mammography in the high-risk population.

Mammography for patients with hereditary risk for breast cancer - technique

• digital mammography;

• homogeneous breast compression;

• automatic exposure control;

• bilateral standard, mediolateral oblique, and craniocaudal positions.

Mammography - Indications

As part of the **population screening program** for women:

- > 50 years without genetic tests but with a greater than 30% probability of being a TP53 carrier;

- > 60 years at high risk of breast cancer but with a 30% or lower probability of being a BRCA or TP53 carrier/ at moderate risk of breast cancer/no genetic testing but a greater than 30% probability of being a BRCA carrier;

- > 70 years with a known BRCA1 or BRCA2 mutation.

Annual mammographic surveillance for women:

- 30-39 years at high risk of breast cancer but with a 30% or lower probability of being a BRCA or TP53 carrier/ a greater than 30% probability of being a BRCA carrier/ known BRCA1 or BRCA2 mutation;
- 50-59 years at moderate risk of breast cancer.

Breast ultrasound for patients with hereditary risk for breast cancer - indications

• In average-risk women under 40 years of age, ultrasound is the initial imaging modality of choice, but in BRCA carriers breast ultrasonography can be considered under 30 years of age, as a screening tool, if MRI is unavailable (ESMO guidelines).

• Also, ultrasound may be considered as an adjunct to mammography at all ages and as an alternative when MRI is not available (at all ages).

• Both breasts should be systematically examined by ultrasound for nodular lesions, as well as axillary and supraclavicular regions, for morphologically abnormal lymph nodes.

Imaging surveillance – sensitivity

Imaging method	Screening interval	Sensitivity	Other advantages
Ultrasound	1 year	42%	No radiation
Mammography	1 year	40%	
MRI	1 year	81%	No radiation

Other imaging modalities for breast cancer

• When breast cancer is diagnosed by one of the screening tools, CT of the thorax, abdomen and pelvis should be considered in patients at high risk of metastatic disease based on the size and grade of the primary tumour.

• If there is clinical suspicion of metastatic disease, the type of imaging will depend on the presentation:

- bone pain: isotope bone scan and MRI;
- neurological symptoms: contrast-enhanced head CT/ MRI;

PET scan is not generally required for breast cancer patients.

Assessment of the risk of other associated cancers

The genes associated with a hereditary predisposition to breast cancer are also associated with other cancers:

- BRCA1 (HBOC syndrome): associated ovarian and pancreatic cancers;
- BRCA2 (HBOC syndrome) - ovarian, prostate, and pancreatic cancers;
- p53 (Li–Fraumeni syndrome) - soft-tissue sarcoma, osteosarcoma, brain tumors, adrenocortical carcinoma, leukemia, colon cancer;
- PTEN (Cowden's disease; Bannayan–Riley–Ruvalcaba syndrome; Proteus syndrome; Proteus-like syndrome) - thyroid, endometrial, and genitourinary cancers;
- STK11/LKB1 (Peutz–Jeghers syndrome) - small-intestine, colorectal, uterine, testicular, and ovarian sex cord cancers;
- CDH1 (Hereditary diffuse gastric carcinoma) - lobular breast and diffuse gastric cancer; other tumors.

Management of the patients with hereditary risk of breast and ovarian cancer

- screening for ovarian cancer in women with *BRCA1/2* mutations;
- risk-reducing surgery: prophylactic oophorectomy;
- chemoprevention;

Take home message

• Magnetic resonance imaging (MRI) has the best sensibility for breast cancer detection in high-risk patients, especially a high diagnostic accuracy for the early stages;

• The combination of the mammography and MRI has demonstrated a sensitivity close to 100%;

• Imaging surveillance protocol is tailored by age and genetic risk;

• Management of the other cancers associated with breast cancer should be taken into consideration.

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https://www.uspreventiveservicestaskforce.org/uspstf/recommendation/brca-related-cancer-risk-assessment-genetic-counseling-and-genetic-testing

II.8.1. Good clinical practice in the management of hereditary risk of colon cancer

Learning objectives

• to know the surveillance and risk reduction measures for colorectal cancer for each category of patients with risk of HCRC;

• to no the guidelines for extracolonic cancer screening for each category of patients with risk of HCRC.

Introduction

• Multidisciplinary team: gastroenterologist, oncologist, surgeon, geneticist, family doctor;

• Screening and surveillance in patients with familial adenomatous polyposis and Lynch syndrome reduce the incidence and mortality of colorectal cancer(CCR);

• Genetic testing for all patients with suspected hereditary colorectal cancer (HCRC) and family members.

ESMO Recommendations - Lynch Syndrome

Screening and surveillance	Method	Age of beginning	Interval	Observations
Colorectal cancer	colonoscopy	MLH1 and MSH2 – 25 years MSH6 and PMS2 – 35 years	1-2 years	Chromoendoscopy is more effective
Uterine cancer	Transvaginal ultrasound Endometrial biopsy	30-35 years	1 year	Prophylactic hysterectomy and oophorectomy can be discussed
Ovarian cancer	Transvaginal ultrasound CA125	30-35 years	1 year	Prophylactic hysterectomy and oophorectomy can be discussed
Gastric cancer	Upper digestive endoscopy	30-35 years	1-3 years	Consider *Helicobacter pylori* eradication
Pancreatic, urinary tract, small bowel cancer				No enough evidences

Lynch syndrome – prevention of CRC

• Quit smoking

• Maintain normal weight

• Aspirin

• **Colon cancer syndrome X: c**olonoscopy every 3-5 years starting 10 years earlier than age of youngest affected relative

• **Constitutional mismatch repair-deficiency syndrome**: semesterly blood work and abdominal ultrasound, annual brain MRI, upper endoscopy and colonoscopy and consideration of annual whole-body MRI (WBMRI) - the lack of robust evidence and the need for more research!

Familial adenomatous polyposis

• Family history of FAP
• Genetic counseling
• Genetic testing (APC)
• Gene carriers or indeterminate cases – flexible sigmoidoscopy every year from puberty
• If polyposis is present consider colectomy!

Screening and surveillance	Method	Onset age	Interval	Observations
Colorectal cancer	Sigmoidoscopy and colonoscopy (if adenomas)	12-15 years	1-2 years	Colectomy planned under 25 years
Duodenum	Upper digestive endoscopy (front and lateral view)	25-30 years	1-5 years	Detection of adenoma – Spigelman classification – surveillance
Liver	Abdominal ultrasound Alfafetoprotein	0.5 years	1 year	
Thyroid	Cervical palpation/ultrasound	25-30 years	1 year	
Desmoid tumors	CT/MRI			

Spigelman classification for duodenal polyposis in familial adenomatous polyposis

Variable	1 point	2 points	3 points
Number of polyps	1-4	5-20	> 20
Polyp size (mm)	1-4	5-10	> 10
Histology	Tubular	Tubulovillous	Villous
Dysplasia	Mild	Moderate	Severe

Stage 0, 0 points; stage I, 4 points; stage II, 5-6 points; stage III, 7-8 points; stage IV, 9-12 points

Surveillance
Stage I - upper endoscopy every 5 years
Stage II - every 3 years
Stage II – every 1-2 years
Stage IV - every 6 months or prophylactic surgery

ESMO recommendations - other polyposis syndromes

Syndrome	Site	Technique	Age (years)	Interval (years)
Attenuated FAP	Colorectal	Colonoscopy	18-20	1-2
	Duodenum	Gastroduodenal endoscopy (front and side view)	25-30	1-5[a]
MAP	Colorectal	Colonoscopy	18-20	1-2
	Duodenum	Gastroduodenal endoscopy (front and side view)	25-30	1-5[a]
PPAP	Colorectal	Colonoscopy	18-20	1-2
	Uterus	TV US	30-35	1
SP	Colorectal	Colonoscopy	45	1-2[b]
PJ	Colorectal	Colonoscopy	8[c]	1-3
	Gastric	Gastroduodenal endoscopy	8[c]	1-3
	Small bowel	Capsule endoscopy or MRI enterography	8[c]	1-3
	Pancreas	Endoscopic ultrasonography or MRI	30	1
Juvenile polyposis	Colorectal	Colonoscopy	15	1-3
	Gastric	Gastroduodenal endoscopy	15	1-3

Take Home Message

- identification of patients with risk of HCRC
- molecular diagnosis
- surveillance colonoscopy
- screening for extracolonic malignancy

} Can reduce the risks in HCRC !

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II.8.2.Good clinical practice in the management of hereditary risk of colon cancer

Learning objectives

At the conclusion of this presentation, participants will be able to identify, differentiate, summarize, evaluate, and apply the following aspects related to the management of the hereditary risk of colon cancer:

- ✓ Prevention and diagnosis of hereditary colorectal cancer (CRC)
- ✓ Surveillance of hereditary CRC and related syndromes

The summarized guidelines aim to highlight the current data on hereditary CRC, bringing useful clinical recommendations for identification and management of patients with hereditary CRC.

Introduction

Hereditary colo-rectal cancer

A. POLYPOSIS

- ✓ Adenomatous Polyposis Syndrome **(**Familial Adenomatous Polyposis - FAP, Gardner, Turcot I syndrome)
- ✓ MUTYH-associated Polyposis (MAP)
- ✓ Hereditary Hamartomatous Polyposis Syndromes– juvenile polyposis syndrome (JPS); PTEN hamartoma tumor syndrome, which includes Cowden syndrome (CS) and Bannayan-Riley-Ruvalcaba syndrome (BRRS); and Peutz-Jeghers syndrome (PJS)

B. Hereditary Nonpolyposis Colorectal Cancer - 2-5% of all colorectal carcinomas

- ▪ Lynch syndrome I (familial colon cancer)
- ▪ Lynch syndrome II (HNPCC associated with other cancers of the gastrointestinal [GI] or reproductive system)
- ▪ Muir-Torre syndrome
- ▪ Turcot II syndrome

• Although approximately 35% of colorectal cancers (CRCs) are considered to be related to heritable factors, only 5-10% are due to high-risk mutations of CRC susceptibility genes, mainly the mismatch repair genes (Lynch syndrome) and adenomatous polyposis coli gene (APC, familial adenomatous polyposis).

• The genes for CRC included in the multigene panels range from well-known susceptibility genes to less validated CRC genes, with less clinical utility.

• Knowledge of the genotypic/phenotypic picture of the patients with Lynch syndrome (LS) or familial adenomatous polyposis (FAP) will promote more targeted and efficient surveillance.

• Because there is no consensus regarding inclusion of CRC genes in multigene panels, the patient counseling and management is still challenging.

• Specialists involved in the management of patients with CRC should know the hereditary CRC syndromes to guide patients to specialized cancer genetic centers for adequate counselling.

Hereditary non-polyposis colorectal cancer syndrome (Lynch syndrome/LS)

The prevalence and the clinical phenotypes of the LS

PREVALENCE PENETRANCE	LS CLINICAL PHENOTYPES
1-3% of CRC diagnosis **Germline mutations of MMR genes and EPCAM** (>70% - MLH1, MSH2, EPCAM mutations in tumors with MSI) **Autosomal dominant inheritance** **30-73% - increased risk for CRC** **30-51% - increased risk for endometrial cancer** **4-15% - increased risk for ovarian cancer** **Up to 18% - increased risk for gastric cancer** **3-5% - increased risk for small bowel cancer** **2-20% - increased risk for urinary tract cancer** **4% - increased risk for pancreatic cancer**	Germline MMR gene pathogenic variants with: CNS tumors **(Turcot syndrome)** Cutaneous gland tumors **(Muir-Torre syndrome)** Homozygous or compound heterozygotes individuals for MMR gene pathogenic variants: **Constitutional/biallelic MMR deficiency (CMMRD)**

Clinical criteria used for identification of individuals at risk of LS

Amsterdam criteria II and revised Bethesda guidelines

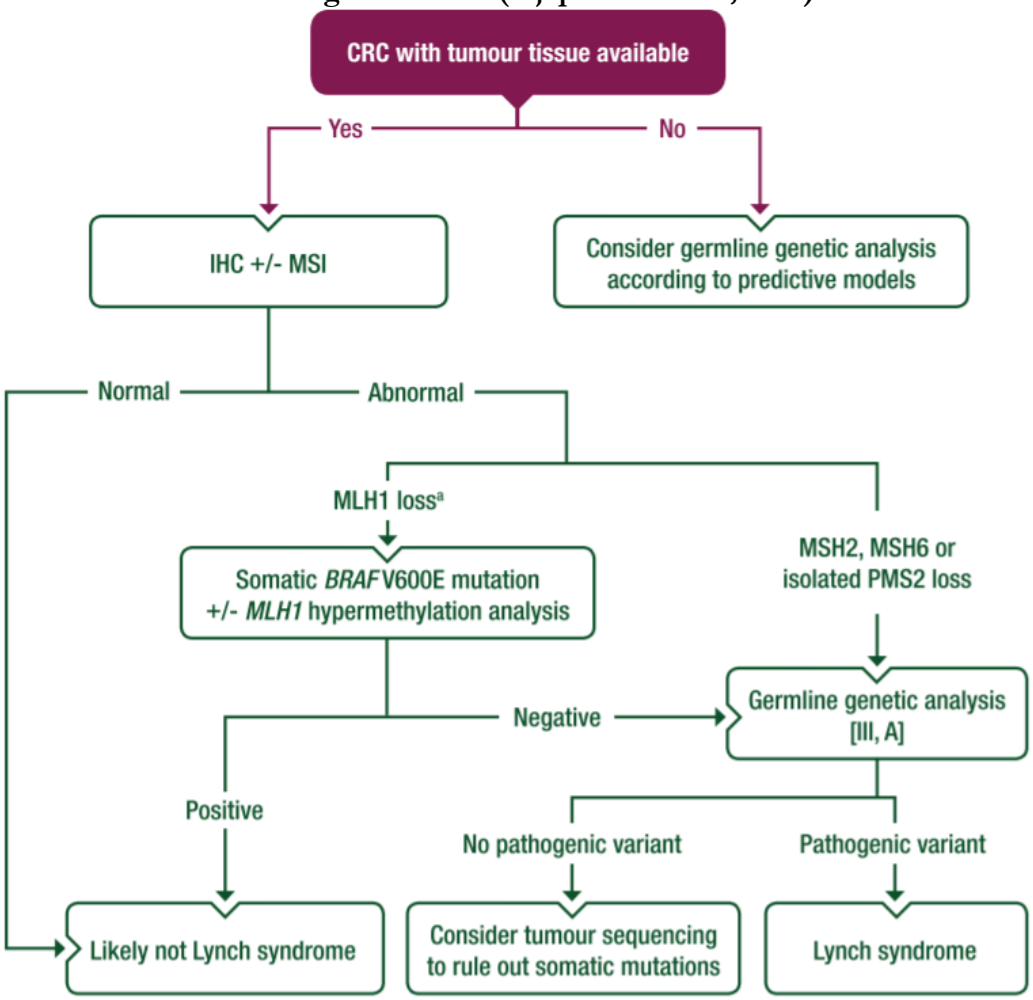
Amsterdam criteria (AC) II	Revised Bethesda guidelines
At least three relatives must have a cancer associated with LS (colorectal, endometrial, small intestine, ureter or renal pelvis cancer); **all of the following criteria** should be present: • One must be a FDR of the other two • At least two successive generations must be affected • At least one relative with a cancer associated with LS should be diagnosed before age 50 • FAP should be excluded in the CRC case(s) (if any) • Tumors should be verified whenever possible	Tumors from individuals should be tested for **MSI** in the following situations: • CRC diagnosed in a patient who is younger than 50 years of age • Presence of synchronous or metachronous colorectal or other LS-related tumours*, regardless of age • CRC with MSI-high histology** diagnosed in a patient who is younger than 60 years of age • CRC diagnosed in a patient with one or more FDRs with an LS-related cancer, with one of the cancers being diagnosed below age 50 • CRC diagnosed in a patient with two or more first- or second-degree relatives with LS-related cancer regardless of age

*LS-related cancers include colorectal, endometrial, gastric, ovarian, pancreas, ureter and renal pelvis, biliary tract, brain (usually glioblastoma), small intestinal cancers, as well as sebaceous gland adenomas and keratoacanthomas.

**Presence of tumour infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet ring differentiation or medullary growth pattern.

CRC, colorectal cancer; FAP, familial adenomatous polyposis; FDR, first-degree relative; LS, Lynch syndrome; MSI, microsatellite instability.

Algorithm for molecular diagnosis of LS (Stjepanovic et al, 2019)



[a]If the loss of expression of MLH1 is concurrent with the loss of expression of MSH2 or MSH6 a germline genetic analysis should be recommended.

CRC, colorectal cancer; IHC, immunohistochemistry; MSI, microsatellite instability

Surveillance and risk reduction of individuals at risk of LS

LS surveillance recommendations

Site	Technique	Age (years)	Interval (years)
Colorectum	Colonoscopy	MLH1/MSH2: 25[*,**] MSH6/PMS2: 35	1-2
Uterus	TV US Endometrial biopsy	30-35	1
Ovaries	CA125 TV US	30-35	1
Stomach	UGI endoscopy[***] Consider testing *Helicobacter pylori*	30-35	1-3
Other LS-associated cancers	None[****]		

[*]Or 5 years before the earliest CRC, if diagnosis <25 years.

[**]Consider later age for MSH6 carriers.

[***]Consider in high-incidence countries or family history of gastric cancer.

[****]Consider pancreatic/urinary tract cancer surveillance if family history.

CA 125, cancer antigen 125; CRC, colorectal cancer; LS, Lynch syndrome; TV, transvaginal; UGI, upper gastrointestinal; US, ultrasound.

Management of Lynch Syndrome

Cancer treatment	Familial colorectal cancer X syndrome	Constitutional MMR-deficiency syndrome	Lynch-like syndrome
Colorectal surgery: extended colectomy may be an option in patients with LS undergoing primary surgery for CRC, especially in younger patients **Systemic treatment:** Pembrolizumab for any MMR deficient solid tumor Nivolumab for colorectal MMR deficient tumors	- up to 40% of families who fulfil the AC for hereditary non-polyposis colon cancer (HNPCC) but do not present a tumor MMR deficiency/or a MMR gene alteration - cancer risk limited to the colorectum - colonoscopy surveillance at 3–5-yrs, starting at 40 yrs/10 years earlier than the age at diagnosis of the youngest case in the family	- childhood cancers - high-incidence of CRC, adenomatous polyposis and small bowel, haematological, brain, endometrium and urinary tract tumors - proposed surveillance approach: semesterly blood work and abdominal US, annual brain MRI, upper endoscopy and colonoscopy and consideration of annual whole-body MRI	- resemble LS because of - MMR deficiency/MSI (excluding MLH1 hypermethylation) - but without germline mutation - rule out a sporadic somatic biallelic inactivation of these genes in relatives considered potentially at risk

Familial adenomatous polyposis (FAP)
Prevalence, clinical diagnosis

The prevalence and the clinical diagnosis of the FAP

	CLINICAL AND MOLECULAR DIAGNOSIS
- autosomal dominant inherited disorder associated with germline mutations in the adenomatous polyposis coli (APC) gene - presence of multiple colorectal adenomas - near 100% risk of developing CRC at an early age if prophylactic colectomy is not carried out – for classical form of FAP - represents < 1% of all cases of CRC - the most frequent cause of polyposis with a known genetic cause	Two main phenotypes: - Classical - > 100 adenomas along the entire colon - Attenuated phenotype - between 10 and 100 adenomas, preferentially in the right colon and with a later onset Associated with extracolonic tumors: children hepatoblastoma, duodenal, pancreatic, thyroid and brain cancers Germline mutation in the APC gene - 80% of the classical FAP - 10% of attenuated cases Full germline genetic testing – DNA sequencing and large rearrangement analysis APC analysis should include large rearrangements Multigene panels genetic testing for multiple genes involved in colorectal adenomatous polyposis (APC, MUTYH, POLE, POLD1, NTHL1)

Surveillance and risk reduction

Classical FAP surveillance guidelines

Site	Technique	Age (years)	Interval (years)
Colorectal	Sigmoidoscopy and colonoscopy (if adenomas)*	12-15	1-2
Duodenum	Gastroduodenal endoscopy (front and side view)	25-30	1-5**
Thyroid	Cervical US or cervical palpation	25-30	1
Liver	Abdominal US 0.5c 1 Serum alpha foetoprotein	0.5***	1
Desmoids	CT/MRI****		

*If adenomas are found at sigmoidoscopy, carry out annual colonoscopies until colectomy.
**Periodicity according to the Spigelman stage.
***Until age 7 years.
****If family history or symptoms. Periodicity is not well-established.
CT, computed tomography; FAP, familial adenomatous polyposis; MRI, magnetic resonance imaging; US, ultrasound.

Other polyposis syndromes surveillance guidelines

Syndrome	Site	Technique	Age (years)	Interval (years)
Attenuated FAP	Colorectal Duodenum	Colonoscopy Gastroduodenal endoscopy (front and side view)	18-20 25-30	1-2 1-5*
MAP	Colorectal Duodenum	Colonoscopy Gastroduodenal endoscopy (front and side view)	18-20 25-30	1-2 1-5*
PPAP	Colorectal Uterus	Colonoscopy TV US	18-20 30-35	1-2 1
SP	Colorectal	Colonoscopy	45	1-2**
PJ	Colorectal Gastric Small bowel Pancreas	Colonoscopy Gastroduodenal endoscopy Capsule endoscopy/MRI enterography Endoscopic ultrasonography/MRI	8*** 8*** 8*** 30	1-3 1-3 1-3 1
Juvenile polyposis	Colorectal Gastric	Colonoscopy Gastroduodenal endoscopy	15 15	1-3 1-3

*Periodicity according to the Spigelman stage.
**FDR: starting at 45 or 10 years earlier than the affected relative. If no polyps, repeat every 5 years.
***Basal colonoscopy at age 8. If negative for polyps, re-start surveillance at age 18.
FAP, familial adenomatous polyposis; FDR, first-degree relative; MAP, MUTYH-associated polyposis; MRI, magnetic resonance imaging; PJ, Peutz–Jeghers;
PPAP, polymerase proofreading-associated polyposis; SP, serrated polyposis; TV, transvaginal; US, ultrasound.

Prevalence and diagnosis

MUTYH-associated polyposis (MAP)

The prevalence and the diagnosis of MAP

PREVALENCE AND PENETRANCE	CLINICAL AND MOLECULAR DIAGNOSIS
- autosomal recessive syndrome - biallelic germline mutations in the MUTYH gene - phenotype of attenuated adenomatous polyposis - lower risk of extracolonic manifestations in comparison with FAP - develops in the second or third decade of life	Heterogenous clinical spectrum of MUTYH germline mutations: - attenuated and classic adenomatous polyposis - CRC without polyposis - Lynch-like syndrome Germline genetic testing should include: - all exons of MUTYH - recommendation for multigene single analysis of the genes involved in colorectal adenomatous polyposis (APC, MUTYH, POLE, POLD1, NTHL1)

Hereditary polyposis colorectal cancer syndromes

Polymerase proofreading-associated polyposis (PPAP)
- autosomal dominant inheritance of two genes associated with multiple adenomas and early onset CRC: POLE and POLD1 - an approach similar to MAP is recommended with regular colonoscopy surveillance (Table slide 11)

Adenomatous polyposis associated with germinal mutation in NTHL1-
- association of biallelic germinal mutation of NTHL1 (16p13.3) with attenuated adenomatous polyposis - - autosomal recessive inheritance - no specific recommendations for the management - recommended similar approach to MAP, with regular colonoscopy surveillance

Serrated polyposis syndrome (SPS)
Prevalence - combination of large and/or numerous serrated lesions spreading throughout the colorectum - 15-30% increased lifetime risk of CRC - while prevalence of SPS remains unknown, this syndrome is emerging as one of the most common CRC polyp syndromes **Clinical and molecular diagnosis** - according to the WHO criteria developed in 2019, SPS is defined as: *Criterion 1*: 5 serrated lesions/polyps proximal to the rectum, all being 5 mm in size, with 2 being 10 mm in size; *Criterion 2*: >20 serrated lesions/polyps of any size throughout the large bowel, with 5 being proximal to the rectum.

- any histological subtype of serrated lesion/polyp is included in the final polyp count, which is cumulative over multiple colonoscopies
- the genetic basis of SPS remains largely unknown – reported biallelic MUTYH mutations, RNF-43 germline mutations

Surveillance and risk reduction
- colonoscopy every 1–2 years (see Table slide 11)
- surgery only for patients with CRC or those who cannot be managed endoscopically
- total colectomy with IRA (ileorectal anastomosis) for patients with severe and recurrent polyposis
- segmental colectomy may be indicated in less severe cases
- recommended 1–2-year surveillance after colectomy

Hamartomatous polyposis

- Peutz–Jeghers syndrome (PJS), juvenile polyposis (JP) syndrome represent rare entities
- diagnostic criteria and surveillance recommendations based on expert consensus (Table slide 11)

Spigelman classification for duodenal polyposis in familial adenomatous polyposis

Variable	1 point	2 points	3 points
Number of polyps	1-4	5-20	> 20
Polyp size (mm)	1-4	5-10	> 10
Histology	Tubular	Tubulovillous	Villous
Dysplasia	Mild	Moderate	Severe

Stage 0, 0 points; stage I, 4 points; stage II, 5–6 points; stage III, 7–8 points; stage IV, 9–12 points

DCBE (double-contrast/air-contrast barium enema)

• Colonic preparation is essential for an optimal examination (laxative);

• Evaluates the colon by coating the mucosal surface with a suspension of barium and distending the colon with air introduced through a flexible catheter inserted into the rectum;

• Radiographies are acquired, with patient in different positions, under fluoroscopic guidance;

• DCBE was adopted as a CRC screening option by the Multi-Society Gastroenterology Consortium and the ACS in1997

• It is also considered appropriate for screening of the average-risk population by the ACR, as well as by Medicare;

• No randomized controlled trials evaluating the efficacy of DCBE as a primary screening modality to reduce incidence or mortality from CRC in average-risk adults;

• No case-control studies evaluating the performance of DCBE.

• The majority of studies showed sensitivity for cancer detection of 85% to 97%.

Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology*†

• Review of the literature concerning the performance of DCBE for polyps = difficult; heterogeneity of study design.

• The target lesion and thresholds considered clinically significant often varied based upon size and/or morphology.

• Studies involving asymptomatic individuals with a history of prior adenoma removal, followed-up by DBCE = sensitivities of 73% for adenomas >1; 75% rate of detection for adenomas with advanced histology.

Limitations

• requirement for extensive colonic preparation; suboptimal preparation can reduce both sensitivity and specificity;

• some discomfort during and after the procedure.

• no opportunity for biopsy or polypectomy; any polyp larger than 6 mm should further be biopsied by colonoscopy.

Quality of the DCBE examination influenced by:

(1) ability to fully evaluate the entire colon due to lack of retained barium or collapse of segments of the colon;

(2) adequacy of the bowel preparation;

(3) patient's ability to stand and be imaged in prone and supine position.

DCBE every 5 years is an acceptable option for CRC screening in average-risk adults aged 50 years and older. No studies for patients with hereditary risk of colon cancer.

Virtual colonoscopy

• A minimally invasive imaging examination of the entire colon and rectum.

• Abdominal-pelvic CT + 2D and 3D-image reconstruction.

• Acquisition of thin slices (1 to 2 mm) of the entire abdomen and pelvis.

• 3D reconstruction – images similar to colonoscopy;

• Polyp detection, characterization of lesion density and location.

• Evaluation of the extracolonic structures as well.

• Adequate bowel preparation + aeric distention of the colon = essential for an optimal examination.

• Native examination/ Intravenous contrast in symptomatic patients.

• No sedation or recovery time needed.

• Efficacy similar to colonoscopy for detection of polyps or CRC.

• Low radiation-dose CT colonography has similar test performance to colonoscopy for CRC.

Limitations:

• Limited efficiency in the identification of serrated or non-polypoid lesions.

• Dose or radiation.

• Pickhardt PJ, Mbah I, Pooler BD, et al. CT colonographic screening of patients with a family history of colorectal cancer: Comparison with adults at average risk and implications for guidelines. Am J Roentgenol 2017;208:794–800.

• Plumb AA, Halligan S, Nickerson C, et al. Use of CT colonography in the English Bowel Cancer Screening Programme. Gut 2014;63:964–73. doi:10.1136/gutjnl-2013-304697

Colonoscopy surveillance: advanced imaging techniques – Lynch Syndrome (LS)

• ESGE (European Society of Gastrointestinal Endoscopy) recommends that individuals with LS should be followed in dedicated units that practice monitoring of compliance and endoscopic performance measures.

• Strong recommendation, low quality evidence, level of agreement 100 %.

• ESGE suggests the use of chromoendoscopy may be of benefit in individuals with LS undergoing colonoscopy; however routine use must be balanced against costs, training, and practical considerations.

• Weak recommendation, moderate quality evidence, level of agreement 89 %.

Non-Invasive surveillance methods for people with FHCC

• There is insufficient evidence to recommend other methods of surveillance than colonoscopy for patients with familial CRC risk, such as MR or CT colonography.

• (GRADE of evidence: low; Strength of recommendation: strong) Consensus reached: 95% agreement.

Guidelines for the management of hereditary colorectal cancer from the British Society of Gastroenterology (BSG)/ Association of Coloproctologists of Great Britain and Ireland (ACPGBI)/United Kingdom Cancer Genetics Group (UKCGG), Friday, 04, October, 2019. Available at https://www.bsg.org.uk/resource/guidelines-for-the-management-of-hereditary-colorectal-cancer.html

Cross-sectional imaging in the management of hereditary risk of colon cancer

• Noninvasive methods based on cross-sectional imaging - **Magnetic resonance (MR)** and **Computed tomography (CT) colonography** - recommended for diagnosing colorectal cancer.

• A compliant systematic review and meta-analysis - MRC and CTC for diagnosing colorectal cancer were associated with higher sensitivity and specificity.

• Compared indirectly, MRC and CTC, CTC was found to be associated with higher positive likelihood ratio (PLR) and area under the receiver operating characteristic (ROC) for diagnosing colorectal cancer, compared with MRC.

• Moreover - sample size, mean age, and percentage of males – can influence PLR of MRC and CTC in diagnosing colorectal cancer.

• The impact of ionizing radiation should not be neglected for MRC and CTC.

The ratios of sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and receiver operating characteristic (ROC) curve were calculated to compare the diagnostic value of MRC versus CTC.

Sun S. et al, 2018

Take Home Message

✓ Colorectal cancer (CRC) is a multifactorial disease, inheritance and environment representing the most important factors.

✓ The diagnosis of Lynch syndrome, familial adenomatous polyposis, or another hereditary CRC associated syndrome can influence the management of patients with CRC and their related family members.

✓ Prompt and efficient identification of persons at risk for hereditary CRC syndromes can improve the prevention, diagnosis and therapy of this disease.

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II.9. Good clinical practice in the management of endocrine tumors and cancers

Learning objectives

- To decide which type of patient, according with clinical sign, personal and family medical history should be tested for hereditary endocrine tumors and cancer.
- To select the optimal hormonal, imagistic and genetic test for each type of syndrome/tumor.
- To select and evaluate the optimal treatment for each type syndrome/tumor.

Introduction

1. The diagnosis of hereditary endocrine tumors is very complex: genetic, hormonal and imagistic.

2. The multidisciplinary team (endocrinologist, oncologist, radiologist) is mandatory in the management of hereditary endocrine tumors.

3. The diagnostic and treatment are sometimes extremely difficult because the syndromic characteristic of endocrine tumors is not evident at the disease onset.

4. The follow-up in patients with hereditary endocrine tumors is permanent in order to have a prompt diagnosis of possible relapses.

MEN 1: characteristic tumours and associated genetic abnormalities

Type (chromosome location)	Tumours (estimated penetrance)	Gene, most frequently mutated codons
MEN1 (11q13)	Parathyroid adenoma (90 %) Enteropancreatic tumour (30-70%): gastrinoma (40%), insulinoma (10%), nonfunctioning and PPoma (20-55%), glucagonoma (<1%), VIPoma (<1%) Pituitary adenoma (30-40%): prolactinoma (20%), somatotropinoma (10%), corticotropinoma (<5%), nonfunctioning (<5%) Associated tumours: adrenal cortical tumour (40%), pheochromocytoma (<1%), bronchopulmonary NET (2%), thymic NET (2%), gastric NET (10%), lipomas (30%), angiofibromas (85%), collagenomas (70%), meningiomas (8%)	MEN1 83/84, 4-bp del (≈4%) 119, 3-bp del (≈3%) 209-211, 4-bp del (≈8%) 418, 3-bp del (≈4%) 514-516, del or ins (≈7%) Intron 4 ss (≈10%)

MEN1 diagnostic

The diagnosis is made according to the following criteria:

1. Association of two or more MEN1-associated tumours (parathyroid adenoma, entero-pancreatic tumour, pituitary tumour).

2. Occurrence of a MEN1-associated tumour in the relative of the 1st degree of a patient with clinical MEN1.

Identification of a MEN1 germline mutation in a patient who may be asymptomatic and has no suggestive biological or imaging changes for tutor syndrome.

MEN1 diagnosis (genetic testing)

Genetic testing for germline mutation gene MEN1 is indicated for:

1. All subjects meeting the criteria of MEN1

2. Suspicion of MEN1 (multiple parathyroid adenomas before the age of 40, recurrent hyperparathyroidism, gastrinomas or multiple pancreatic neuroendocrine tumours at any age).

3. Atypical MEN1 (concurrent parathyroid and adrenal tumours).

4. Their relatives of 1st degree (with or without manifestations of MEN1).

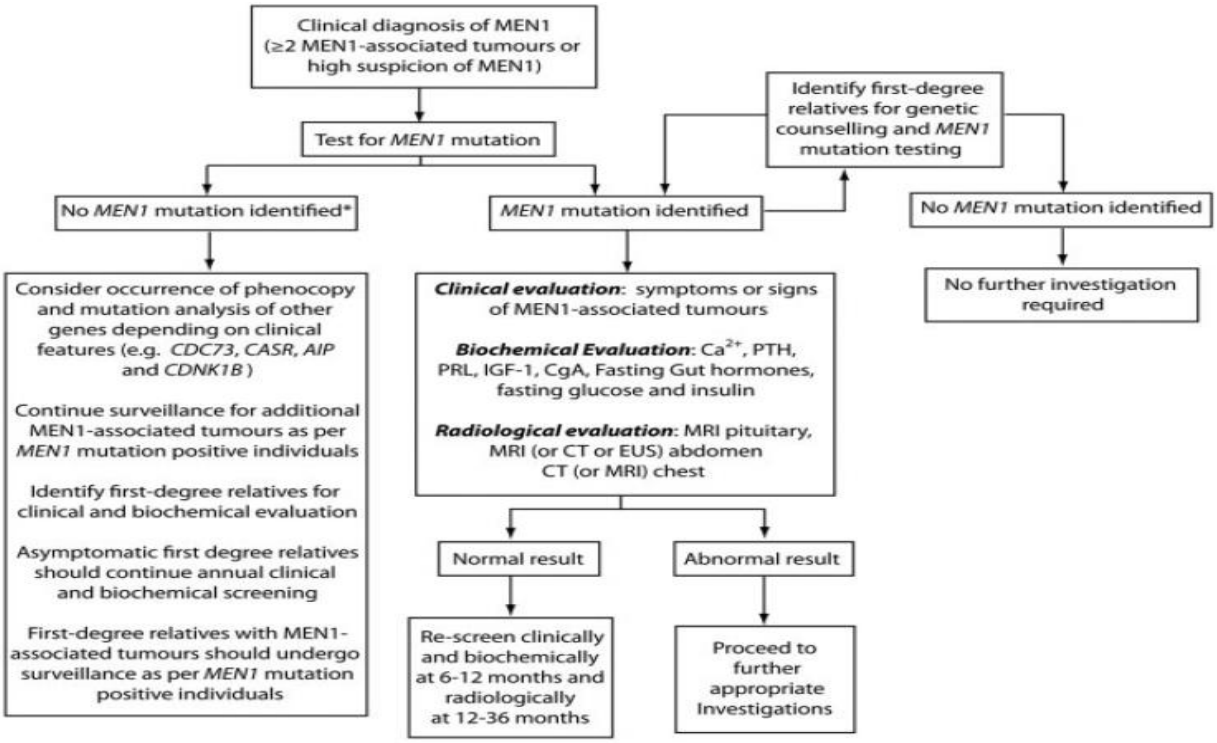
5. Testing should be done as early as possible: before the age of 5 or 10 (depending of the authors) for asymptomatic patients.

Screening in MEN1 according to age and affected organ

Tumour (estimated frequency)	Age to begin screening (yrs)
Parathyroid adenoma (90%)	10
Gastrinoma (40%)	10
Insulinoma (10-30%)	10
Other enteropancreatic tumours (30-70 %)	10
Anterior pituitary tumours (30-40%)	10
Thymic and bronchial carcinoids (~3%)	18
Adrenal lesions (20%)	10

Source: https://eced.squarespace.com/s/2017-02-01-MEN1-Screening.docx

An approach to screening in MEN1



Thakker RV et al. (2012)

Screening in MEN1 - The test for MEN1 mutation is indicated in clinical diagnosis of MEN1 or high suspicion of MEN1

No MEN1 mutation present:	MEN1 mutation identified:
1. Consider analysis of other genes depending of clinical features (CDC73, CASR, AIP, CNDK18). 2. Continue surveillance for additional MEN1-associated tumors. 3. Identify first-degree relatives for clinical and biochemical evaluation. 4. Annual clinical and biochemical screening for asymptomatic firs-degree relatives. First degree relatives with MEN1 associated tumors will be monitored as per MEN1 positive patients.	1. Identify first-degree relatives for genetic counselling and MEN1 mutation testing. 2. If the results of clinical, biochemical and radiological evaluation are normal, re-screen clinically and biochemically at 6-12 months and radiologically at 12-36 months. If abnormal results are present, is necessary to proceed to further appropriate investigations.

MEN 1: hyperparathyroidism diagnostic

• calcium dosage (adjusted for albumin)

• parathormone dosage (PTH)

Cervical ultrasound

- high-frequency linear transducers;

- Search for pathological parathyroid tissue (well-circumscribed, oval or oblong mass, hypoechoic compared with the adjacent thyroid gland) along the posterior margin of the thyroid lobes or near their lower poles.

99mTc-sestamibi SPECT or SPECT/CT

• intravenous administration of technetium (99mTc) sestamibi;

• Images acquired with a gamma camera 10 min (early phase) and 120 min (late phase) after tracer injection.

• There is a different tracer washout of the hyperactive parathyroid tissues compared with the thyroid (the uptake becomes increasingly evident during the late phase);

MRI/ CT

• second-line localization methods, especially for ectopic parathyroid (retropharyngeal , mediastinum);

• most parathyroid adenomas (92.7%) are hyper-enhancing.

MEN 1: diagnosis of pancreatic neuroendocrine tumours, pulmonary, thymic and gastric

Name	Biologically active peptide(s) secreted	Incidence per million per year	% MEN1	Main symptoms/signs
Insulinoma	Insulin	1-3	4-5	Hypoglycaemic symptoms (100%)
ZES	Gastrin	0.5-2	20-25	Pain (79-100%) Diarrhoea (30-75%) Oesophageal symptoms (31-56%)
VIPoma	VIP	0.05-0.2	6	Diarrhoea (90-100%) Hypokalaemia (80-100%) Dehydration (83%)
Glucagonoma	Glucagon	0.01-0.1	1-20	Rash (67-90%) Glucose intolerance (38-87%) Weight loss (66-96%)
GRHoma	GHRH	Unknown	16	Acromegaly (100%)
Somtatostatinomas	Somatatostatin	Rare	45	Diabetes (63-90%) Cholelithiasis (65-90%) Diarrhoea (35-90%)

VIP vasoactive intestine peptide; GHRH growth hormone releasing hormone

Source: https://eced.squarespace.com/s/2017-02-01-MEN1-Screening.docx

Screening for pancreatic neuroendocrine tumors includes biological balance (hormonal profile: gastrin, glucagon, vaso-intestinal peptide, pancreatic polypeptide, chromogranin A, insulin).

Pancreatic tumours can be visualized with nuclear magnetic resonance imaging, computerized tomography or endoscopic ultrasound.

CT or thoracic MRI to detect lung and thymic tumors. Gastric endoscopy with biopsy (in those with hypergastrinemia) to detect peptic peptic ulcer and gastric carcinoid type II.

Scintigraphy with somatostatin

MEN 1: pituitary adenoma diagnostic

• Exploration to detect secretory pituitary tumours includes hormonal balance (prolactin, IGF1) which also adapts to the clinical picture.

• Imagistically, the nuclear magnetic resonance, especially if there are hormonal and clinical arguments (headaches, vision disorders, clinical signs suggestive of pituitary dysfunction).

• Hormonal tests can be extended if a pituitary tumour is identified (secreting or not) in order to exclude a possible pituitary insufficiency.

MEN1 - pituitary tumour - imaging

MRI:

- the fundamental preoperative and postoperative imaging modality.

- sagittal and coronal images - small field of view; thin sections (≤ 3 mm);

Post-gadolinium enhanced sequences are obtained with fat saturation to improve contrast between pathology and the basi-cranium.

MEN 1: Pituitary microadenoma- imaging

• T1: iso (10%) or slightly hypointense

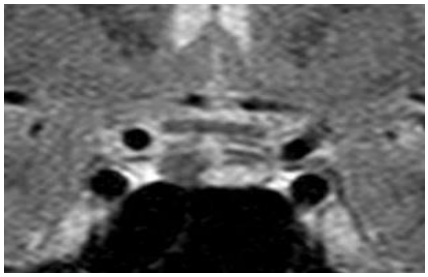
• T2: iso/slightly hypo (GH)/slightly hyperintense

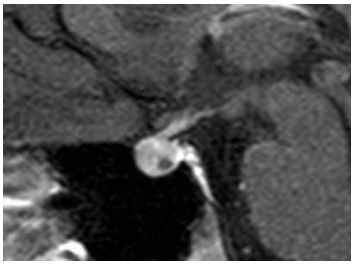
• T1 +C: hypointense early/ hyperintense delayed – precise location in surgical candidates

• Rare – necrosis (hypointense T1, hyperintense T2)

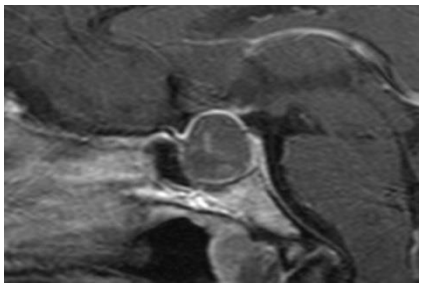
• Indirect signs: deviation of the infundibulum, asymmetric convexity, mild down-sloping of the roof of the sphenoid sinus

• Differential diagnosis: Rathke's cleft cyst

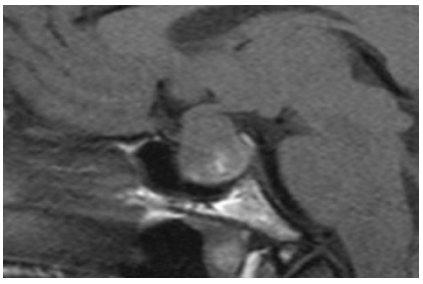


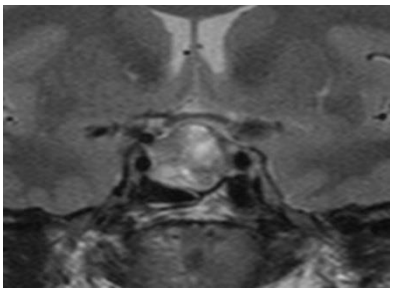


MEN 1: Pituitary macroadenoma- imaging



Solid, soft (indent at the diaphragma sellae), with necrosis and hemorrhage, first expand the sella, then grow upwards;
T1: iso or hypointense/heterogeneous
T2: iso/slightly hypo (GH)/hyperintense
T1 +C: intense enhancement



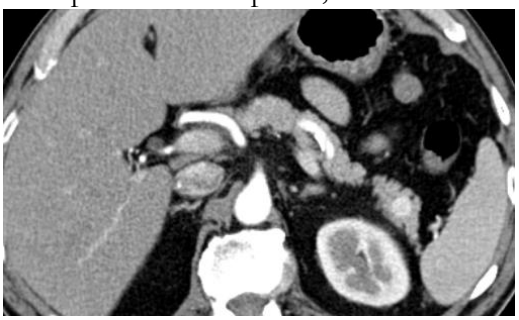


Necrosis (cystic degeneration):
T1: hypo, iso or hyperintense
T2: hyperintense +++
T1 +C: ring enhancement

Haemorrhage:
T1- hyperintense/blood-fluid level

MEN 1: diagnosis of pancreatic neuroendocrine tumours - imaging

- Imaging pancreatic neuroendocrine tumours:
- MDCT: multiphasic: unenhanced (to identify calcifications or hemorrhage) + iodinated intravenous contrast at 4-5 mL per second + arterial late phase + portal venous phase; slice thickness of 2-3 mm for diagnostic review and at 0.625mm images for coronal and sagittal multiplanar reconstructions;



- lesions are hyper-vascularized compared to pancreatic tissue in late arterial phase; mean sensitivity of 73% and specificity of 96%.
- Gastrinomas are the most common functioning pancreatic neuroendocrine tumor in the MEN 1 syndrome, and they are frequently manifested by multifocal duodenal involvement.

• Nuclear studies - 111In-octreotide (somatostatin analogue) SPECT; sensitivity for PNET of approximately 70-90%.

FDG PET/Ct is used as a complementary technique, since PNET's don't typically demonstrate sufficient uptake unless they are poorly differentiated;

New PET/CT agents: gallium labeled somatostatin analogs: DOTA-TOC; DOTA-NOC; DOTATATE, GaTate);

• Endoscopic ultrasound

- mean detection rate for neuroendocrine tumors of 90%.

Tests schedule in MEN1

Tumour (estimated frequency)	Annual biochemical tests	Imaging tests
Parathyroid adenoma (90%)	Calcium (esp. iCa^{2+}) PTH	Neck US and sestamibi, if calcium elevated and surgery is proposed
Gastrinoma (40%)	None, unless clinical suspicion or imaging identifies tumour(s)	2 yearly MRI abdomen*
Insulinoma (10-30%)	None, unless clinical suspicion or imaging identifies tumour(s)	2 yearly MRI abdomen*
Other entero-pancreatic tumours (30-70%)	None, unless clinical suspicion or imaging identifies tumour(s)	2 yearly MRI abdomen*
Anterior pituitary tumours (30-40%)	Prolactin, IGF1	2 yearly non-contrast MRI pituitary*
Thymic and bronchial carcinoids (~3%)	None (typically non-secretory, but have malignant potential)	Low dose CT chest at age 18 or at time of diagnosis (if later) Low dose CT chest age 40 2. yearly MRI chest* when CT chest not performed
Adrenal lesions (~20%)	None unless symptoms develop or identified tumour > 1 Renin, aldosterone, U&Es 24hr UFC, overnight dexamethasone suppression test 24 urinary metanephrines Total testosterone DHEA-S	2 yearly MRI abdomen*

Source: https://eced.squarespace.com/s/2017-02-01-MEN1-Screening.docx

MEN2: characteristic tumors and associated genetic abnormalities

MEN2 (10 cen-10q11.2)		
MEN2A	MTC (90%)	RET
	Pheochromocytoma (50%)	634, missense
	Parathyroid adenoma (20-30%)	e.g. Cys → Arg (~85%)
MTC only	MTC (100%)	RET
		618, missense (>50%)
MEN2B (also known as MEN3)	MTC (>90%)	RET
	Pheochromocytoma (40-50%)	918, MET→ Thr (>95%)
	Associated abnormalities (40-50%)	
	Mucosal neuromas	
	Marfanoid habitus	
	Medullated corneal nerve fibres	
	Megacolon	

Source: Thakker RV et al. (2012)

Genotype to phenotype correlation. ATA risk classification in MEN2

ATA risk	Mutation	MEN subtype	Phenotype
A	G321R[106, 107]	MEN2A, FMTC	MTC
	531/9 base pair duplication[106]	MEN2A, FMTC	MTC
	532 duplication[109]	MEN2A, FMTC	MTC
	C515S[110]	MEN2A, FMTC	MTC
	G533C[111,112]	MEN2A, FMTC	MTC, PHE
	R600Q[113, 114]	MEN2A, FMTC	MTC
	K603E[115]	MEN2A, FMTC	MTC
	Y606C[107, 116]	MEN2A, FMTC	MTC
	635/insertion ELCR; T636P[107]	MEN2A, FMTC	MTC, PHE
	S646L[117, 110]	MEN2A, FMTC	MTC, HPT
	K666E[107]	MEN2A, FMTC	MTC, PHE
	E768D[119]	MEN2A, FMTC	MTC, PHE, HPT
	N777S[120]	MEN2A, FMTC	MTC
	L790F[121]	MEN2A, FMTC	MTC, PHE, HPT
	Y791F[121]	MEN2A, FMTC	MTC, PHE, HPT
	V804L[119]	MEN2A, FMTC	MTC, PHE, HPT
	V804M[119]	MEN2A, FMTC	MTC, PHE, HPT
	G819K[117]	MEN2A, FMTC	MTC
	R833C[122]	MEN2A, FMTC	MTC
	R844Q[123]	MEN2A, FMTC	MTC
	R866W[124]	MEN2A, FMTC	MTC
	S891A[117, 125, 126]	MEN2A, FMTC	MTC, PHE, HPT
	R912P[117]	MEN2A, FMTC	MTC
B	C609F/R/G/S/Y[119]	MEN2A, FMTC	MTC, PHE, HPT, HSC
	C611R/G/F/S/W/Y[119]	MEN2A, FMTC	MTC, PHE, HPT, HSC
	C618R/G/F/S/Y[119]	MEN2A, FMTC	MTC, PHE, HPT, HSC
	C620R/G/F/S/W/Y[119]	MEN2A, FMTC	MTC, PHE, HPT, HSC
	C830R/F/S/Y[127]	MEN2A, FMTC	MTC, PHE, HPT
	D631Y[123]	MEN2A, FMTC	MTC
	633/9 base pair duplication[120]	MEN2A, FMTC	MTC, HPT
	634/12 base pair duplication[129]	MEN2A, FMTC	MTC, HPT

	V804M+V7781[120]	MEN2A, FMTC	MTC
C	C834R131	MEN2A	MTC, PHE, HPT, CLA
	C624G/F/S/W/Y[121]	MEN2A, FMTC	MTC, PHE, HPT, CLA
D	V804M+E805K[122]	MEN2B	MTC, PHE
	V804M+Y806C[122]	MEN2B	MTC, PHE
	V804M+S904C[124]	MEN2B	MTC, PHE
	A883F[125,126]	MEN2B	MTC, PHE
	M918T[119]	MEN2B	MTC, PHE

Source: Krampitz and Norton (2014)

Diagnostic MEN2

- Genetic testing to identify specific mutations
- In those with identified mutations it is necessary to perform a biological profile:

1. Calcitonin dosage (thyroid medullary carcinoma);
2. Calcium and parathormone (PTH) dosage (primary hyperparathyroidism);
3. Methanephrine dosage and urinary catecholamines (pheochromocytoma).

Genetic exam - oncogene RET

• Genetic testing for detecting mutations of RET oncogene is performed at: relatives of 1st degree of the index patient, parents of children with classic MEN2B phenotype, patients with cutaneous amyloidosis, infants or young children with Hirschsprung disease, patients with medullary carcinoma

• Initial testing for MEN2A: exon 10 (codons 609, 611, 618, and 620), exon 11 (codons 630 and 634), exons 8, 13, 14, 15 and 16.

• Initial testing for MEN2B: exon 16 (codon M918T), if mutation is absent exon 15 (codon A883F) is tested. If these two mutations are not identified, the entire RET region must be investigated.

• Depending on the mutations identified, patients can be classified into 4 risk categories: highest risk **D** (M918T + all MEN2B mutations) and lowest risk **A**.

MTC diagnosis

• Secreted thyroid C cells: ACTH, MSH, chromogranin, neurotensin, calcitonin, carcinoembryonic antigen (CEA).

• Of all these calcitonin and CEA secretion products, they are the most valuable tumor markers for thyroid medullary carcinoma.

• Their serum concentration is directly related to the tumor cell mass of C, the dosage being useful both as a diagnostic method and as a means of tracking the relapses.

• Calcitonin values> 100 pg/ml has a positive predictive value of 100% for thyroid medullary carcinoma.

1. Thyroid ultrasound with fine needle biopsy aspiration for nodules selected according to TI-RADS (thyroid imaging reporting and data system) criteria.

2. If the biopsy result is not conclusive or only suggestive for MTC, calcitonin and aspirate dosing and even immunohistochemistry can be performed for markers of the type: calcitonin, ACE, chromogranin.

3. In the patient with the thyroid nodule with histological result (following the fine needle puncture) of thyroid medullary carcinoma and increased calcitonin values is indicated for germline mutation RET.

4. For those with mutation (-), strictly follow the explorations necessary for the surgical treatment.

5. In those with mutation (+), scans are performed for pheochromocytoma and primary hyperparathyroidism.

Diagnosis test in MTC according to ATA risk level

ATA Risk Level	Genetic Testing	Neck Ultrasound	Serum Calcitonin	Thyroidectomy
A	< 3-5 y	> 3-5 y	> 3-5 y	May delay beyond age 5 if normal annual calcitonin and neck ultrasound, indolent MTC history, family preference
B	< 3-5 y	> 3-5 y	> 3-5 y	Consider before age 5
C	< 3-5 y	> 3-5 y	> 3-5 y	Before age 5
D	Immediately	Immediately	Immediately	Immediately

Source: Krampitz and Norton (2014)

Pheochromocytoma diagnosis

• Testing for a possible pheochromocytoma should be performed in patients with MTC.

• Regardless of whether MEN2A or MEN2B, the diagnosis of a possible pheochromocytoma is very important before any therapeutic manoeuvre on the endocrine tumours associated in the syndrome.

• Dosage of plasma metanephrines or fractionated urinary metanephrines.

• For localization, it is recommended to perform computer tomography, except in cases where this method is contraindicated when nuclear magnetic resonance is recommended.

• Iod123-metaiodobenzylguanidine (MIBG) scintigraphy is recommended in patients with metastatic pheochromocytoma or prior to I-MIBEG radiotherapy.

• 18F-fluorodeoxyglucose (18FFDG) positron emission tomography (PET)/CT scanning in patients with metastatic pheochromocytoma.

Differential diagnosis of syndromic pheochromocytoma

DISORDER	GENE	CLINICAL FEATURES
Neurofibromatosis type 1	NF1	Café au lait macules Axillary & inguinal freckling Neurofibromas
Von Hippel–Lindau disease (VHL)	VHL	Hemangioblastomas Renal, pancreatic, epididymal, & broad ligament cysts Renal cell carcinoma Pancreatic neuroendocrine tumors
SDHX-relaxed syndromes	SDHD (PGL1), SDHB (PGL4), SDHC (PLG3), SDHAF2 (PGL2), SDHA (PLG5)	Head and neck, thoracic, abdominal paragangliomas Pituitary adenoma Papillary thyroid carcinoma Renal cell carcinoma Gastrointestinal stromal tumors (GIST)

Pheochromocytima - Imaging

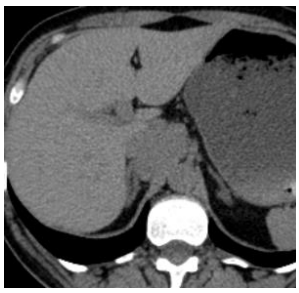
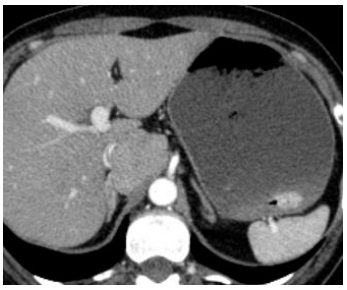
• Multi-detector computer tomography (MDCT): localization and characterization of the lesion

• Non-enhanced CT:

- varied appearance: solid/ complex or cystic.
- hemorrhage increase the density;
- calcifications in 10% of pheochromocytomas

Contrast-enhanced CT: homogeneous or variable/ avid enhancement of the solid components

Contrast washout in pheochromocytomas may overlap with both benign lesions, such as adenomas, and malignant lesions.

MRI

• On T2-weighted imaging: a "light-bulb" bright lesion comparable to the signal intensity of CSF - classic appearance only in 11% to 65% of pheochromocytomas.

- 35% of cases - low signal intensity.

• On T1-weighted imaging: isointense to muscle and hypointense to liver;

• Rare: microscopic fat resulting in signal loss on chemical-shift MRI (dual-echo in and opposed-phase T1-weighted imaging), mimicking adenomas.

• On contrast-enhanced sequences: avid gadolinium enhancement/ variable depending on the presence of cystic-necrotic areas.

• **^{131}I-MIBG/ 123 Iod-metaiodobenzylguanidine (MIBG) scintigraphy** = functional imaging; recommended in metastatic pheochromocytoma or prior to I-MIBEG radiotherapy.

• MIBG = norepinephrine analog that localizes first to presynaptic adrenergic nerves and sympathomedullary tissue and then into cytoplasmic storage vesicles.

• The uptake is proportional to the number of neurosecretory granules within the tumor => the characteristic appearance of a pheochromocytoma is unilateral focal uptake.

• The sensitivity of ^{123}I MIBG range from 77% to 90% with a specificity of 95–100%.

PET/CT

• **^{18}F-Fluorodopamine, ^{18}F-Dihydroxyphenylalanine** DOPA (a precursor to dopamine) - high sensitivity for metastatic disease (up to 100%). Fluorine-18-fluorodopamine has a sensitivity of up to 100% for primary pheochromocytomas and has depicted pheochromocytomas that yielded negative findings on MIBG imaging.

• **^{18}F-FDGFDG** (a glucose analog that becomes trapped within cells) is used for PET-CT, in distinguishing between benign and malignant adrenal lesions.

• 68-Ga-DOTATATE PET CT is a more sensitive modality to detect somatostatin receptor positive disease, especially in individuals with metastatic disease.

• Octreotide scintigraphy, a technique that measures tumor uptake of a somatostatin analog radioisotope, may be used in addition to MIBG scintigraphy as some MIBG-negative tumors are positive with octreotide scintigraphy. The sensitivity is fairly low. Octreotide scintigraphy has been largely replaced by 68-Ga-DOTATATE PET CT, where available, because of the significantly higher sensitivity.

Treatment of pancreatic tumours

• In the case of functional pancreatic tumours, symptomatic surgery is recommended when possible.

• For improvement of clinical symptoms (hyperacidity, diarrhea), proton pump inhibitors (somatostatin analogues) are recommended.

• Surgical treatment in non-functional pancreatic tumours is controversial, being recommended only in the case of tumours over 1 cm and/or with significant growth within 6-12 months.

• In the case of non-resectable tumours, somatostatin analogues, biological therapy, chemotherapy, radiotherapy are recommended.

• Sunitinib and everolimus are indicated in inoperable or metastatic forms of well differentiated pancreatic tumours.

Treatment of neuroendocrine pulmonary, thymic and gastric tumours

• Surgical treatment (when possible) for bronchial carcinoid tumours and thymus tumours.

• When the disease is advanced, chemotherapy and radiotherapy can be used.

• In the case of gastric carcinoids of type II if they are below 10 mm endoscopic is monitored and the largest ones require endoscopic resection with total or partial gastrectomy.

Treatment of pituitary tumors

• Resection of pituitary adenoma through transsphenoidal pituitary surgery

• Gamma Knife Radiotherapy

• Dopaminergic agonists used in the medical treatment of prolactinomas, somatostatin analogue in somatotropin adenoma.

MTC treatment

- Surgical treatment (total thyroidectomy) with metastasis resection depending on the extent of the disease.

- Prophylactic thyroidectomy is performed in patients at extremely high risk in the first year of life, in those at increased risk before the age of 5 and for those at moderate risk when calcitonin levels start to rise.

- After total thyroidectomy, thyroid hormone replacement treatment (levothyroxine).

- In cases of advanced disease: systemic therapy with tyrosine kinase inhibitors vandetanib or cabozantinib.

Prophylactic thyroidectomy for MTC

NANETS Risk Level for MTC	Most common codon mutations	Age at Prophylactic thyroidectomy
Level 1 (High)	609	By 5-10 y of age
	630	
	768	
	790	
	791	
	804	
	891	
Level 2 (Higher)	611	By 5 y of age
	618	
	620	
	634	
Level 3 (Highest)	883	Within the first 6 mo of life (preferably in the first month of life)

ATA Risk Level	Thyroidectomy
A	May delay beyond age 5 if normal annual calcitonin and neck ultrasound, indolent MTC history, family preference.
B	Consider before age 5
C	Before age 5
D	Immediately

Krampitz & Norton, Cancer 2014; 120:1920–31.

Pheochromocytoma treatment

• Surgical treatment that must precede surgical treatment of MTC or hyperparathyroidism.

• Subtotal adrenalectomy for preservation of adrenal function and avoidance of adrenal cortical insufficiency requiring replacement therapy.

• In bilateral forms, bilateral subtotal adenalectomy will be performed (as far as possible).

• In the event of the installation of the cortico-adrenal insufficiency, substitution treatment with acetate hydrocortisone and fludrocortisone is instituted.

Treatment in PHEO/PGL

	Scenario	**Intervention**
Staging and blockade	Elevated plasma or urine normetanephrine and/or metanephrine	a. alpha-adrenoceptor blocker, eg. doxazosin 1-2 mg, increase 2-4 mg weekly to maximum tolerated dosage for ≤ 30 mg/d b. Localisation studies CT, MRI or PET/CT.
Localised stage	Thoracic or absominal/pelvic	Curative resection, if safe
	HN	Surgery, external beam radiation, locoregional therapy, or watchful waiting. If not possible, follow algorithm for malignant disease 5.3.1
Metastatic stage	Elevated plasma or urine normetanephrine and/or metanephrine	a. Palliative doxazosin 1-2 mg, increase 2-4 mg weekly. Balance maximum tolerated dosage to quality of life.
	Confined disease	b. Before start of any treatment, doxazosin according to 1.1. Surgery, external radiation, or locoregional therapy if safe and with acceptable morbidity. If not, proceed to 3.3.1
	Disseminated disease	Medical treatment to alleviate hormone or mass effect alternatively at disease progression. Perform ^{123}I-MIBG scintigraphy and ^{68}Ga-DOTATATE PET/CT
	First-line ^{131}I-MIBG or ^{68}Ga-DOTATATE positive [a]	^{123}I-MIBG = ^{68}Ga- DOTATATE choose ^{131}I-MIBG. ^{123}I-MIBG > ^{68}Ga- DOTATATE choose ^{131}I-MIBG ^{123}I-MIBG < ^{68}Ga- DOTATATE choose ^{177}Lu- DOTATATE
	Second-line or first-line ^{123}I-MIBG/^{68}Ga- DOTATATE	Priority I. Re-challenge ^{123}I-MIBG or ^{68}Ga- DOTATATE Priority II. CVD, [a] if WHO performance status > 1 or wish for non-hospitalization, proceed to 3.5.3. Priority III. Temozolomide. Tyrosine kinase inhibitor or experimental therapy.

Source: Crona J et al. Endocr (2017)

Treatment of hyperparatiroidism

The surgical options include:

• exploration and removal of all 4 parathyroid glands

• 3.5 gland parathyroidectomy

• removal of ipsilateral glands if a single abnormal gland is seen in imaging

The pros and cons of each approach should be discussed with the patient

Concurrent thymectomy should be considered at the time of parathyroid surgery, especially in men and those with a family history of thymic carcinoid.

Take home message

• Tumoral markers in hereditary endocrine tumors are very useful in diagnostic and further for the therapy follow-up.

• For each type of tumor is recommended to test the hormone or hormones secreted by the specific endocrine gland involved.

• The imagistic tests are necessary for the correct diagnostic and vary from the simple ultrasound with fine needle biopsy aspiration (as in MTC) to CT-scan, IRM, MIBG or FDG-PET (as in malignant pheochromocytoma).

• Besides the surgical removal, the therapy must block the hormonal hypersecretion by medical resources.

• The therapy prioritization in the case of multiple tumors is mandatory especially in syndromic pheochromocytoma.

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II.10. Good clinical practice in the management of rare hereditary syndromes (like Li Fraumeni)

Learning objectives

• learn about the best clinical practice in the multidisciplinary management of rare hereditary cancer syndromes (RHS)

• understand the molecular basis and heterogeneity of the genetic susceptibility to develop cancer Rare Genetic Disorders: Learning About Genetic Disease Through Gene Mapping,

• be familiar with the main hereditary cancer syndromes and refer patients to specialised cancer genetic units for adequate genetic counselling and to address specific concerns associated to each genetic susceptibility.

• learn how to recognize, diagnose, treat and provide prevention recommendations to patients with a germline genetic susceptibility:

- Li Fraumeni syndrome
- Cowden disease
- Peutz-Jegers syndrome
- Von Hippel Lindau syndrome

Introduction

• A cancer syndrome or family cancer syndrome is a genetic disorder in which inherited genetic mutations in one or more genes predispose the affected individuals to the development of cancers and may also cause the early onset of these cancers.

• Cancer syndromes often show not only a high lifetime risk of developing cancer, but also the development of multiple independent primary tumors.

• Many of these syndromes are caused by mutations in tumor suppressor genes, genes that are involved in protecting the cell from turning cancerous. Other genes that may be affected are DNA repair genes, oncogenes and genes involved in the production of blood vessels (angiogenesis).

• Common examples of inherited cancer syndromes are: Li Fraumeni syndrome, Cowden disease, Peutz-Jegers syndrome and Von Hippel Lindau syndrome.

Li-Fraumeni syndrome

• Li-Fraumeni syndrome (LFS) is an inherited condition that is characterized by an increased risk for certain types of cancer.

• Affected people often develop cancer at an earlier age than expected and may be diagnosed with more than one cancer during their lifetime.

• LFS is primarily associated with sarcomas (cancers of muscle, bone or connective tissue), breast cancer, brain tumors, leukemia and adrenocortical carcinoma; however, many other types of cancer have been reported in people with this condition.

• It is caused by changes (mutations) in the *TP53* gene and is inherited in an autosomal dominant manner. Management may include high-risk cancer screening and/or prophylactic surgery.

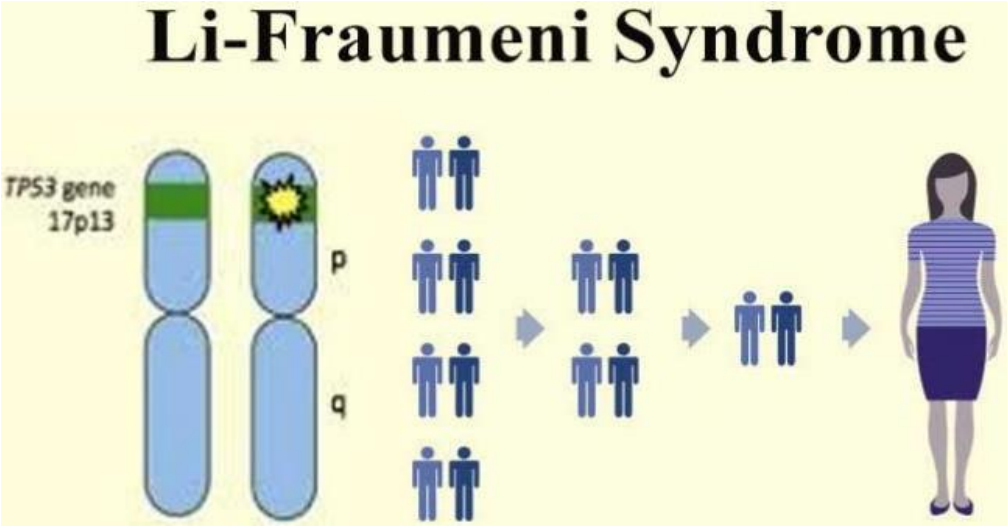
• Individuals with LFS have an approximately 50% risk of developing cancer by age 40, and up to a 90% percent chance by age 60, while females have nearly a 100% risk of developing cancer in their lifetime due to their markedly increased risk of breast cancer. Many individuals with LFS develop two or more primary cancers over their lifetimes.

How is LFS inherited?

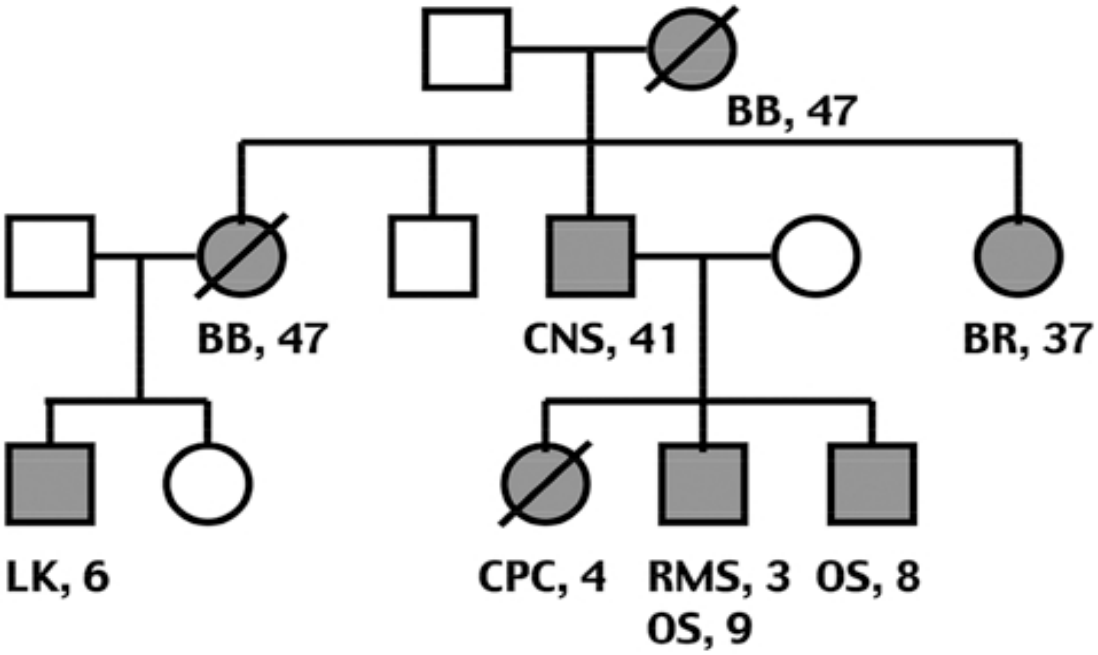
• Normally, every cell has 2 copies of each gene: 1 inherited from the mother and 1 inherited from the father. LFS follows an autosomal dominant inheritance pattern. That means that even if a mutation happens in only 1 of the 2 copies of the TP53 gene, that person will have LFS.

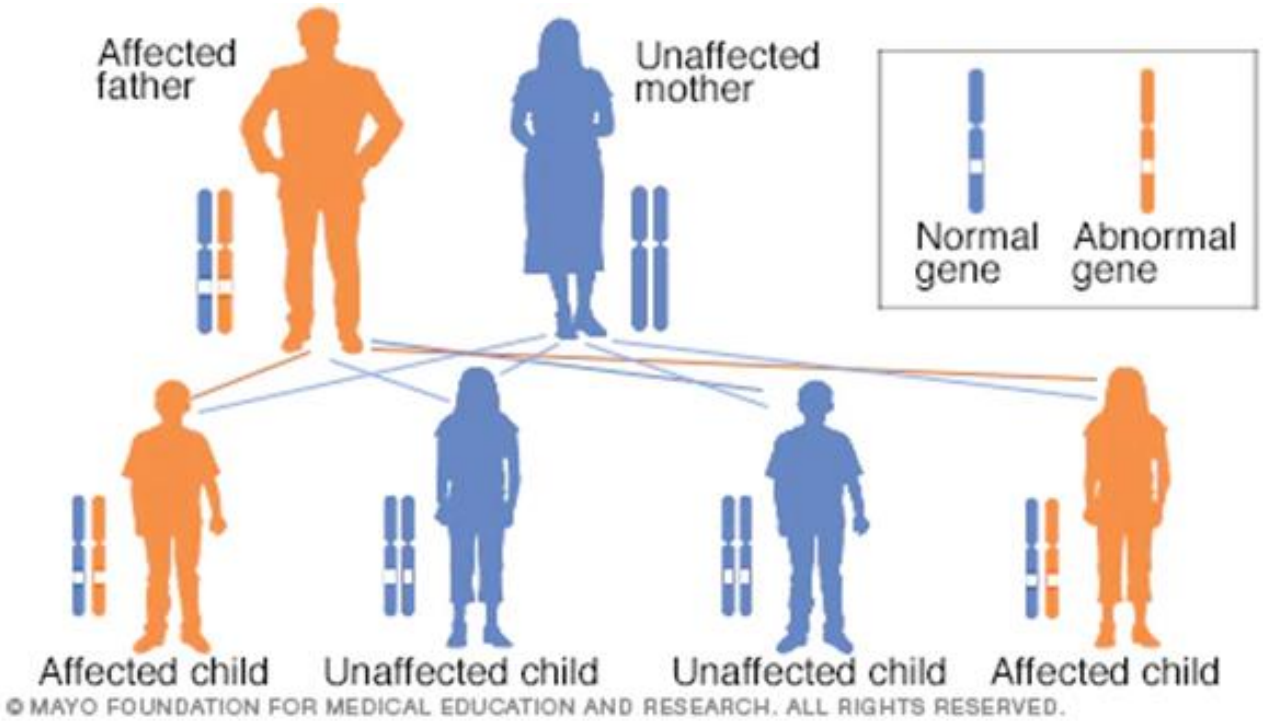
• Most people with LFS have 1 normal copy of TP53 and 1 mutated (altered) copy of TP53, most often because they have inherited the mutated copy of TP53 from a parent who was also affected by LFS. However, it is estimated that 25% of people with LFS do not have any family history of the condition; they have a de novo (new) mutation in the TP53 gene.

• Regardless of whether a person inherits a mutation or the mutation occurs for the first time in a person, that person has a 50% chance of passing on the normal copy of the TP53 gene and a 50% chance of passing on the mutated copy of the gene to his/her child. A brother, sister, or parent of a person who has a mutation also has a 50% chance of having the same mutation.



Li Fraumeni-autosomal dominance inheritance





Pedigree of a family with Li-Fraumeni syndrome. Filled circles/squares represent affected members; slashes represent deceased family members. Numbers represent age at diagnosis.

BB = bilateral breast cancer;
CNS = brain tumor;
BR = unilateral breast cancer;
LK = leukemia;
CPC = choroid plexus carcinoma;
RMS = rhabdomyosarcoma;
OS = osteosarcoma.

How is LFS inherited?

• Approximately 80% of families with the features of Li-Fraumeni Syndrome (LFS) have an identifiable change (mutation) in the *TP53* gene

• *P53* is a tumor suppressor gene, which means that it encodes a protein that stops cells from growing and dividing too rapidly or in an uncontrolled way.

• Mutations in *TP53* result in a defective protein that is unable to carry out its normal role. This contributes to the development of the many different types of tumors found in LFS.

Signs and symptoms

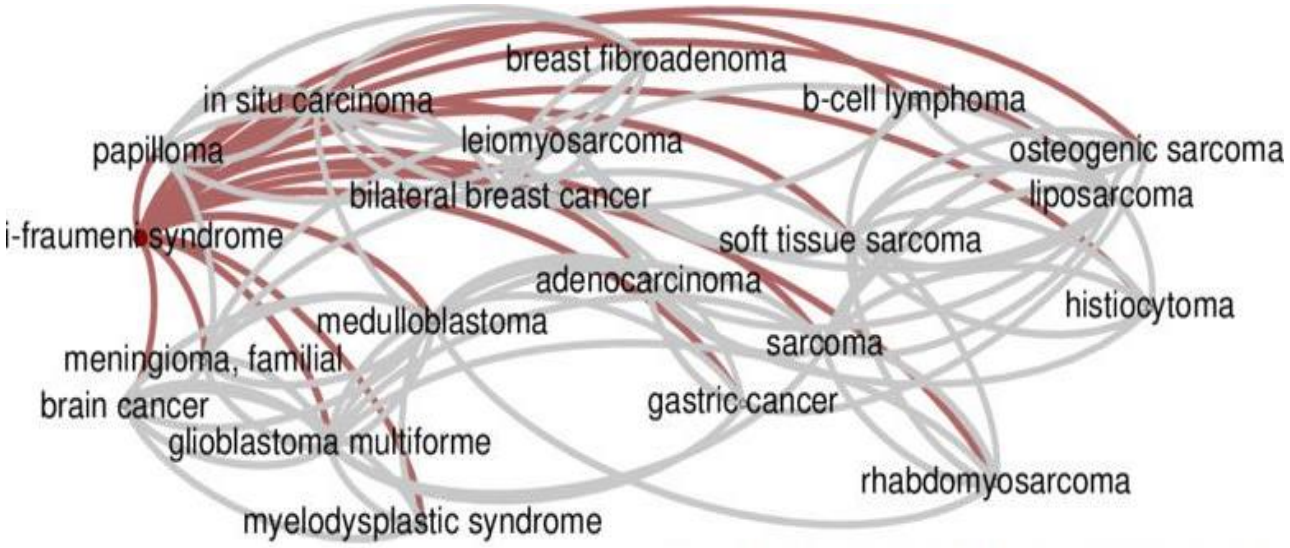
• LFS may be suspected if someone has a personal or family history of cancers featured in LFS.

• There are certain rare cancers that are characteristic of the syndrome that should alert clinicians to the potential of a diagnosis of LFS.

• Cancers most closely associated (core cancers) with LFS include:

- Soft tissue sarcoma, lung adenocarcinoma, thyroid, gonadal germ cells (ovarian, testicular), prostate
- Osteosarcoma, melanoma, kidney, adrenal carcinoma
- Breast cancer, gastro-intestinal tumours (colon, pancreas)
- Brain and CNS tumours (glioma, choroid plexus carcinoma, SHH subtype medulloblastoma, neuroblastoma), acute leukemia.

Li Fraumeni syndrome include also core cancer such as renal cancer, prostate cancer, thyroid…



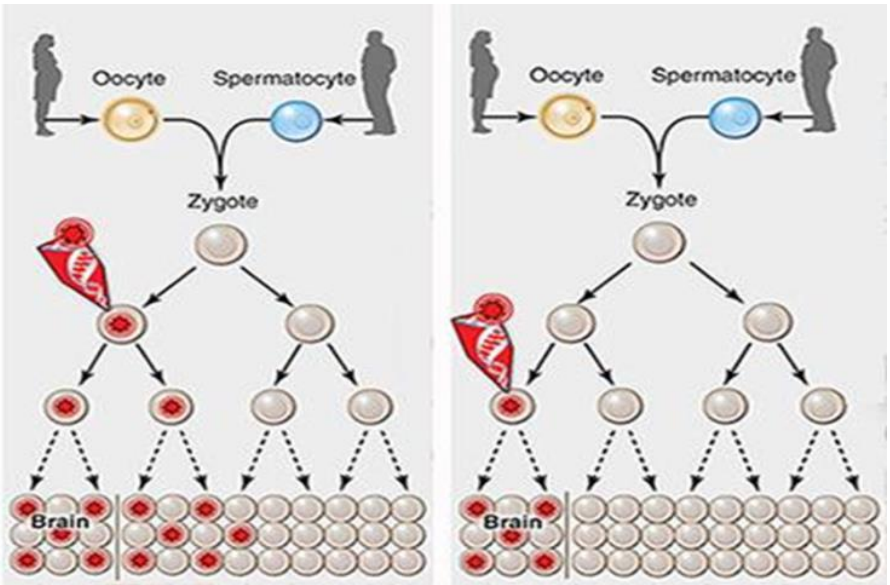
Li Fraumeni is associated very frequently with mosaicism

• In genetics, a mosaic, or mosaicism, involves the presence of two or more populations of cells with different genotypes in one individual who has developed from a fertilised egg. Mosaicism has been reported to be present in as high as 70% of cleavage-stage embryos and 90% of blastocyst-stage embryos derived from in vitro fertilization.

• Genetic mosaicism can result from many different mechanisms including chromosome nondisjunction, anaphase lag, and endoreplication.

• The most common form of mosaicism found through prenatal diagnosis involves **trisomies.**

• Mosaicism: test of parents and sibling is not useful but test of children of mutation carrier is mandatory (and pre-implantary diagnosis needs to be proposed).



Li-Fraumeni syndrome - criteria for diagnosis

• Li-Fraumeni syndrome associates: sarcomas, osteosarcomas, tumors brain tumors, breast, leukemia, and adrenal carcinomas.

• This cancer predisposition syndrome is inherited as an autosomal dominant disorder and is associated with abnormalities in the p53 protein (TP53). Responsible for ~ 1% breast cancers.

Table 2 – Clinical criteria for Li-Fraumeni like syndrome (Birch)

Proband with any childhood cancer or sarcoma, brain tumour, or adrenocortical tumour diagnosed before 45 years of age

AND

First- or second-degree relative with a LFS cancer (sarcoma, breast cancer, brain tumour, adrenocortical tumour, or leukaemia) at any age

AND

First- or second-degree relative with any cancer under the age of 60

Table 1. 2009 Chompret Criteria for Germline TP53 Mutation Screening

Criterion

I. Proband with tumour belonging to LFS tumour spectrum (e.g., soft tissue sarcoma, osteosarcoma, brain tumour, premenopausal breast cancer, adrenocortical carcinoma, leukaemia, lung bronchoalveolar cancer) before age 46 years AND at least one first- or second-degree relative with LFS tumour (except breast cancer if proband has breast cancer) before age 56 years or with multiple tumours; OR

II. Proband with multiple tumours (except multiple breast tumours), two of which belong to LFS tumour spectrum and first of which occurred before age 46 years; OR

III. Patient with adrenocortical carcinoma or choroid plexus tumour, irrespective of family history.

Abbreviation: LFS, Li Fraumeni syndrome.

Li-Fraumeni patients - avoid X-Rays

– Li-Fraumeni presentation/management requires indication of avoiding X-Rays (TDM for example) and radiotherapy if possible. Indeed, radiotherapy is the first cause of second cancer in mutation carrier.

– In general radiation therapy should be avoided in patients with Li-Fraumeni syndrome because individuals are at high risk of radiation-induced secondary cancers. Similarly, exposure to CT scans or X-rays should be avoided.

– In LFS patients who received RT for their primary cancer, 40% developed a secondary malignancy in the RT field. Soft tissue sarcoma was the predominant type of secondary cancer in the RT field, while breast cancer was most common outside of the RT field.

– So analysis to be done urgently for diagnosis and therapeutic adaptation upstream of radiotherapy if possible!

Find a specialist !

• If you need medical advice, you can look for doctors or other healthcare professionals who have experience with this disease. You may find these specialists through advocacy organizations, clinical trials, or articles published in medical journals. You may also want to contact a university or tertiary medical center in your area, because these centers tend to see more complex cases and have the latest technology and treatments.

• If you can't find a specialist in your local area, try contacting national or international specialists. They may be able to refer you to someone they know through conferences or research efforts. Some specialists may be willing to consult with you or your local doctors over the phone or by email if you can't travel to them for care.

Who to test?

• individuals at high risk for Li-Fraumeni syndrome based upon their clinical features

• Chompret criteria

• Women with early onset breast cancer (less than age 30 years) and without a detectable BRCA1 or BRCA2 mutation.

• Individuals with adrenocortical carcinoma regardless of age or family history.

• Individuals with choroid plexus carcinoma regardless of age or family history.

• Individuals from a family with a known TP53 mutation.

• Prenatal testing may be considered for at-risk pregnancies in situations where a specific TP53 mutation has been identified.

Methods for Screening Li-Fraumeni Syndrome - Adults

• General assessment/Complete physical exam every 6 months

• Prompt assessment with primary care physician for any medical concerns

• Breast cancer/Breast awareness (age 18 years and forward)

• Clinical breast exam twice a year (age 20 years and forward)

• Annual breast MRI screening (ages 20-75) – ideally, alternating with annual whole-body MRI (one scan every 6 months)

• Consider risk-reducing bilateral mastectomy

(Note that the use of ultrasound and mammography has been omitted)

• Brain tumor (age 18 years and forward)

• Annual brain MRI (first MRI with contrast – thereafter without contrast if previous MRI normal)

• Soft tissue and bone sarcoma (age 18 years and forward)

• Annual whole-body MRI

• Ultrasound of abdomen and pelvis every 12 months

• Gastrointestinal cancer (age 25 years and forward)

• Upper endoscopy and colonoscopy every 2-5 years)

• Melanoma (age 18 years and forward)

• Annual dermatologic examination

• Also noted, for families in which breast cancer has already made an appearance at or around age 20 – awareness and screening can be considered 5 to 10 years before the earliest age of onset known. The same is recommended for gastrointestinal cancers – consider screening 5 years before the earliest known onset of a gastrointestinal cancer in the family.

Li–Fumeni syndrome - screening

• For families in which breast cancer has already made an appearance at or around age 20 – awareness and screening can be considered 5 to 10 years before the earliest age of onset known.

• The same is recommended for gastrointestinal cancers – consider screening 5 years before the earliest known onset of a gastrointestinal cancer in the family.

Screening Li-Fraumeni syndrome-children (birth to age 18 years)

• General assessment
• Complete physical exam every 3-4 months
• Prompt assessment with primary care physician for any medical concerns
• Adrenocortical carcinoma
• Ultrasound of abdomen and pelvis every 3-4 months
• In case of unsatisfactory ultrasound, blood tests every 3-4 months
• Brain tumor
• Annual brain MRI (first MRI with contrast – thereafter without contrast if previous MRI normal with and no new abnormality)
• Soft tissue and bone sarcoma
• Annual whole body MRI

Cowden syndrome - definition

• Autosomal dominant disorder with facial tricholemmomas, acral keratosis, oral mucosal papillomas, small intestinal and colorectal polyps
• Also called multiple hamartoma syndrome
• Increased risk of malignancy (breast and thyroid cancer) but not in polyps
• Polyps have same histology as mucosal prolapse syndromes (colitis cystica profunda)
• Histology - hamartomatous features with disorganization and proliferation of muscularis mucosa
• **PTEN** (phosphatase and tensin homolog) hamartoma tumor syndrome includes Cowden disease (multiple hamartoma syndrome), Bannayan-Riley-Ruvulcaba syndrome (BRRS), **Proteus syndrome,** and **Proteus-like syndrome**, which all have PTEN mutations 1.200.000 persons
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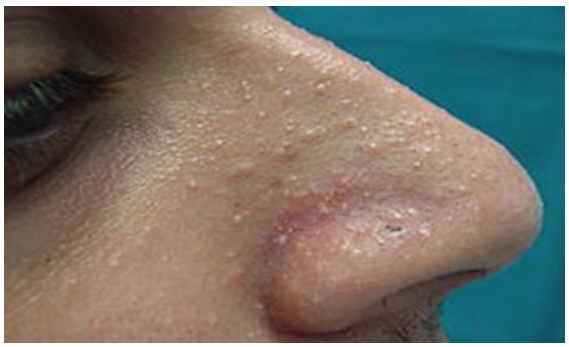
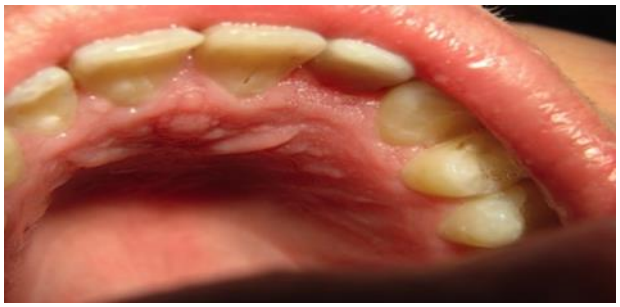




Proteus syndrome

• Proteus syndrome is a rare disorder characterized by overgrowth of various tissues of the body. The cause of the disorder is a mosaic variant in a gene called *AKT1*. Disproportionate, asymmetric overgrowth occurs in a mosaic pattern (i.e., a random "patchy" pattern of affected and unaffected areas). Affected individuals may experience a wide variety of complications that may include progressive skeletal malformations, benign and malignant tumors, malformations of blood vessels (vascular malformations), bullous pulmonary disease, and certain skin lesions. In some people, life-threatening conditions relating to abnormal blood clotting may develop including deep vein thrombosis and pulmonary embolism.

• This condition is characterized by various cutaneous and subcutaneous lesions, including vascular malformations, lipomas, hyperpigmentation, and several types of nevi. Cerebriform nevi are thought to be characteristic of the disorder. Progressive, asymmetrical limb overgrowth is pathognomonic, and patients have an unusual body habitus.

Diagnostic criteria for Proteus syndrome

Major findings

• Distorting, progressive overgrowth, typically of postnatal onset often resulting in asymmetric distortion of the skeletal architecture., hemimegencephaly can be prenatal.

• Cerebriform connective tissue nevi (CCTN), a specific type of connective tissue nevus that is characterized by deep grooves and gyrations as seen on the surface of the brain.

• Linear verrucous epidermal nevus (LVEN), a streaky, pigmented, rough nevus that often follows the lines of Blaschko and can be present anywhere on the body.

• Adipose dysregulation including lipomatous overgrowth and lipoatrophy.

Other:

• Vascular malformations including cutaneous capillary malformations, prominent venous patterning or varicosities, and lymphatic malformations

• Overgrowth of other tissues, most commonly spleen, liver, thymus, and gastrointestinal tract

• Tumors, most commonly meningiomas. Ovarian cystadenomas, breast cancer, parotid monomorphic adenoma, mesothelioma, and others have also been reported.

• Bullous pulmonary degeneration

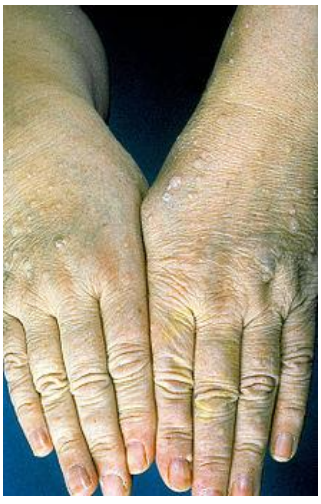
• Dysmorphic facial features including dolichocephaly, long face, down-slanting palpebral fissures, and/or minor ptosis, depressed nasal bridge, wide or anteverted nares, and open mouth at rest

Proteus like syndrome

• Proteus like syndrome describes patients who do not meet the diagnostic criteria for Proteus syndrome but who share a multitude of characteristic clinical features of the disease. The prevalence is unknown.

• The main clinical features include skeletal overgrowth, hamartomatous overgrowth of multiple tissues, cerebriform connective tissue naevi, vascular malformations and linear epidermal naevi. Mutations in the PTEN gene are found in 50% of Proteus-like syndrome cases, making them a part of the PTEN hamartoma syndrome group.

Cowden syndrome



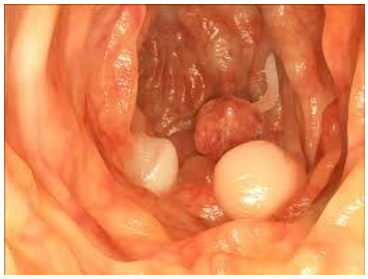
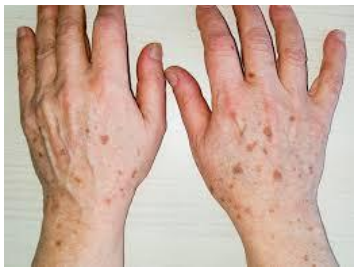
• Cowden syndrome (also known as Cowden's disease and multiple hamartoma syndrome) is an autosomal dominant inherited condition characterized by benign overgrowths called hamartomas as well as an increased lifetime risk of breast, thyroid, uterine, and other cancers.

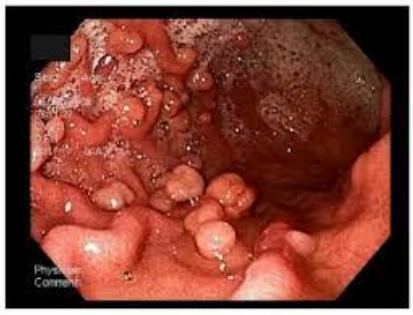
• It is associated with mutations in PTEN on 10q23.3, a tumor suppressor gene otherwise known as phosphatase and tensin homolog, that results in dysregulation of the mTOR pathway leading to errors in cell proliferation, cell cycling, and apoptosis.

• Germline PTEN mutations are rare and highly penetrant.

• The most common malignancies associated with the syndrome are adenocarcinoma of the breast (25-50%), followed by ginecological-uterus (5-28%), adenocarcinoma of the thyroid (7%), renal, squamous cell carcinomas of the skin (4%), and the remaining from the colorectal, upper gastrointestina, skin and brain/cognitive.

Peutz Jegers syndrome




Peutz Jeghers syndrome - diagnostic

• **Peutz-Jeghers syndrome** is an autosomal dominant disorder in which hamartomatous polyps can occur throughout the gastrointestinal tract. These polyps are histologically distinctive for Peutz-Jeghers syndrome and most patients also have characteristic mucocutaneous pigmentation. There is an elevated risk of many cancers including a 39% lifetime risk of colorectal cancer.

• **Genetic testing** is indicated to confirm the diagnosis and in relatives of known mutation carriers. Over 90% of patients meeting the clinical criteria for Peutz-Jeghers syndrome have an identifiable pathogenic mutation in the STK11 gene[2] In 38–50% of cases pathogenic mutations are de novo rather than inherited.[2]. Many are deletions which are not picked up on sequencing, this requiring MLPA.

Individuals with at least 2 of the following characteristics may be considered to have PJS:

• At least 2 Peutz-Jeghers type hamartomatous polyps in the small intestine

• Characteristic freckling of the mouth, lips, fingers, or toes

• At least 1 relative diagnosed with PJS

Individuals who meet these criteria are recommended to have genetic testing to look for an inherited mutation in the STK11 gene. More information is presented below.

Follow-up for men and women with Peutz-Jeghers syndrome (STK11 mutations)

Breast and ovarian cancer screening for women

• Clinical breast exam every 6 months, beginning at age 30

• Annual breast MRI and mammogram, beginning at age 30

• Annual pelvic exam and PAP smear, beginning at age 18-20

• Consider transvaginal ultrasound, beginning at age 18-20

Cancer screening for men

- Annual testicular exam

Guidelines for men and women with Peutz-Jeghers

• Colonoscopy every 2-3 years beginning in late teens

• Upper endoscopy every 2-3 years beginning in late teens

• Small bowel CT, MRI or video capsule endoscopy starting at age 8-10 with a follow-up by age 18, and then every 2-3 years

• Magnetic resonance cholangiopancreatography with contrast or endoscopic ultrasound every 1-2 years beginning age 30-35

Reproductive options

• For patients of reproductive age, advise about options for prenatal diagnosis and assisted reproduction including pre-implantation genetic diagnosis.

Risk to relatives

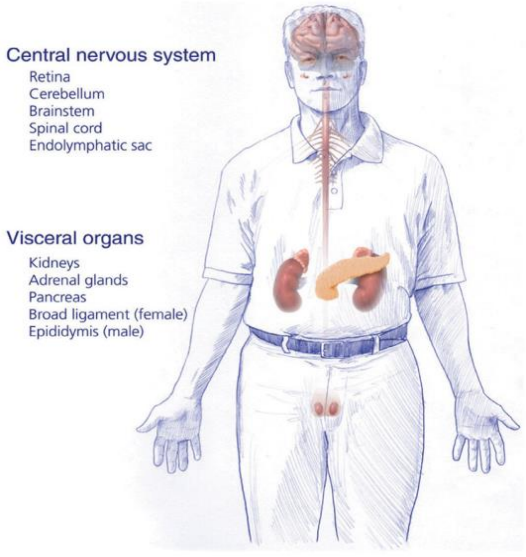
• Advise about possible inherited cancer risk to relatives, options for risk assessment, and management.

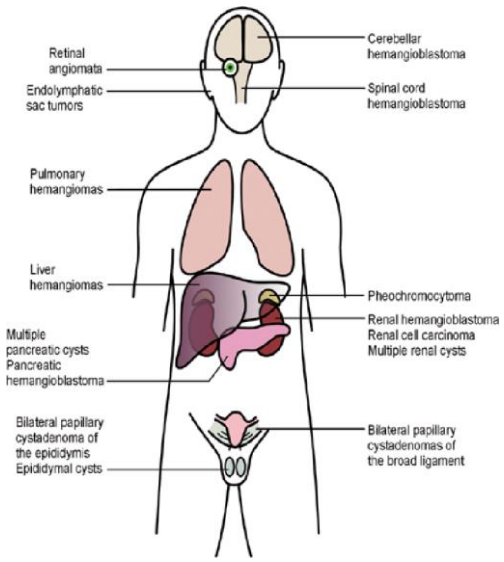
• Recommend genetic counselling and consideration of genetic testing for at-risk relatives.

What is Von Hippel Lindau disease?

• **Von Hippel Lindau syndrome (VHL)** is an autosomal dominant condition, associated with mutations of the VHL gene. This gene is located on chromosome 3 and encodes a protein involved in the ubiquination and depredation of hypoxia-inducible-factor (HIF).

• The lack of **HIF** degradation drives overexpression of vascular-endothelial-growth factor F (VEGF) The commonest clinical features are retinal and central nervous system haemangioblastomas but it is also frequently associated with renal cell carcinomas, phaeochromocytomas and paragangliomas and occasionally with pancreatic neuroendocrine tumours.





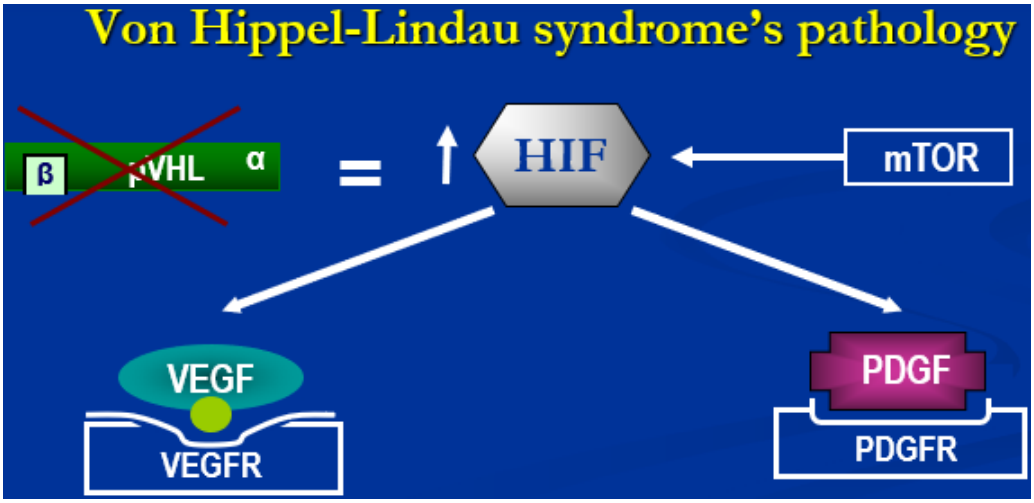
The initial manifestations of VHL disease

The initial manifestations of disease can occur in childhood, adolescence, or adulthood, with a mean age at initial presentation of approximately 26 years. The spectrum of VHL-associated tumors includes:

- Hemangioblastomas of the brain (cerebellum) and spine
- Retinal capillary hemangioblastomas (retinal angiomas)
- Clear cell renal cell carcinomas (RCCs)

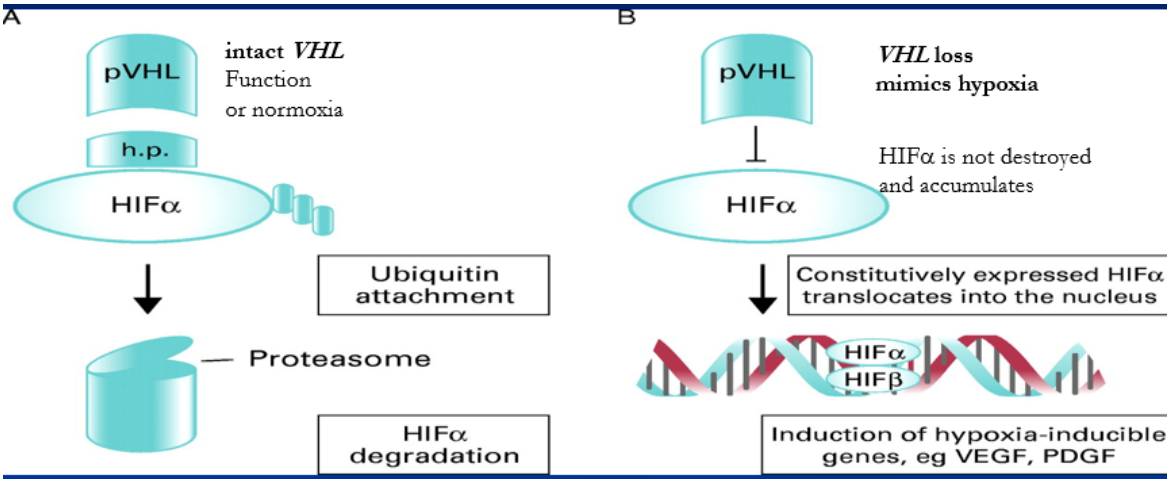
Von Hippel-Lindau syndrome

- Von Hippel-Lindau syndrome (VHL) is a hereditary condition associated with tumors arising in multiple organs.
- Tumors in VHL include hemangioblastomas, which are blood vessel tumors of the brain, spinal cord, and eye. The eye tumors are also called retinal angiomas. People with VHL also have an increased risk of developing clear cell renal cell carcinoma, which is a specific type of kidney cancer; pheochromocytoma, which is a tumor of the adrenal gland; and a type of pancreatic tumor known as pancreatic neuroendocrine tumor. Other features of VHL include kidney cysts, which are closed sacs usually filled with fluid; pancreatic cysts, epididymal cystadenomas, which are tumors near a man's testicles; and endolymphatic sac tumors, which are tumors of the ear that may cause hearing loss.



Source: Kaelin WG. *Nat Rev Cancer* 2002; 2:673–682

Aberrant functioning of the von Hippel-Lindau (VHL) gene regulates angiogenesis



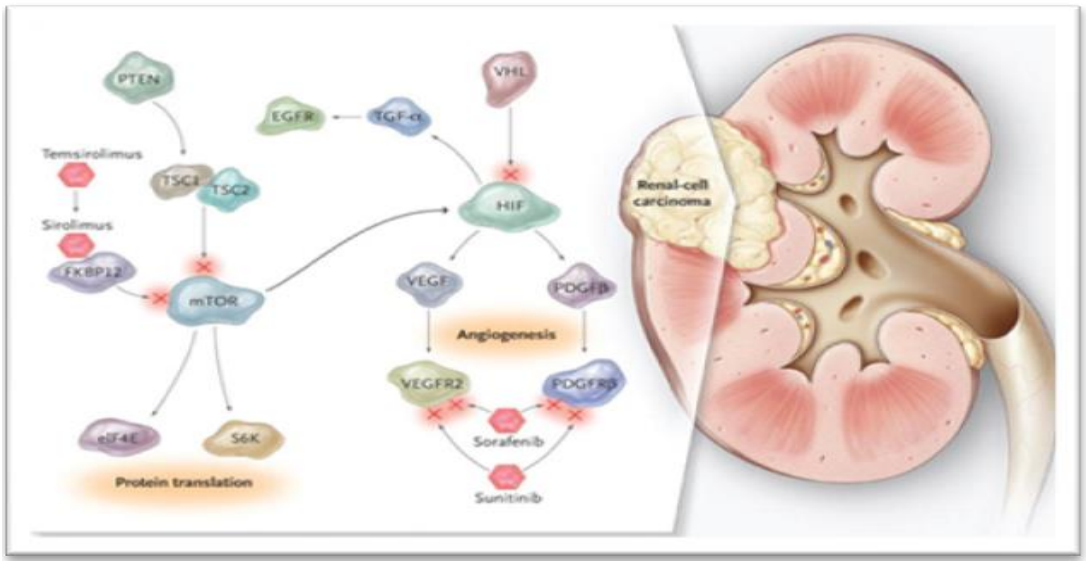
Rini BI *et al. J Clin Oncol* 2005; 23:1028-1043 Copyright © American Society of Clinical Oncology

Rationale for the Use of Anti-Angiogenic Agents in mRCC

- RCC: tumours of different epithelial origin
- Result of different genetic abnormalities
- Different morphological features
- **Most common type: clear-cell type: loss/mutation of the VHL-gene**
- Enables transcription of hypoxia-inducible genes

Arvid Lindau (1926)

Eugen von Hippel (1904)



Latif et al. (1993) describe the relationship between VHL mutation and cc-RCC

Source: Zambrano NR et al. J Urol 1999; 162:1246-58

Follow-up recommendations for asymptomatic patients (I)

> the age of 5 years old

- abdominal ultrasound
- dilated Fundus Examination
- urinary and plasma metanephrines
- audiogram (every 2 years)

} **annually**

Follow-up recommendations for asymptomatic patients (II)

> the age of 15 years old

- abdominal ultrasound
- dilated Fundus Examination
- urinary and plasma metanephrines

to be continued annually

- audiogram (every 2 years)
- first systematic CNS MRI (encephalus + spine) this image is to be renewed every 2 years in the absence of lesions, or every year in the event of lesions to be monitored
- the frequency can be adapted according to the size and the number of lesions and the opinion of the referring neurosurgeon

Follow-up recommendations for asymptomatic patients (III)

> the age of 18 years old

- first systematic abdominal MRI, then alternating annually with an abdominal ultrasound in the absence of lesions

If one or more lesions are detected, the MRI frequencies can be annualy or to be adapted according to the size and number of lesions;

An abdominal scan is only to be performed in the event of a better characterized lesion or before a possible surgical intervention;

- dilated Fundus Examination, urinary and plasma metanephrines, audiogram (every 2 years), first systematic CNS MRI (encephalus + spine) as recommended before

Source: Reseau National de Reference pour Cancers Rares d'Adulte PREDIR et Association VHL France

Treatment

• The two main treatment options are photocoagulation on smaller lesions and cryotherapy for larger ones. Multiple treatments with photocoagulation or cryotherapy are needed to fully obliterate the tumor. Observing smaller lesions may be an option, as some have been known to remain stable for years or even to regress.

• It is now known that VHL cause an upregulation of, or are sensitive to, many growth factors, including VEGF and PDGF. Antiangiogenic agents, such as bevacizumab (Avastin) have therefore been used both intravenously and intravitreally.

• So far, limited studies, including the institutional experience, have found that anti-VEGF therapy reduces the amount of exudation and may improve vision but does not change the size of the lesion. Of note, both systemic anti-VEGF therapy and photodynamic therapy combined with intravitreal anti-VEGF therapy for cerebellar hemangioblastoma have been tried with some success.

• Small lesions are best managed with early photocoagulation. Large lesions respond better to cryotherapy, but usually carry a poor prognosis.

Take home message

• Rare ereditary syndrome (RES) like Li –Fraumeni syndrome may have a profound psychological and emotional impact on patients and may be further complicated by relationships with parents and other family members

• For RES family members who were offered genetic testing and counseling, it was observed that greater cancer- specific distress was associated with having a lower quality of life, a higher perceived risk of having a *TP53* mutation, no personal history of cancer, and a greater number of first-degree relatives affected with cancer.

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II.11.1 Chances of prophylactic surgery in the personalized oncogenetic monitoring program – Breast and ovarian cancer

Learning objectives

• knowledge of the primary role of prophylactic surgery in preventing breast and ovarian cancer in high risk patients

• indications, advantages and disadvantages of prophylactic surgery

• knowledge of the main types of surgery, the complications and the possibilities of reconstruction after mastectomy

Introduction

• Once detected the carriers of gene mutations, genetics departments must recommend either monitoring methods or even treatments to reduce the risk of developing the disease to which the carrier is prone.

• Genetic factor is involved in the occurrence of 5-10% of breast cancer. The main genetic disorders that increase the risk of breast cancer - BRCA1 or BRCA2 mutation the most widely recognized:

lifetime risk for breast cancer is 50% to 85% among BRCA1 carriers and approximately 45% among BRCA2 carriers.

women of Ashkenazi Jewish descent are known to be at high risk for the BRCA mutation, although they may also have higher rates for other mutations.

• Most inherited cases of breast cancer have been associated with two genes: BRCA1 and BRCA2. Women with an abnormal BRCA1 gene seems to have a worse prognosis than women with an abnormal BRCA2 gene.

• A positive test result indicates that a person with a BRCA1 or BRCA2 mutation has an increased risk of developing certain cancers. However, women who inherit a BRCA1 or BRCA2 mutation may never develop breast or ovarian cancer.

Management of BRCA carriers

• Surveillance/screening recommendation:
  - Digital mammography with or without digital breast tomosynthesis annually beginning at the age at 30
  - breast MRI should be performed annually beginning at age 25 to 30.
• Chemoprevention
• Bilateral salpingo-oophorectomy
  - no evidence for ovarian cancer screening efficacy
• Bilateral prophylactic mastectomy

What is "prophylactic surgery"?

According to National Cancer Institute,

- "Prophylactic surgery is a form of surgery whose purpose is to minimize or prevent the risk of developing cancer in an organ or gland that has yet to develop cancer and is known to be at high risk of developing cancer."

"Surgery to remove an organ or gland that shows no signs of cancer, in an attempt to prevent development of cancer of that organ or gland. **Prophylactic surgery** is sometimes chosen by people who know they are at high risk for developing cancer."

What is "prophylactic mastectomy"?

• **"Prophylactic** mastectomy is **surgery** to remove one or both breasts to reduce the risk of developing **breast** cancer. According to the National Cancer Institute, **prophylactic** mastectomy in women who carry a BRCA1 or BRCA2 gene mutation may be able to reduce the risk of developing **breast** cancer by 95%".

• **"Prophylactic mastectomy** can reduce the chances of developing breast cancer in women at high risk of the disease: For women with the BRCA1 or BRCA2 mutation, **prophylactic mastectomy** reduces the risk of developing breast cancer by 90 to 95 percent."

Prophylactic mastectomy

• It is expected to cause an increase in life expectancy compared to screening; at the same time, the anxiety of developing the neoplasia is eliminated, which can influence the quality of life in BRCA carriers.

• There are many technical variants for prophylactic mastectomy. Of all the variants, nipple-sparing mastectomy became favourite - this is an intervention that removes the mammary gland but preserves the areola, nipple and the skin lining the mammary gland. This intervention has cosmetic advantages and is performed either through an axillary incision, in the submammary crease or a radial one. This surgical procedure does not compromise the oncological/prophylactic outcome.

• In order to perform a nipple sparing mastectomy in good conditions and with good results, the surgical team must have an important experience. Particular attention should be paid not to leave macroscopic breast tissue, especially at the extremities of the gland, extensions (e.g.: axillary tail), submammary fold or in the proximity of the nipple and areola.

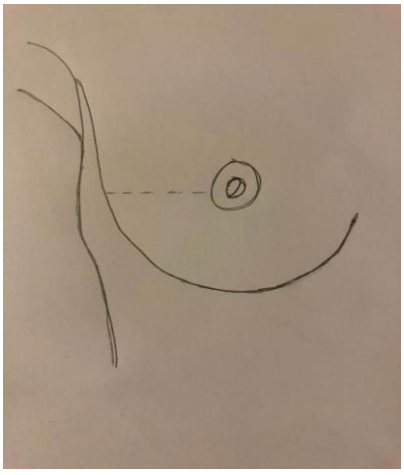
• Also, the dissection must be performed accurately, the cutaneous flaps and the nipple complex must be meticulously prepared in order not to compromise the vascularization.

• All patients who benefited from prophylactic bilateral mastectomy may also benefit from breast reconstruction, in the absence of contraindications. Negative impact of mastectomy is also reduced, both physically and psychologically.

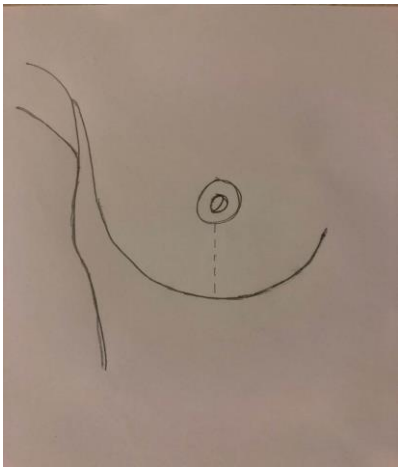
• It is indicated to perform the complete imaging investigations (breast ultrasound, mammography and breast MRI) whenever a patient chooses bilateral prophylactic mastectomy. The purpose of these investigations is to minimize the risk of occult cancers detection at the final pathological examination.

• Sentinel lymph node biopsy in prophylactic mastectomy is controversial but is not recommended for a potential occult carcinoma.

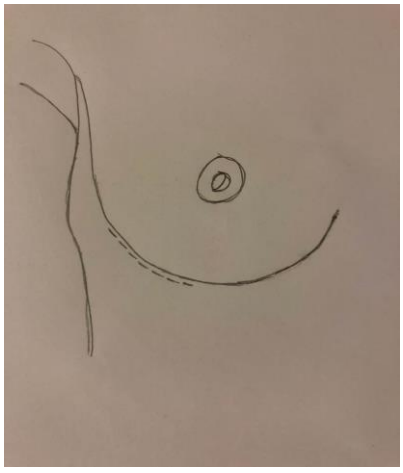
Types of incision – nipple sparing mastectomy



horizontal incision



vertical incision



inframammary fold incision

Prophylactic mastectomy

There are some issues to discuss with the patient before deciding to perform a prophylactic mastectomy:

• In about 5% of cases, there is a risk of developing neoplastic disease on ectopic or restant breast tissue.

• Morbidity and surgical complications occur in 15-20% of cases. These include ischemia or necrosis of the skin or of the nipple, haematoma, seroma, infections as well as complications of breast reconstruction that can go on to implant removal.

• In a considerable proportion of cases, re-interventions to correct the imperfections or due to complications are required

• The presence of sequelae such as lack of sensitivity to areola and nipple, paresthesia or pain sensation

• The need to readjust to a new body image

• Breast reconstruction after prophylactic mastectomy may be performed using:

- Implant based breast reconstruction
- Autologous tissue breast reconstruction

• There were no differences in health-related quality of life between these two groups

• Patients in whom breast reconstruction used implants were significantly less satisfied than those in whom the reconstruction was performed using autologous tissue.

• The benefits of prophylactic mastectomy seem to be greater in younger women than older women. This is because younger women have more years of life ahead.

• For a 30-year old woman who has a *BRCA1* or *BRCA2* gene mutation, prophylactic mastectomy may add 3-5 years to her lifespan;

• For women 60 years and older, the gain in lifespan after a prophylactic mastectomy is small.

• Contralateral breast cancer – contralateral prophylactic mastectomy

• BRCA carriers with breast cancer have a higher risk of contralateral breast cancer, 25% compared with non-carriers, 3-6 %.

• The risk is higher for BRCA 1 carriers

• Contralateral prophylactic mastectomy reduces the risk of contralateral breast cancer with 91-93%.

• Benefits on survival – none/some studies suggest benefits in second decade of follow up after first breast cancer was diagnosed.

Prophylactic salpingo-oophorectomy

• Meta-analysis shown that prophylactic salpingo oophorectomy significantly reduces the risk of ovarian cancer in patients with BRCA 1 and BRCA 2 mutations

• Hormone replacement treatment used after prophylactic salpingoophorectomy does not increase breast cancer risk

• Prophylactic salpingoophorectomy can be performed by laparoscopic approach (even through trans-mammary access) at the same time with mastectomy and breast reconstruction. Advantages: good cosmetic results without increasing the complication rate.

• The NCCN recommends women who have a *BRCA1* or *BRCA2* mutation have prophylactic oophorectomy between ages 35-40 (or after childbearing is complete)

• Women with a *BRCA2* mutation tend to be diagnosed with ovarian cancer at a later age than women with a *BRCA1* mutation. So, women with a *BRCA2* mutation who have had bilateral prophylactic mastectomy may delay oophorectomy until age 40-45.

Primary peritoneal cancer after prophylactic surgery:

Incidence 1-2 %

The interval from prophylactic surgery to peritoneal cancer varies from 12 to 84 months

Ovarian and primary peritoneal cancers are histologically similar to the Mullerian epithelium.

Compared to ovarian cancer, primary peritoneal cancer is characterized by loss of heterozygosity at chromosomal loci and overexpression of the HER2 oncogene on immunohistochemistry

Peritoneal cancer after prophylactic surgery

The possible mechanisms that could explain the origin of peritoneal cancer after prophylactic salpingo-oophorectomy:

the presence of the precancerous lesion/serous tubal intraepithelial carcinomas during salpingectomy and the possibility that lesion would have metastasized to the peritoneum before the prophylactic procedure

the salpingo-oophorectomy was incomplete, meaning that there might be an ovarian or tubal remnant postoperatively.

the primary origin of the peritoneal cancer/appendix origin

Prophylactic surgery

National Cancer Institute in France presented the benefits in survival after prophylactic surgery (KURIAN 2010):

- prophylactic salpingoophorectomy performed at 40 years old -

15% for women with BRCA1 mutation

6% for woman with BRCA2 mutation

- Prophylactic mastectomy performed at 30 years old

13% for women with BRCA1 mutation

8% for women with BRCA2 mutation

Take Home Message

• Prophylactic mastectomy can be followed, in most cases, at the same time with breast reconstruction - a very important psychological element

• Prophylactic salpingoophorectomy can be performed at the same time with prophylactic mastectomy, with minimal scars or psychological impact

• Prophylactic mastectomy does not involve removal of the sentinel lymph node or axillary lymphadenectomy, avoiding the shortcomings of these additional procedures

• Prophylactic surgery does not entirely reduce the risk of developing breast or ovarian cancer

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II.11.2.Chances of Prophylactic Surgery in the Personalized Oncogenetic Monitoring Program – Colorectal cancer

Learning objectives

- To know which is the optimal surgical approach in patients with familial adenomatous polyposis
- To know which is the optimal surgical approach in patients with Peutz-Jeghers Syndrome
- To know which is the optimal surgical approach in Lynch Syndrome

Introduction

• Highly penetrant syndromes such as Lynch syndrome (LS), familial adenomatous polyposis (FAP) and other polyposis syndromes account for 3-5% of all CRC diagnoses, although heritable factors account for approximately 35% of colorectal cancer (CRC) risk;

• advances in genetic diagnosis, endoscopic or surgical control, as well as lifestyle interventions = opportunities for CRC prevention and effective treatment in susceptible individuals.

• risk-reducing interventions include endoscopic surveillance, as well as preventive surgical approaches;

• Different strategies according to type of hereditary risk.

The optimal surgical approach in patients with familial adenomatous polyposis (FAP)

• Colon cancer will inevitably develop in patients with FAP if the colon is not removed.

• Proctocolectomy will prevent colon cancer in FAP patients.

• Prophylactic surgery can usually be planned at a time which is suitable to the patient, based on the risk of cancer as assessed colonoscopically.

• The timing and choice of surgical procedure should take into account the educational, social, family planning and emotional development of the patient and their reliability for attending follow-up evaluations.

The optimal surgical approach in patients with FAP - the choice of surgery

Colonoscopic surveillance enables assessment of adenoma burden and distribution, guiding the timing of and type of prophylactic surgery required according to:

1. rectal polyp number,
2. size of polyps;
3. presence of high-grade dysplasia,
4. genotype
5. functional consequences of the surgical procedure.
6. compliance with follow-up surveillance.

Total colectomy with ileorectal anastomosis (IRA)

• patients with relative rectal sparing (<20 polyps) if all rectal adenomas are < 5mm in diameter and any polyps >5mm can be endoscopically removed.

• The decision to retain the rectum is made based on future rectal cancer risk, polyposis phenotype in the rectum, on functional considerations and on genotype.

Proctocolectomy and ileal pouch anal anastomosis (IPAA)

• rectal cancer,

• large rectal polyp burden (>20 synchronous adenomas, adenoma with high-grade dysplasia, large (> 10 mm) adenomas),

• severe phenotype (> 1000 synchronous adenomas)

• patients with poor compliance with follow-up surveillance.

Total proctocolectomy with end ileostomy

- for patients with:

1. poor sphincter function,
2. incontinence,
3. distal rectal cancer,
4. cancers requiring radiation,
5. to avoid the functional consequences of an ileoanal pouch.

• Bowel frequency, nocturnal defecation and use of incontinence pads are more frequent with the ileal pouch, although fecal urgency is reduced.

• Sexual dysfunction, dietary restriction, or postoperative complications are not significant different between the two techniques (IRA and IPAA).

• An extensive examination to check for extracolonic manifestations is recommended prior to colorectal resection.

• The fecundity of women with FAP:

- before operation and after colectomy with ileorectal anastomosis has been reported to be similar to that of the general population.

- dropped to 54 per cent following proctocolectomy with ileal pouch-anal anastomosis.

- postoperative fertility problems appear to be more common among women who had their first surgical procedure at a younger age.

It is recommended that the significant reduction in female fecundity after ileal pouch-anal anastomosis should be communicated to young women with FAP prior to surgery.

Source: Nieuwenhuis MH, Douma KF, Bleiker EM, et al. Female fertility after colorectal surgery for familial adenomatous polyposis: A nationwide cross-sectional study. Ann Surg 2010; 252:341–4

Indications for rectal excision following IRA:

1. the development of rectal cancer,
2. Polyps >10 mm diameter,
3. polyps with high-grade dysplasia,
4. marked increases in polyp number between examinations.

Functional outcomes after conversion of an IRA to IPAA is similar to primary IPAA procedures. Complication rates and pouch failure rates are reported to be similar, but conversion to IPAA will not be possible in a small percentage of patients.

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The optimal surgical approach in patients with FAP - Risk of neoplasia and cancer in the pouch

• a very small risk of adenocarcinoma after an IPAA, developed in residual rectal or in the anal transitional zone (ATZ) mucosa (75%), and within the ileal component of the pouch (25%).

• Currently nearly all pouches are constructed with the use of stapling devices which results in the ATZ mucosa being preserved.

• The risk of adenoma of the IPAA at 10 years is 45- 51% after stapled IPAA.

• The cumulative risk of developing a pouch carcinoma at 10-year follow-up is 1%.

• A Mayo clinic study - median time to development of dysplasia = 149 months.

• Thus, although the risk of severe dysplasia and cancer is low, annual endoscopic surveillance of any remaining rectal mucosa, ATZ mucosa and ileal pouch are recommended for life.

The optimal surgical approach in patients with Peutz-Jeghers Syndrome

• elective polypectomy to prevent polyp related complications.

• Small bowel polyps greater than 1.5-2cm in size (or smaller if symptomatic) should be considered for elective resection to prevent intussusception.

(GRADE of evidence: low; Strength of recommendation: weak)

The optimal surgical approach in Lynch Syndrome (LS)

• Patients meeting Amsterdam criteria for diagnosis of hereditary nonpolyposis colorectal cancer (HNPCC) have a lifetime colorectal cancer risk approaching 50%, and a metachronous cancer rate of approximately 25%;

• **No Prophylactic surgery**;

• **Surveillance colonoscopy** (the age of onset should be stratified according to the LS-associated gene).

• **For patients with LS who develop cancer - surgical resection** represents the front-line therapy.

• The two surgical procedures taken into account are segmental colectomy (treating the malignant lesion) versus total colectomy (treating the malignant lesion and as a secondary prevention of the metachronous cancer);

• Total colectomy (TC) is the preferred operation for primary colon cancer by some authors, particularly in younger affected individuals and those with more severe phenotypes;

• Stupart DA et al.(Colorectal Dis. 2011 Dec;13(12):1395-9) compared survival after TC with segmental colectomy (SC) in HNPCC in a prospective cohort study; after 6 years follow up, metachronous colon cancer occurred in 21% of SC patients and in none of the TC patients. The risk of developing metachronous cancer after SC was 20% at 5 years.

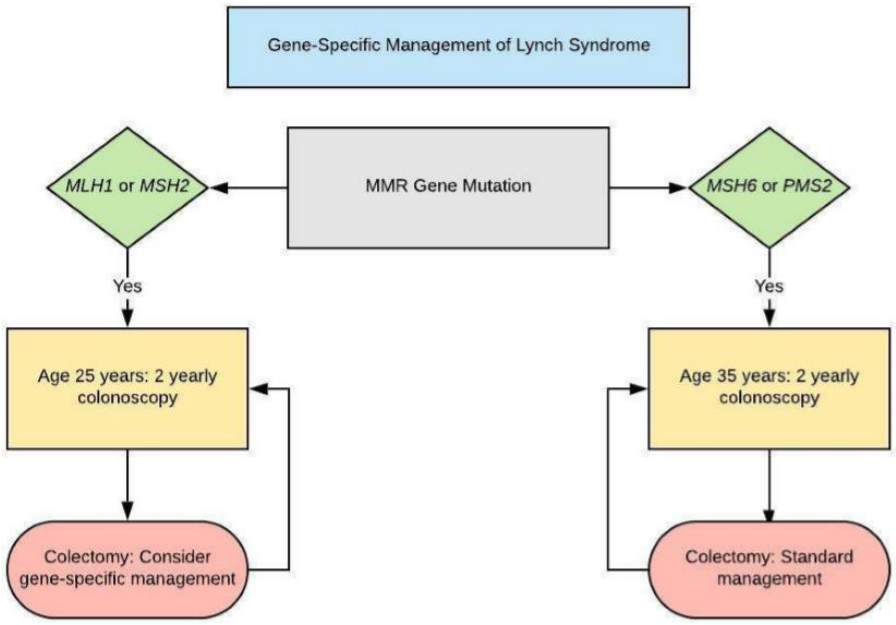
• However, there is no clear evidence that more extensive surgery confers a survival benefit, and it does impart a greater risk of chronic diarrhea and/or incontinence.

The optimal surgical approach in Lynch Syndrome (LS) – the choice of surgery

Influenced by:

- the risks of metachronous cancer,
- tumor location (two-thirds of colon cancers occur in the proximal colon),
- the functional consequences of surgery,
- the patient's age,
- the patient's wishes.

The optimal surgical approach in Lynch Syndrome (LS)



in LS patients with MLH1 or MSH2 mutations who develop colon cancer or colonic neoplasia not amenable to endoscopic control:

segmental vs total/near total colectomy

• for LS patients with MSH6 or PMS2 mutations there is insufficient evidence for oncological benefit of extended colectomy over segmental resection.

• When abdominal-perineal excision can be avoided, a standard low anterior resection is a reasonable option to treat rectal cancers in LS patients, even though the residual colon is at high-risk of metachronous neoplasia.

• There is an associated high risk of endometrial cancer (second most common), ovary, stomach, small intestine, hepatobiliary tract, upper urinary tract, brain, and skin cancer.

• Prophylactic hysterectomy and salpingo-oophorectomy performed after the completion of childbearing have potentially life-saving benefits.

Take Home Message

• The subtotal or total colectomy represents a currently preferred recommendation for individuals with hereditary risk for colon cancer;

• However, the decision with respect to extent of surgery should consider the patient's risk of additional cancers, surgical risk with additional resection, and patient preferences.

• The moment of the surgery is decided according to the risk of cancer based on colonoscopy findings.

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II.12.1. Monitoring of patients diagnosed with hereditary cancer and their families

Learning objectives

Learn about:

- What is genetic screening, genetic counseling and testing?
- Who is a candidate for genetic counseling?
- What would a positive genetic test result tell about the risk for a cancer?
- Monitoring hereditary breast and ovarian cancer.
- Monitoring hereditary colo-rectal cancers.
- What is chemoprevention?

Introduction

• Hereditary cancer syndrome -a type of inherited disorder in which there is a higher-than-normal risk of certain types of cancer.

• Majority of cancers are sporadic. Hereditary cancer syndromes are caused by mutations (changes) in certain genes passed from parents to children. In a hereditary cancer syndrome, certain patterns of cancer may be seen within families.

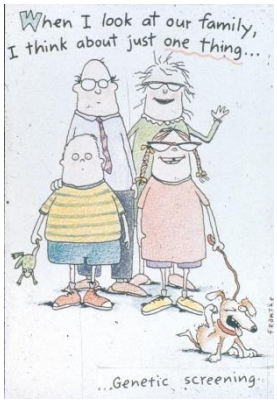
• These patterns include having several close family members (such as a mother, daughter, and sister) with the same type of cancer, developing cancer at an early age, or having two or more types of cancer develop in the same person.

• Examples of hereditary cancer syndromes are: hereditary breast and ovarian cancer syndrome, Li-Fraumeni syndrome, Cowden syndrome, and Lynch syndrome, also called *family cancer syndrome* and inherited cancer syndrome

Cancer is sporadic, familial or hereditary ?

How can we know?

1. Family history - history
2. Genetic testing
3. The genetic advice (counseling)



What features of a person's personal family history suggest hereditary cancer?

• Young age at diagnosis (tipc <50 years): breast, colon and uterus

• Multiple family members from the same family, with the same cancer

• Several cancers in the family known to be caused by a single gene mutation (e.g., breast / ovarian; pancreatic; colon / uterine / ovarian; colon / polyps / desmoid tumors / osteomas)

• Multiple cancers in the same person: breast, ovary, pancreas, colon, polyposis

• Bilateral cancers (on both sides)

• Rare cancers (i.e. breast cancer in men, thyroid cancer, retinoblastoma)

• Ethnicity (Ashkenazi Jewish, 1/40 BRCA mutation)

• Particular pathology (triple negative), cancer <60; bone marrow cancers are more common in

women with hereditary breast and *ovarian cancer*, colon with irosatellite instability (MSI +), or immunohistochemistry (IHC), which increases the risk of hereditary colon cancer syndromes.

What is genetic screening, genetic counseling and testing ?

Because gene mutations that cause hereditary and familial cancers are rare in the population (<1%), family history is the best way to identify people who have mutations in hereditary and genetic predisposition cancers.

What is genetic counseling for hereditary cancer?

During genetic counseling, a genetic counselor meets with you to:

• Review your personal and family history of cancer
• Create a family tree, also known as a pedigree
• Provide a personalized cancer risk assessment
• Discuss whether genetic testing is recommended
• Provide recommendations for increased cancer screening and prevention
• Provide psychological support and follow-up

• Having limited information about cancer diagnoses in family members or a small family size may make it more difficult to accurately assess your chances of hereditary cancer.

However, that shouldn't stop you from seeing a genetic counselor.

• hysterectomies.

Screening must systematically be adapted to family history! (for example, if an endometrial cancer is described at 30 years of age in the family, hysterectomy could be performed before post-menopausal state).

Who are the candidate for genetic screening?

• A personal history of a cancer diagnosis before the age of 50
• At any age, a personal history with:
  - Epithelial ovarian cancer
  - Male breast cancer
  - Pancreatic cancer
  - Metastatic prostate cancer

• The same or related cancers in 2 or more close family members, such as breast cancer in a mother and her daughter

• Multiple primary cancers in 1 person, such as colon and stomach cancer or breast cancer that occurs in both breasts

• Particular ancestry, such as Ashkenazi Jewish ancestry with a personal or family history of breast, ovarian, or pancreatic cancer

Who is a candidate for genetic counseling?

• Only 5-10% of most cancers are due to a single driver mutation in the dominant genes susceptible to cancer

• The key to the clinician is to identify patients who are at increased risk for hereditary mutation.

• The first factor, the young age suggests even in the absence of a family history of increased risk of germline mutation in several types of cancer.

• The second factor is the presence of the same cancer in several relatives of the same part of the pedigree

• The third factor is the agglomeration of various cancers known to be caused by the mutation of a single gene in a family (e.g., breast, ovary / pancreas or colon / ovary / uterus).

• The fourth risk factor is the presence of multiple primary cancers in a single individual

• Fifth, the presence of an unusual cancer for one sex (eg, breast cancer in men)

• Sixth, ethnicity (e.g., hepatitis has a high incidence of BRCA1 / 2 mutations) and the latter, histology (e.g., breast cancer in women with BRCA 1 a and triple negative mutations)

• Very important although rare, the presence of *a hereditary malformation* associated with rare hereditary syndromes (e.g. autism, large cranial circumference, desmoid tumors)

Genetic Counseling and Testing

• Genetic counseling involves a **discussion** of your personal or family history of cancer. It is typically recommended for individuals or families with multiple cases of cancer diagnosed at unusually young ages.

• Genetic counselors will tell you about the scientific concepts that relate to **genetic testing** and help you decide what genetic tests, if any, might be useful for you.

• Genetic testing involves **a simple blood test** and may be used to obtain a more precise estimate of your cancer risk. In some cases, genetic testing can be done on stored tissue samples from deceased relatives.

• Genetic testing is not required for a **cancer risk assessment**. However, it may, in some cases, help you and your physician make important **decisions** about your medical care.

• Deciding whether to undergo genetic testing is a personal choice that can be made at the time of the counseling session or at a future date. Genetic **counseling** does not require genetic testing, and genetic testing may not be useful for everyone receiving genetic counseling

What are the types of genetic tests?

• Genetic testing can provide information about a person's genes and chromosomes. Available types of testing include:

• **Newborn screening** is used just after birth to identify genetic disorders that can be treated early in life (e.g. test infants for phenylketonuria and congenital hypothyroidism (a disorder of the thyroid gland).

• **Diagnostic testing** is used to identify or rule out a specific genetic or chromosomal condition. In many cases, genetic testing is used to confirm a diagnosis when a particular condition is suspected based on physical signs and symptoms. Diagnostic testing can be performed before birth or at any time during a person's life, but is not available for all genes or all genetic conditions.

• **Carrier testing** is used to identify people who carry one copy of a gene mutation that, when present in two copies, causes a genetic disorder. This type of testing is offered to individuals who have a family history of a genetic disorder and to people in certain ethnic groups with an increased risk of specific genetic conditions.

• **Prenatal testing** is used to detect changes in a fetus's genes or chromosomes before birth. This type of testing is offered during pregnancy if there is an increased risk that the baby will have a genetic or chromosomal disorder.

What types of genetic tests are available?

• A genetic counselor can review with you what type of genetic testing is recommended based on your personal and family history and any earlier genetic testing you may have had.

• Types of genetic testing include:

- A multi-gene panel: This type of testing allows for the analysis of many genes at 1 time. Because several different genes can cause the same or related cancers, this type of testing is often recommended.

- Site-specific testing: This type of testing may be recommended if a family member had genetic testing that identified a specific mutation.

Genes to test

• **BRCA1/2:** Breast and Ovarian

• **MLH1, MSH2, MSH6, PMS2**: Colon/Endometrial/ Stomach/Ovarian

• **PTEN:** Breast/Endometrial/Thyroid

• **P53 (ECAM)**: Breast, Brain, Sarcoma, Leukemia, Adrenal Cortical Cancer

• **APC/MYH**: Colon, colon polyps

• **CDH1**: Breast/Stomach

• **P16**: Melanoma/Pancreatic

• And others!

What are the possible genetic test results?

• **Positive:** This means that testing found a gene mutation known to increase the risk for developing a certain type or types of cancer.

• If your genetic test result is ***positive, your family members are at risk for having inherited the mutation.***

• **Negative:** This means that testing did not find a mutation in any of the genes included in the test.

• **Inconclusive or a variant of uncertain significance:** This means that testing found a change in 1 or more of the genes included on the test, but it's not clear if that change causes an increased risk for cancer or not.

• If you previously had genetic testing that only included **1 or a few genes** and your result was negative or inconclusive, additional genetic testing may be recommended.

Consequences of hereditary cancer risk prediction

Different medical behavior

- Monitoring a HEALTHY population, with a major risk of illness
- **Early detection**: reducing cancer mortality
- **Prevention**: reducing incidence of cancers
- **Germ transmission**: counseling; no over-risk in half of the families that did not inherit the mutation

Steps in genetic counseling

• Information by genetic counseling

• **Family history**

• Search for dysmorphologies (congenital anomalies, benign tumors, dermatological abnormalities)

• Genetic testing - risk assessment:

- what is the chance for the person to develop cancer?

- what is the chance that a cancer in the family will be caused by a single gene mutation?

- what is the chance to identify the mutation of that gene with the means we have available (knowledge + laboratory techniques)?

• Risk monitoring and mitigation options

Who should consider genetic testing for cancer risk?

• People who are concerned about whether their family history puts them at risk for cancer should consult with a genetic counselor.

• ***Suggesting a hereditary cancer syndrome*** includes:

• Cancer was diagnosed at an unusually **young age**

• ***Several different types of cancer*** occurred in the same person

• Cancer in ***both organs*** in a set of paired organs (e.g. both kidneys or both breasts)

• Several first-degree relatives (the parents, siblings, or children of an individual) have the same type of cancer (for example, a mother, daughter, and sisters with breast cancer); family members with breast or ovarian cancer; family members with colon cancer and endometrial cancer

• Unusual cases of a specific cancer type (for example, breast cancer in a man)

• The **presence of birth defects** that are known to be associated with inherited cancer syndromes, such as certain noncancerous (benign) skin growths and skeletal abnormalities associated with neurofibromatosis type 1.

• Being a member of a racial or ethnic group that is known to have an increased risk of having a certain inherited cancer susceptibility syndrome and having one or more of the above features as well

• Several family members with cancer

What is the role of genetic counseling in genetic testing for a hereditary cancer syndrome?

This counseling should be performed by a trained genetic counselor or other health care professional who is experienced in cancer genetics. Genetic counseling usually covers many aspects of the testing process, including:

• A hereditary cancer risk assessment based on an individual's personal and family medical history

Discussion of:

o The appropriateness of genetic testing and potential harms and benefits of testing

o The medical implications of positive, negative, and uncertain test results

oThe possibility that a test result might not be informative (that is, it might find a variant whose effect on cancer risk is not known)

o The psychological risks and benefits of genetic test results

oThe risk of passing a variant to children

o The impact of testing for the family

oThe best test to perform

• Explanation of the specific test(s) that might be used and the technical accuracy of the test(s) and their interpretation

What would a positive genetic test result tell about risk of cancer?

- If you haven't previously been diagnosed with cancer, you may learn that you have an increased risk for developing a certain type(s) of cancer.
- If you previously had cancer, you may learn that you're at increased risk for developing another cancer.

Also, if you've had cancer, your positive genetic test result may help your doctor determine what type of treatment may be the most effective for you. Research on these types of targeted treatments is ongoing.

Managing the breast cancer risk

• If your test result is **positive,** you have a range of options to manage your risk. Risk-reducing surgery is not the only option.

• Ultimately, there's no right or wrong answer about what you should do – it's a decision only you can make.

• **Regularly examining your breasts**

• If you have the faulty BRCA1/2 gene, it's a good idea to be aware of changes in your breasts.

• This advice applies to men with a faulty BRCA2 gene too, as they are also at increased risk of breast cancer (although to a lesser extent).

What are some of the benefits of genetic testing for inherited cancer susceptibility syndromes?

There can be benefits to genetic testing, regardless of whether a person receives a positive or a negative result.

• An informative negative test can provide the person with **peace of mind** that a harmful gene variant was not inherited.

• A **positive** test result provides the person an opportunity to understand and, in some cases, manage their cancer risks.

• For people who are already diagnosed with a cancer, results of genetic testing may help them make decisions about their treatment and understand their risk for other cancers.

• Genetic testing provides an opportunity for family members to learn about their own cancer risks.

Genetic testing can have potential emotional, social, and financial harms, including:

• Psychological stress of learning that one has a genetic variant that increases cancer risk and having to decide whether to share those findings with blood relatives

• An uninformative test results, such as a report of a variant of uncertain significance (VUS), increases uncertainty and may increase stress until results are clarified

• Survivor guilt upon learning that one doesn't have a harmful variant that is present on other members of the family

• Cost of testing itself and additional follow-up testing, if not covered by insurance

• Privacy and discrimination issues

• Incorrect or misleading information provided by DTC or clinical genetic testing

What do the results of genetic testing mean?

• **Positive result**. A positive test result means that the laboratory found a genetic variant that is associated with an inherited cancer susceptibility syndrome. A **positive** result may:

• For a person who has cancer, confirm that the cancer was likely due to an inherited genetic variant and help guide treatment choices.

• Indicate an increased risk of developing certain cancer(s) in the future and guide future management to lower that risk.

• Provide important information that can help other family members make decisions about their own health care, such as whether to have genetic testing to see if they have also inherited the variant.

• People who have a positive test result that indicates that they have an increased risk of developing cancer in the future may be able to take steps to lower their risk of developing cancer or to find cancer earlier, including:

• Being checked at a younger age or more often for signs of cancer.

• Reducing their cancer risk by taking medications or having surgery to remove "at-risk" tissue. (These approaches to risk reduction are options for only a few inherited cancer syndromes.)

• **Changing personal behaviors** (like quitting smoking, getting more exercise, and eating a healthier diet) to reduce the risk of certain cancers.

• Getting help to guide decisions about fertility and pregnancy.

• **Negative result.** A negative test result means that the laboratory did not find the specific variant that the test was designed to detect.

• Such a test result is called a **true negative**. A true negative result does not mean that there is no cancer risk, but rather that the risk is probably the same as the cancer risk in the general population.

• When a person has a strong family history of cancer but the family has not been found to have a known variant associated with a hereditary cancer syndrome, a negative test result is classified as an **uninformative negative** (that is, it typically does not provide useful information).

• In the case of a **negative test** result, it is important that the person's doctors and genetic counselors ensure that that person is receiving appropriate cancer screening based on that person's personal and family history and any other risk factors they may have. Even when the genetic testing is negative, some individuals may still benefit from increased cancer surveillance.

Breast Cancer

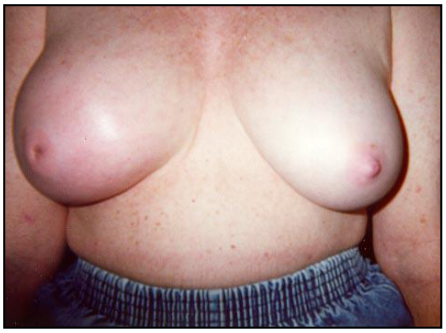
A.O., female, aged 32, Iasi

• Presented with global enlargement, induration of the skin and peau d'orange aspect of right breast (occurred in February 2006, during breast-feeding).

Fine needle aspiration biopsy:

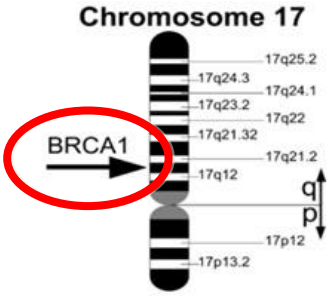
- malignant cytology, with aspects of ductal invasive carcinoma (G3)

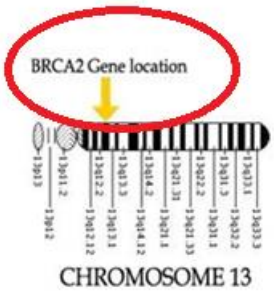
Breast and ovarian cancers

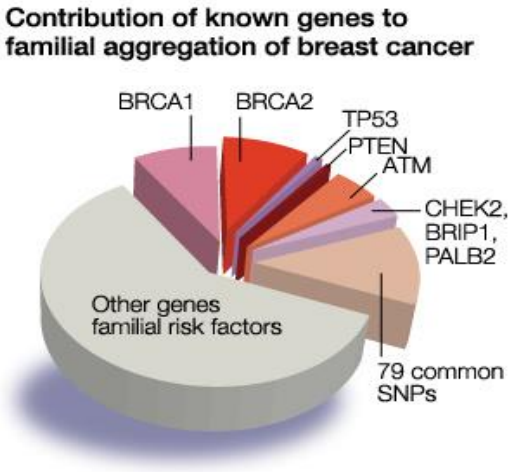


★ The majority (90%) of hereditary breast and ovarian cancers are associated with mutations in two genes: breast cancer type 1 and 2 susceptibility **genes (BRCA1 and BRCA2).**

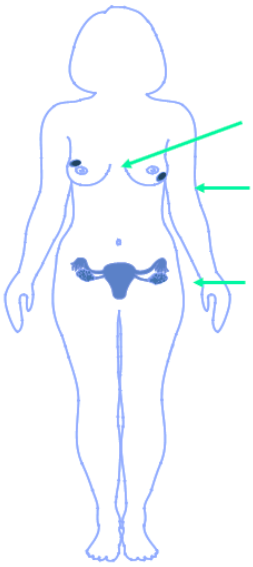
★ Less commonly, breast cancer is due to other hereditary cancer syndromes, such as **Li-Fraumeni** and **Cowden syndromes**, which are related to mutations in the **TP53** and **PTEN** genes (phosphatase and tensin homolog)







Cancers associated with BRCA1 / 2 mutations: Lifetime Risk



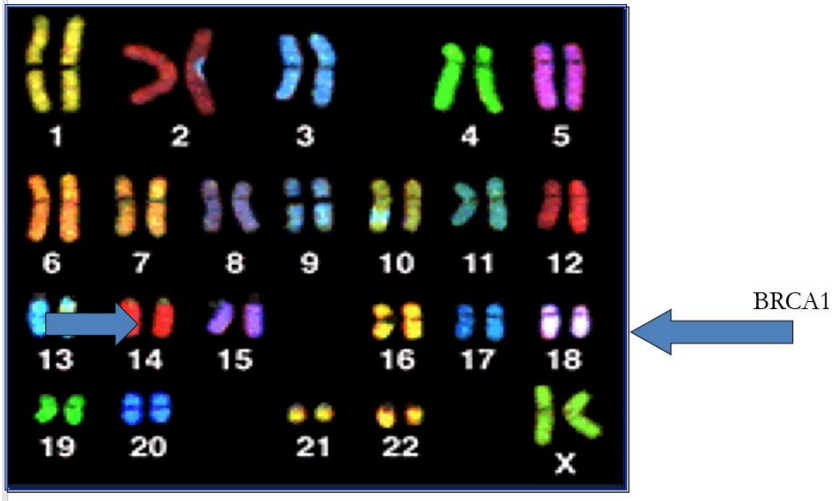
Breast Cancer 60-80%

Second Primary Breast Cancer 40-60%

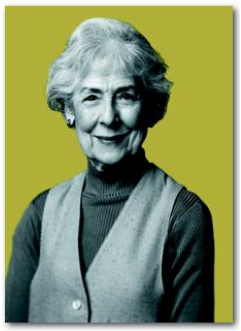
Ovarian Cancer 30-45%

Other types of Cancer:
Colorectal: 10-15%
Male with Breast Cancer: 1-5%
Pancreas: 2-3%

Each cell contains 2 copies of the BRCA1 and BRCA2 genes



BRCA1 / 2 are equally important in the occurrence of sporadic cancers in breast and ovarian cancers by losing alleles in 30-70%; 5-10% with genetic predisposition

	40 ani	50 ani	70 ani
			
Risk in the general population	0.5%	2%	7%-12%
Hereditary risk	10%-20%	33%-50%	**56%-87%**

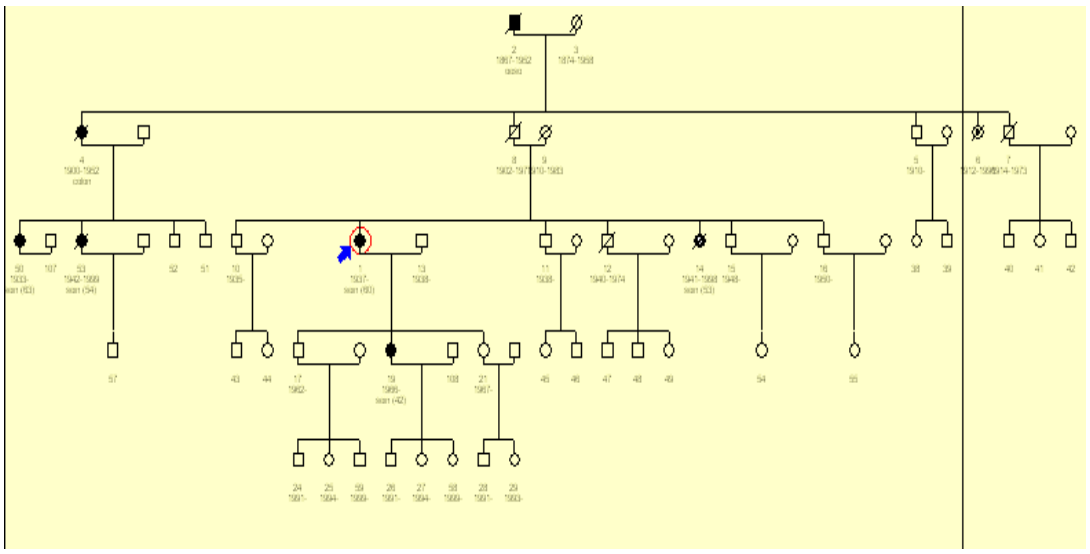


Table 4. Estimates of Risk (Penetrance) of Breast Cancer for *BRCA1/2* Carriers Classified by Proband Characteristics

	Estimated Cumulative Risk (95% CI), %, by Age, y		
	50	70	80
Breast cancer proband			
Unilateral	20 (11-32)	40 (26-58)	50 (31-71)
Contralateral	32 (20-44)	51 (36-69)	57 (42-78)
BRCA1 proband			
Unilateral	30 (16-46)	36 (21-58)	58 (30-80)
Contralateral	38 (23-54)	48 (30-67)	58 (38-81)
BRCA2 proband			
Unilateral	9 (2-22)	47 (25-100)	47 (25-100)
Contralateral	22 (6-40)	59 (30-84)	60 (34-92)
Proband age group, y			
<35	34 (16-56)	52 (29-100)	95 (39-100)
35-44	32 (20-44)	50 (35-66)	54 (38-75)
≥45	14 (5-24)	36 (18-56)	44 (24-68)

Abbreviation: CI, confidence interval.

Indications for testing

A. Individual from a family with a known deleterious BRCA1/BRCA2 mutation

B. Personal history of breast cancer plus one or more of the following:

- Diagnosed age ≤45 years
- Diagnosed age ≤50 years with ≥1 first, second, or third-degree blood relative with breast cancer ≤50 years and/or ≥1 first, second, or third-degree blood relative with epithelial ovarian/fallopian tube/primary peritoneal cancer at any age
- Two breast primaries when first breast cancer diagnosis occurred ≤50 years
- Diagnosed ≤60 years with a triple negative breast cancer
- Diagnosed ≤50 years with a limited family history
- Diagnosed at any age with ≥2 first, second, or third-degree blood relatives with breast and/or epithelial ovarian/fallopian tube/primary peritoneal cancer at any age
- Diagnosed at any age with ≥2 first, second, or third-degree blood relatives with pancreatic cancer at any age
- First, second, or third-degree male blood relative with breast cancer

- For an individual of ethnicity associated with higher mutation frequency (e.g., Ashkenazi Jewish), no additional family history may be required

C. Personal history of epithelial ovarian/fallopian tube/primary peritoneal cancer

D. Personal history of male breast cancer

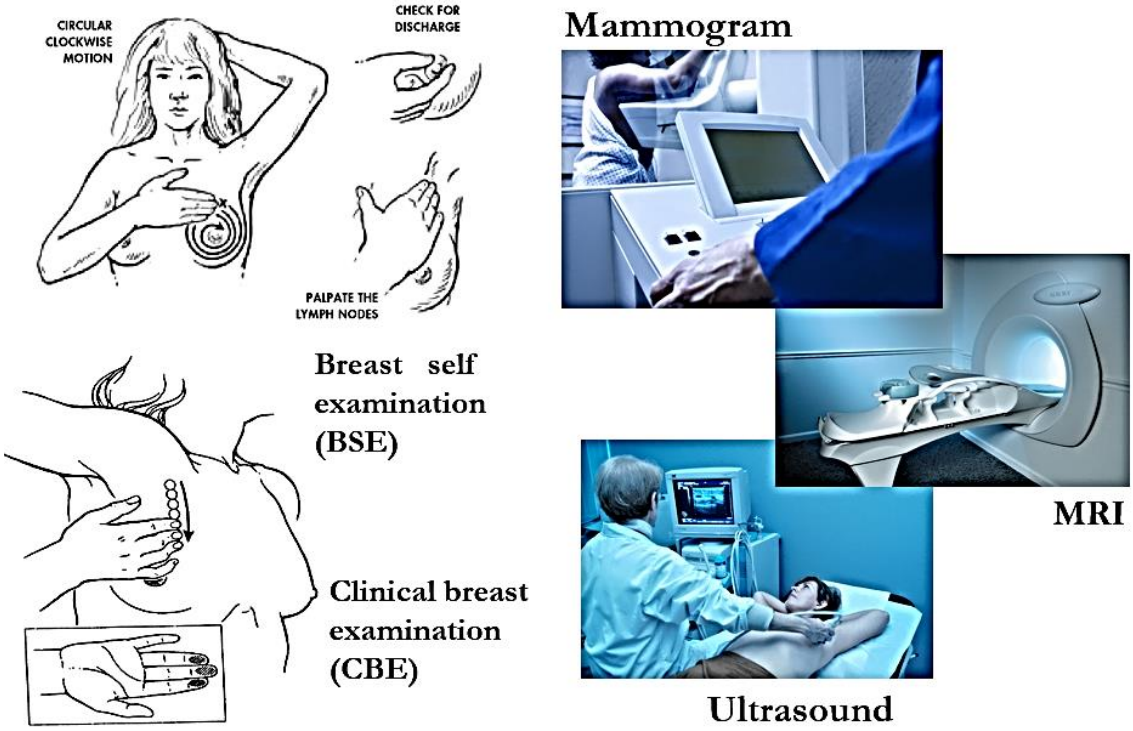
E. Personal history of pancreatic cancer at any age with ≥2 first, second, or third-degree blood relatives with breast and/or ovarian cancer and/or pancreatic cancer at any age

F. Family history only:

- First or second-degree blood relative meeting any of the above criteria
- Third-degree blood relative with breast cancer and/or ovarian/fallopian tube/primary peritoneal cancer with ≥2 first, second, or third-degree blood relatives with breast cancer (at least one breast cancer ≤50 years) and/or ovarian/fallopian tube/primary peritoneal cancer.

G. Hereditary disease with a risk of breast cancer (Cowden, Werner, Bloom, Fanconi, Peutz-Jeghers etc.)

Breast cancer: screening



Monitoring of people with BRCA1 / 2 gene mutations

Procedure	Age To Begin	Frequency
Self breast exam	18 years	Monthly
Clinical breast exam	30 years	Twice/year
Mammography	30 years	Yearly
MRI	25 years	Yearly

www.nccn.org
Cancer 2004
NEJM 2004

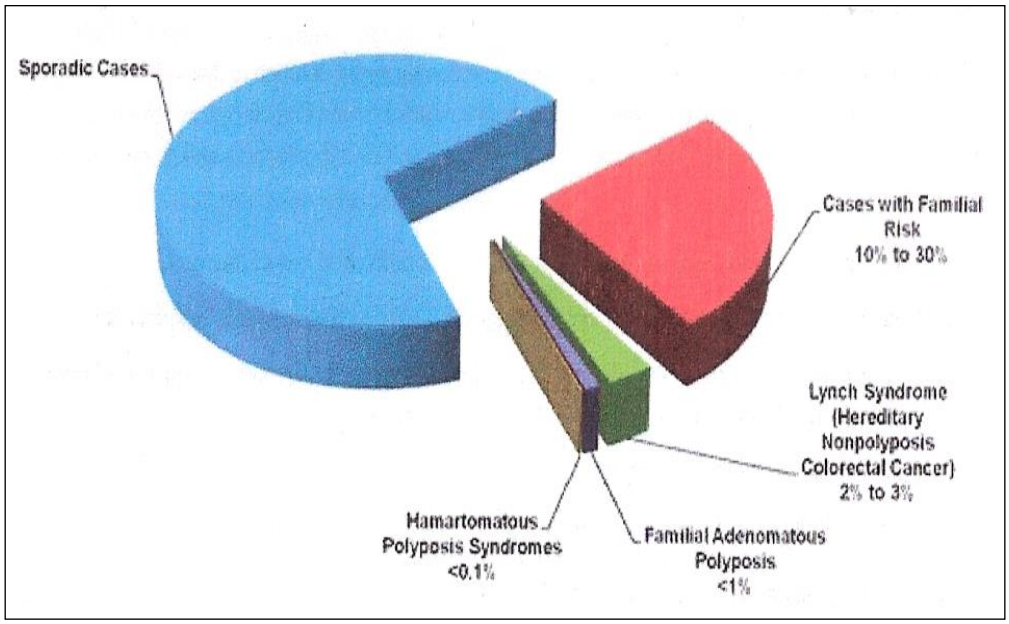
Colo-rectal cancer

Genetic and epidemiological classification of CRCs

1. **Hereditary CRC syndromes** (5-10%)

Lynch (HNPCC) syndrome
FAP syndrome
Familial CRC (25-30%)

2. **Sporadic CRC** (60-70%)



Colorectal cancers arising in various family risk setting *(National Cancer Institute, USA, 2014)*

Familial colo-rectal cancers

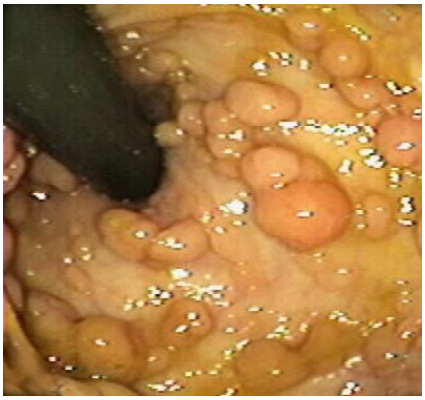
• **Approximately 5% -6%** of RCCs are associated with germline mutations that confer a hereditary predisposition to family RCCs.

• The family CCR syndromes are:

- Lynch Syndrome

- Syndromes associated with APC mutations: classical FAP / Attenuated FAP (AFP) / MUTYC-associated polyposis (MAP) / Type X family CCR

• Rare hereditary entities: Peutz Jaggers Syndr ome



Polyposis colon cancer syndromes

• Familial colonic polyposis (FAP) is caused by a germline mutation of the **APC** (adenomatous colon polyposis) gene that occurs in 1 in 10,000 individuals.

Familial adenomatous polyposis (FAP)

• Incidence: 1 in 5000-10,000 1% of all CCR Estimated penetration for adenomas> 90% .

• Extracolonic tumor risk (upper GI, desmoids, osteoma, thyroid, brain, other)

Hallmark:> 100 to 1000 adenomatous in colorectum Untreated polyposis leads to 100% cancer risk

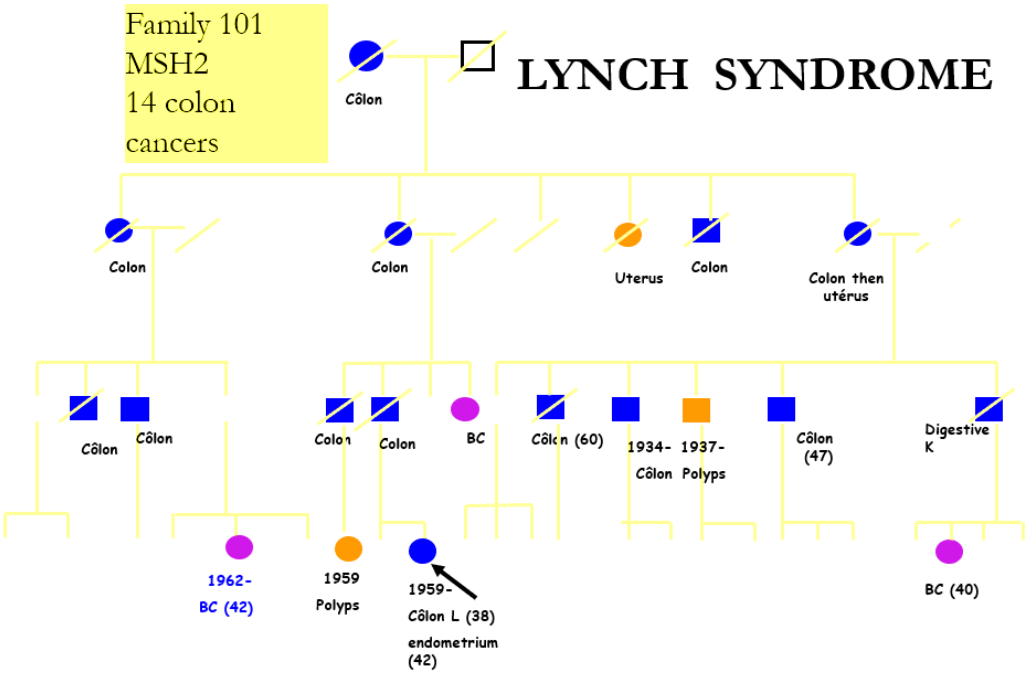
Polyposis syndromes

• AP classic adenomas at the colon and rectum <1% of CCR associated with germline mutations of APC

• Attenuated FAP (AFAP)> 20 colo-rectal adenomas but <100 adenomas with mutations of APC and MUTYH- have an increased risk of CCR.

• MUTYH associated FAP (MAP) at age 50 <100 adenomas.

• Type X family CRC



Non-poylposis hereditary cancers (HNPCC)

✳ Non-polyposis hereditary cancer (HNPCC) refers to Lynch syndrome and is a dominant autoimmune genetic disease that has an increased risk of colon cancer (2-3%), as well as other types of cancer, including: endometrium, ovary, stomach, small intestine, hepato-biliary tract, urinary tract, brain and skin .

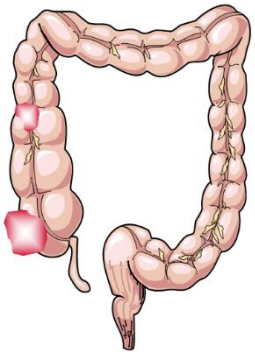
✳ Defect HNPCC in DNA repair asymmetry leads to microsatellite instability also known as MSI-H, which is the HNPCC brand. Mutations in 6 mating defect repair **(MMR) genes: MLH1, MSH2, MSH6 and PMS2 and or EPCAM / TACSD1 (ASCO addition)**

✳ Microsatellites are pieces of DNA sequences in which a single nucleotide or nucleotide group is multiplied multiple times.

✳ Increased MSI = MSI-H

✳ Three major groups of MSI-H (MSI, MicroSatelite instability) cancers can be recognized by histopathological criteria: (1) right localization, poorly differentiated cancers (2) mucinous cancers (3) adenocarcinomas in any location showing any measurable level of intraepithelial lymphocytes (TIL)

Clinical features of non-polyposis colon cancer (HNPCC)



• HNPCC cancers can be recognized by histopathological criteria:

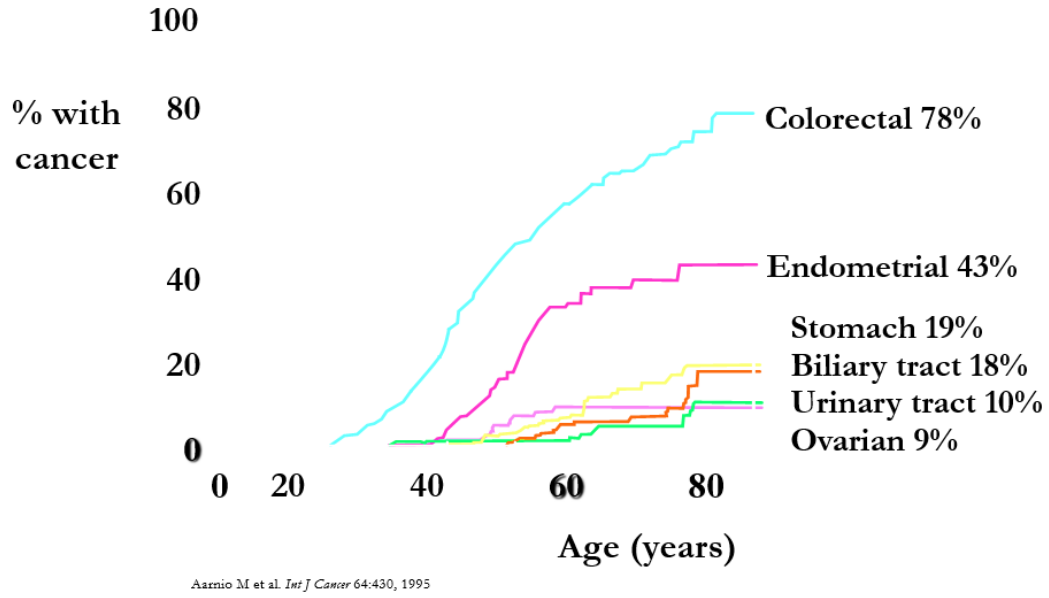
• (1) right location, poorly differentiated cancers

• (2) mucinous cancers

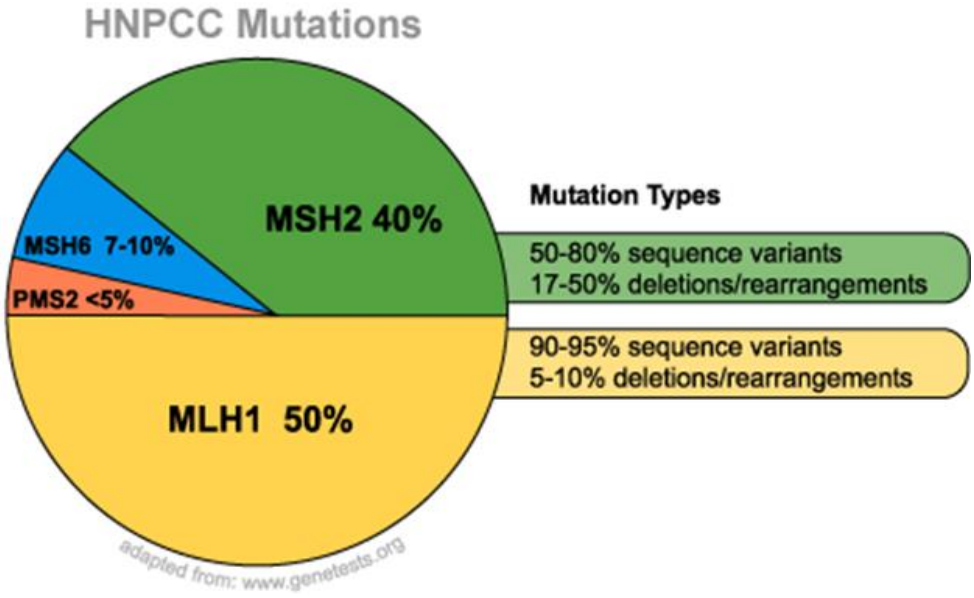
• (3) adenocarcinomas at any location showing any measurable level of intraepithelial lymphocytes (TIL)

• Extracolonic cancers: endometrium, ovary, stomach, pancreas, kidney and ureter, small intestine, bile, urinary and brain

Cancer risk in HNPCC



Aarnio M et al. *Int J Cancer* 64:430, 1995



Revised Bethesda Guidelines for testing Colorectal tumors 1997 (revised in 2004)

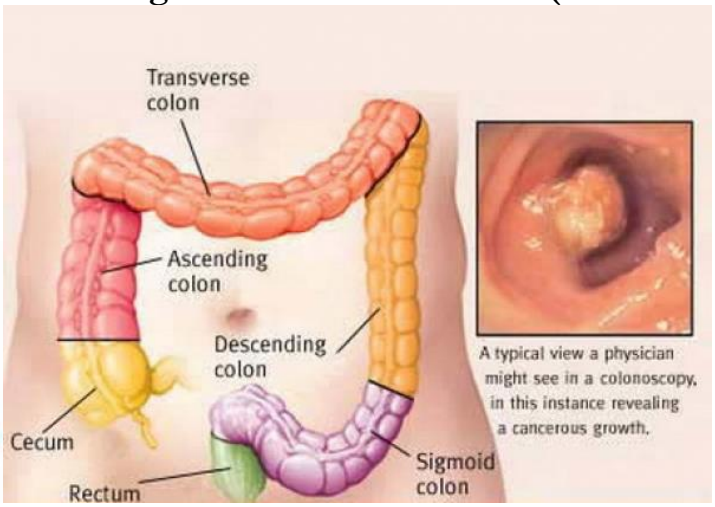


Table 1

Revised Bethesda Guidelines for Testing Colorectal Tumors for Microsatellite Instability

Tumors should be tested for microsatellite instability in the following situations:

1. Colorectal cancer diagnosed in a patient who is less than 50 years of age
2. Presence of synchronous, metachronous colorectal, or other HNPCC-associated tumors,[a] regardless of age
3. Colorectal cancer with the MSI-H[b] histology[c] diagnosed in a patient who is less than 60 years of age[d]
4. Colorectal cancer diagnosed in one or more first-degree relatives with an HNPCC-related tumor, with one of the cancers being diagnosed under age 50 years
5. Colorectal cancer diagnosed in two or more first- or second-degree relatives with HNPCC-related tumors, regardless of age

[a] HNPCC-related tumors include colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, and brain (usually glioblastoma as seen in Turcot syndrome) tumors, sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome, and carcinoma of the small bowel.

[b] MSI-H in tumors refers to changes in two or more of the five National Cancer Institute–recommended panels of microsatellite markers.

[c] Presence of tumor-infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern.

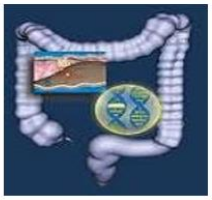
[d] There was no consensus among the Workshop participants on whether to include the age criteria in guideline 3 above; participants voted to keep less than 60 years of age in the guidelines.

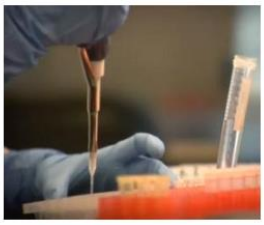
HNPCC = hereditary nonpolyposis colorectal cancer; MSI-H = microsatellite instability–high.

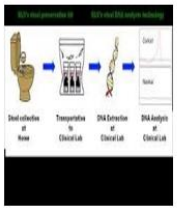
Adapted from Umar A et al.[37]

Faeces DNA Tests

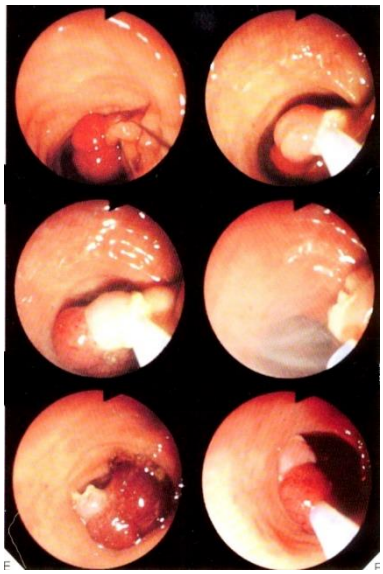
- Non-invazive
- No bowel preparation
- No dietary restrictions
- Based on single stool sample
- Detects proximal and distal neoplasms equally well
- Screening studies on-going





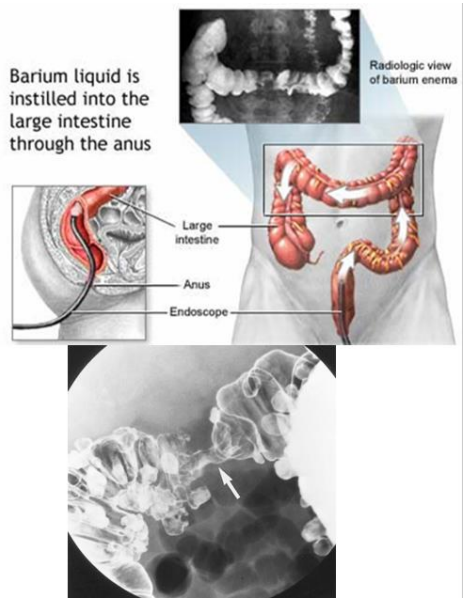


Colonoscopy



- **The best screening method**
- **It visualizes in 90% of cases the entire lining of the colon**
- **Biopsy / Polypectomy / lesion marking**
- **The standard in polygons**
- **Basis - risk stratification for screening**
- **Rare severe complications**
- **High cost**
- **In 2012- "Flexible sigmoidoscopy screening reduces deaths through CCR!**
- **A large study of 154,000 patients followed for 11.9 years showed a 21% reduction in the incidence of RCC and deaths by 26%**

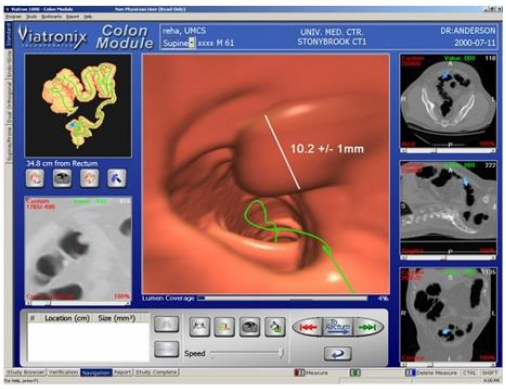
(N. Engl J Med 2012; 366; 2345-2357)

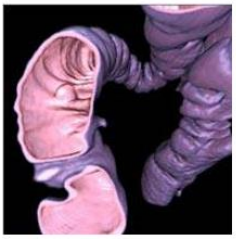


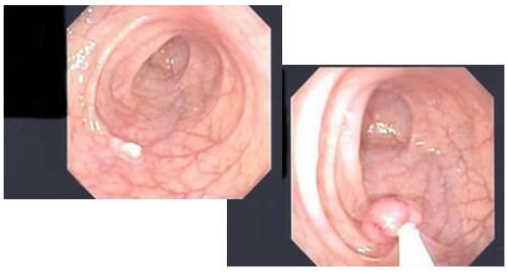
Double-Contrast Barium Enema (DCBE)

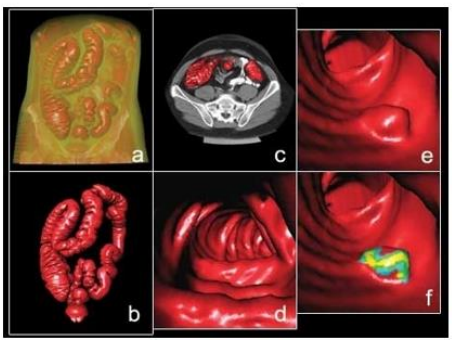
- Low sensitivity
- Extensive bowel preparation
- High cost
- Lack of well designed studies

"Classic" & Virtual Colonoscopy

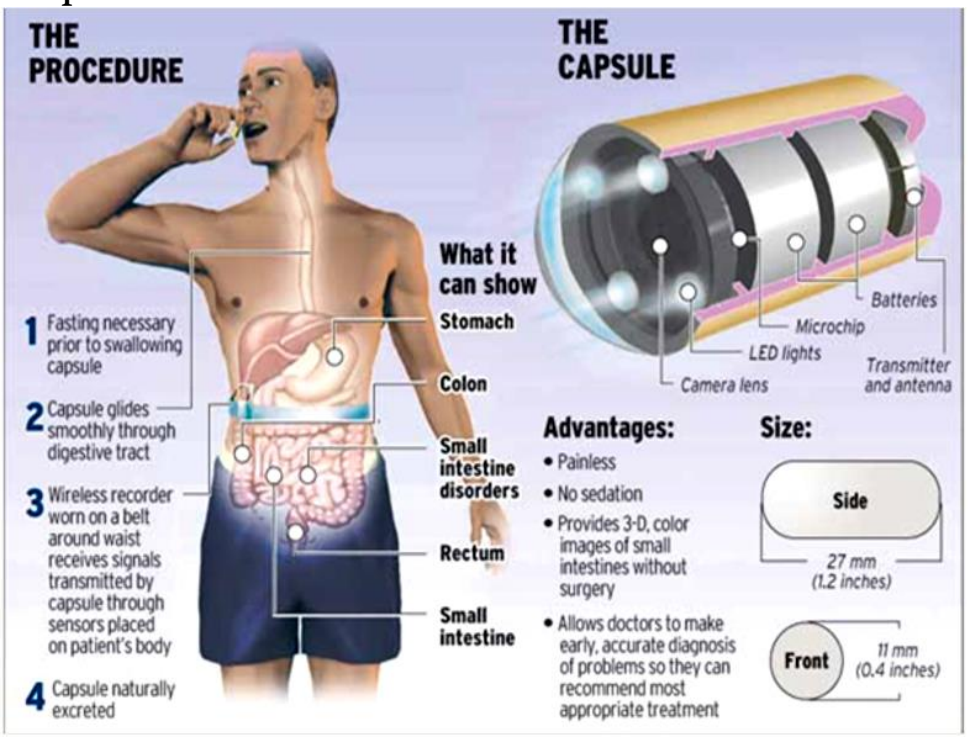








Endoscopic capsule



A colorectal cancer-Screening recommendations

• CCR screening starts at age 50

• In people with a family history of colon cancers that occurred in predecessors at the age of <60 years, it is recommended to initiate ascension at 10 years older than the youngest age at which the relative of grade I appeared.

Screening

• CR: colonoscopy every 1-2 years, starting from 20-25 years or 5 years before the youngest case in the family. No age limit!

• Breast and ovarian cancer: e.g. gynecological, pelvic and pelvic ultrasound (No CA-125) and aspiration biopsy every year from the age of 30 to 35 years. Hysterectomy and salpingo-ovarectomy can be considered when children are not desired.

• Gastric cancer: detection of the presence of Helicobacter pylori and its eradication in mutation carriers. In people with high incidence of CG, some people recommend fibroscopy every 1-3 years.

• Other Lynch cancers: Periodic surveillance is NOT recommended due to reduced sensitivity and specificity

Lynch Syndrome-screening

• **CCR:** colonoscopy every 1-2 years, starting from 20-25 years or 5 years before the youngest case in the family. No age limit!

• **Breast and ovarian cancer**: e.g. gynecological, pelvic and pelvic ultrasound (No CA-125) and aspiration biopsy every year from the age of 30 to 35 years. Hysterectomy and salpingoovarectomy can be considered when children are not desired.

• **Gastric cancer**: detection of the presence of *Helicobacter pylori* and its eradication in mutation bearers. In people with high incidence of CG, some people recommend fibroscopy every 1-3 years.

• **Other Lynch cancers**: periodic surveillance of other cancers based on familial history (ex. urinary tract cancers).

Chemoprevention

• Chemoprevention measures are not included in the ESMO guide!

• Recent data from the Colo Rectal Adenoma / Carcinoma Prevention Program indicated a 60% reduction in the risk of RCC associated with Lynch syndrome if Aspirin 600 mg / and at least 2 years randomized trial is administered. The optimal dose is not known.

• There is not enough evidence to recommend the Aspirin routine!

• For FAP the absence of effective chemoprevention data.

• Data on the oil NSAIDs, Sulindac and Celecoxib can be considered as a treatment for reducing the number of polyps in the colon and not for duodenal! Cardiac side effects!

Take Home Message

• Cancer is not usually inherited, but some types – mainly breast, ovarian, colorectal and prostate cancer – can be strongly influenced by genes and can run in families.

• Genetic testing is now available for some hereditary cancers.

• Even if a cancer susceptibility variant is present in a family, it does not necessarily mean that everyone who inherits the variant will develop cancer.

• Genetic testing involves a simple blood test and may be used to obtain a more precise estimate of your cancer risk.

• Genetic counseling for hereditary cancer is important.

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• Shaman KM, Chitenden A. Genetic testing by cancer site: breast. In Matloff ET (ed) Cancer Principles and Practice of Oncology-Handbook of clinical cancer genetics. Wolters Kluwer/Lipincott Williams&Wilkins.2013: 71-92.

• Anna P. Sokolenko, Evgeny N. Imyanitov Molecular Diagnostics in Clinical Oncology.*Biology,MedicinePublishedMol.Biosci.*2018, 2:10.3389/fmolb.2018.00076

II.12.2. Monitoring of patients with hereditary colorectal cancers (adaptation of medical care - personalized medicine)

Learning objectives

• understand the management in hereditary colorectal cancer
• know the surveillance of individuals at increased risk of developing hereditary CCR (HCRC)
• Lynch syndrome
• Hereditary Polyposis Syndrome
• know the general information regarding post-treatment monitoring and the particularities in the monitoring of the patient diagnosed with hereditary colorectal cancer (HCRC)

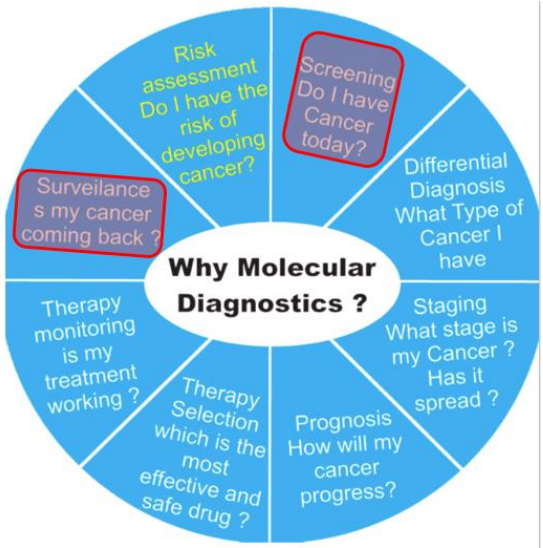
Introduction

Personalized medicine tailors medical management to a patient's personal history, genomic profile and/or specific biomarkers.

The genetics and epigenetics of colon cancer are well characterized, and biomarkers for the early detection are known. All these facts provide an opportunity for a preventive cancer approach.

I. Management in hereditary colorectal cancer

- Determining the individual risk of hereditary colorectal cancer
- **Surveillance of high risk individuals**
- Early diagnosis of cancer
- Staging of the identified tumor
- Screening of extra-colonic cancers
- Establishing the prognosis of the neoplastic disease
- Selection of appropriate therapy
- Monitoring the oncological treatment
- **Post-treatment follow-up with the purpose of early identification of possible tumor recurrence / metastasis**



II. Surveillance recommendations for individuals at high risk (healthy mutation carriers) - NPHCRC: Lynch Syndrome-

TYPE OF CANCER	SURVEILLANCE	
	methods	protocol
Colorectal cancer	- colonoscopy every 1–2 years - chromoendoscopy with indigo carmine added to the standard colonoscopy is more effective than colonoscopy alone	- starting at age 20-25 years or starting 5 years before youngest case in the family - no upper limit is set for stopping the surveillance.

Endometrial and ovarian cancer	- gynaecological examination, - transvaginal ultrasound (TV US) + • cancer antigen 125 (CA 125) analysis for ovarian cancer • hysteroscopy for endometrial surveillance endometrial biopsy	- Regular gynaecological surveillance visits and prophylactic surgery are recommended - Surveillance for endometrial and ovarian cancer must systematically be adapted to family history! yearly, starting at age 30-35 years. - Discuss in a multidisciplinary consultation team about prophylactic hysterectomy with bilateral oophorectomy for mutation carriers who have completed child-bearing or are postmenopausal • if an endometrial cancer is described at 30 years of age in the family, hysterectomy could be performed before post-menopausal state

TYPE OF CANCER	**SURVEILLANCE**	
	methods	**protocol**
Gastric cancer	- testing and treating Helicobacter pylori (H. pylori) in mutation carriers. - upper digestive endoscopy	- upper endoscopy every 1–3 years, starting at the age of 30–35 years (in regions with high GC incidence and in families with a history of gastric neoplasms)
Other cancers associated with sdr Lynch	- Screening for other cancers associated with sdr Lynch must systematically be adapted to family history! - surveillance is not routinely recommended due to low sensitivity and specificity. - to be considered: - *pancreatic surveillance* - annual magnetic resonance imaging (MRI) and/or endoscopic ultrasound (EUS) surveillance - *urinary tract* in case of family history (1st degree relative with cancer) - cancers of the upper urinary tract and the urinary bladder are predominantly linked to MSH2 mutations (surveillance should be targeted at individuals with mutations herein).	
Lynch syndrome families without mutation could have Lynch screening (1st degree relatives).		

II. Surveillance recommendations for individuals at high risk (healthy

mutation carriers) - NPHCRC : Familial CRC X syndrome -

Type of cancer	Cancer risk	SURVEILLANCE	
Colorectal cancer	- Moderate, only CRC	- Colonoscopy 3-5 years	- Starting at age 40 years or starting 5-10 years before youngest case in the family

II. Surveillance recommendations for individuals at high risk (healthy mutation carriers) - HPCRC: hereditary adenomatous polyposis –

TYPE OF CANCER	SURVEILLANCE	
	methods	protocol
Colorectal cancer	Flexible sigmoidoscopy Colonoscopy	- *In patients with classical FAP* - flexible sigmoidoscopy /colonoscopy should be carried out every 2 years, starting at age 12–15 years until diagnosis of adenomas. - once adenomas are detected, colonoscopy should be carried out every 1–2 years until colectomy is planned. - *In patients with APC-attenuated FAP (AFAP)* - colonoscopic surveillance should be done every 1–2 years, starting at the age of 18–20 years - once adenomas are detected, colonoscopy should be carried out yearly until colectomy is planned.

TYPE OF CANCER	SURVEILLANCE	
	methods	protocol
Duodenal adenomas	Upper digestive endoscopy	- every 5 years starting at 25–30 years of age or at the time of diagnosis of colonic polyposis for both classical FAP and AFAP patients - If adenomas are detected, surveillance is guided by the Spigelman classification: - Spigelman stage I - every 5 years, Spigelman stage II - every 3 years, Spigelman stage III - every 1–2 years, Spigelman stage IV every 6 months or prophylactic surgery for - additional side-viewing endoscopic surveillance is recommended for patients with Spigelman stages III and IV and/or papillary involvement.
Thyroidian cancer	annual thyroid palpation and/or US	- yearly, starting at age 25-30 years
Desmoid tumours	CT/MRI	- in case of positive family history for desmoid tumors

Other cancers associated with PAF	**For other cancers localisations than colon, duodenal, thyroid, desmoid, associated to spectrum, the screening must be systematically adapted to family history!** **Screening for other extracolonic cancers is not justified due to their low prevalence and / or limited clinical impact** ***biallelic *MUTYH* mutation carriers are at increased risks of developing *urinary bladder* and *ovarian cancers* and monoallelic carriers are at increased risks of *gastric, liver, breast*, and *endometrial cancers*.**

Spigelman classification for duodenal polyposis in familial adenomatous polyposis (FAP)

Variable	1 point	2 points	3 points
Number of polyps	1–4	5–20	>20
Polyp size (mm)	1–4	5–10	>10
Histology	tubular	tubulovillous	villous
Dysplasia	mild	moderate	severe
Stage 0 - 0 points; Stage I - 4 points; Stage II - 5–6 points; Stage III - 7–8 points; Stage IV - 9–12 points.			

III. Post-treatment monitoring of the patient diagnosed with colorectal cancer- overview-

- Post-treatment monitoring plan
- objectives:
- detection and prevention of adverse effects of therapy
- early detection of the recurrences
- providing psychological support

- The protocol for monitoring the patient diagnosed with colorectal cancer consists of regular medical visits and specialized investigations
  - it is adapted (personalized) to the type of cancer, the stage of the disease and the type of treatment administered
  - evaluation visits must include:
    - anamnesis
    - complete clinical examination
    - determining the carcinoembryonic antigen (ACE) useful in detecting recurrences
    - determining other markers depending on the type of the cancer (eg CA 125)

- **standard sigmoidoscopy / colonoscopy or chromoendoscopy**
- **imaging investigations (ultrasound, CT, MRI, chest x-ray) in order to detect the eventual progression or recurrence of the primary tumour or the identification of metastases**

IV. Particularities in the post-treatment monitoring of the patient diagnosed with hereditary non-polyposis colorectal cancer

SYNDROME	DIAGNOSIS OF INDEX CASE (with cancer)	TREATMENT	**FOLLOW-UP**
Lynch	- Clinical: Amsterdam, Bethesda - Molecular screening (tumour tissue) MSI and/or IHC for MMR proteins - Germline genetic testing: MLH1,MSH2, MSH6,PMS2, EPCAM	- tumor resection - other treatments depending on the stage	**Yearly endoscopy of the remnant colon or rectum**
Familial CRC X	- Clinical: Amsterdam, Bethesda - Molecular screening (tumour tissue) No MMR deficiency - Germline genetic testing: unknown	- tumor resection - other treatments depending on the stage	**As average population**

SYNDROME	**DIAGNOSIS OF INDEX CASE (with cancer)**	**TREATMENT**	**FOLLOW-UP**
Familial adenomatous polyposis - complete form (FAP)	- **Colonoscopy** > 100 adenomas - **Molecular screening** (tumor tissue) - none - **Germline genetic testing**: APC	- Total or subtotal colectomy when adenomas occur - Endoscopic removal of duodenal adenomas	- After subtotal colectomy: rectal examination q 6-12 m - After total colectomy – pouch exam q 1-2 years - Duodenoscopy from 6 months to 5 years according to Spigelman stage - Thyroid examination yearly
Atenuated familial adenomatous polyposis (aFAP)	- **Colonoscopy** - a. 2 relatives 10-99 adenomas (> 30 years of age) - b. 1 relative of CRC patient with 10-99 adenomas (> 30 years of age) - **Germline genetic testing**: APC	- Total or subtotal colectomy when adenomas occur - Endoscopic removal of duodenal adenomas	- After subtotal colectomy: rectal examination q 6-12 m - After total colectomy – pouch exam q 1-2 years - Duodenoscopy from 6 months to 5 years according to Spigelman stage
APC – adenomatous poliposys coli, MSI – microsatellite instability, MMR – mismatch repair proteins, FAP - Familial adenomatous polyposis , aFAP - Atenuated familial adenomatous polyposis, MAP- MUTIH associated polyposis			

SDR	**DIAGNOSIS OF INDEX CASE (with cancer)**	**TREATMENT**	**FOLLOW-UP**
MAP	**Clinical**: biallelic MUTYH mutations should be suspected **in patients:** - with FAP or aFAP with a recessive pattern of inheritance. - diagnosed with CRC before	**Endoscopic treatment** - Resection of all identified polyps if possible **Surgery** when the polyps cannot be controlled	**Colorectal surveillance:** - After surgery is carried out, it is recommended to continue with 1–2-year surveillance intervals of the remaining colorectal segment **Gastric and small bowel**

	the age of 50 years - with >10 colonic polyps (adenomatous / serrated). **Colonoscopy** - first colonoscopy with chromoendoscopy at the age of 20, then at 25 and 30 years in case of normality and at least every 2 years from this age. **Germline genetic testing:** - multigene single analysis of the genes involved in colorectal adenomatous polyposis (APC, MUTYH, POLE, POLD1, NTHL1) (overlap of the clinical phenotype of polyposis syndromes).	endoscopically: - colectomy with ileorectal anastomosis should be considered in absence of rectal involvement; - if rectal involvement is substantial, a total proctocolectomy with ileo-anal anastomosis is indicated (when sphincter preservation is possible).	**surveillance**: - the surveillance strategy with upper digestive endoscopy with chromoendoscopy is determined based on the monitoring of duodenal polyps, carrying out a first endoscopy at at the age of 25 years, then, in case of normality, at 30 years and finally at a rate depending on the existence and the severity of the duodenal involvement assessed using the Spigelman score **Skin** - Dermatological examination for the identification and treatment of "sebaceous" lesions;
APC – adenomatous poliposys coli, FAP - Familial adenomatous polyposis , aFAP - Atenuated familial adenomatous polyposis, MAP- MUTIH associated polyposis			

Take Home Message

- For individuals with hereditary colorectal cancer, prevention and early detection by active surveillance can increase survival and improve quality of life.
- Specialists involved in the care of patients with gastrointestinal cancer should be familiar with the main hereditary cancer syndromes and refer patients to specialised cancer genetic units for adequate genetic counselling and to address specific concerns associated to each genetic susceptibility.
- The surveillance protocol may be tailored according to the genetic alteration and family history of cancer

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Hereditary non-polyposis colorectal cancer syndrome

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II.12.3. Monitoring of patients with endocrine hereditary cancers (adaptation of medical care-personalized medicine) Endocrine tumours

Learning objectives

- **Understanding the necessity of life-long follow-up of patients with MEN syndrome.**
- **To be able to select the patients for monitoring according the age and MEN type.**
- **To be able to plan the monitoring visits and tests necessary for each specific patient.**

Introduction

• Monitoring in hereditary endocrine tumors aims to follow the patient after diagnosis from the clinical, imaging and hormonal point of view.

• Hormonal dosages are basal and dynamic (inhibition tests) and are performed at time intervals depending on the type of tumour, age and treatment.

• Imaging exploration is variable and depends on the gland being explored, the type of tumour (aggression, extension, multiple touch) and the method used (ultrasonography, computer tomography, nuclear magnetic resonance imaging, scintigraphy, PET-CT).

Contents

• patient monitoring with MEN1
• MEN1 monitoring in mutation carrying children
• monitoring the patient with MEN2
• monitoring MEN2 children
• pheochromocytoma MEN2 monitoring
• take home message

Monitoring the patient with MEN1

The follow-up team will be multidisciplinary:

- endocrinology
- gastroenterology
- oncology
- radiology (nuclear medicine)
- surgery (endocrine, cardio-thoracic, pituitary, hepato-biliary)
- anatomopathologist (staging and grading)
- clinical genetics

• Regular checks even at 6-12 months and depending on the symptomatology, the patient remaining under lifelong supervision in a centre with experience.

• Relatives of asymptomatic, 1st degree carriers require clinical, biological and imaging control.

Screening patients with MEN1

• Individuals at high risk for MEN1 (i.e. mutant gene carriers) undergo biochemical screening at least once per annum and also have baseline pituitary and abdominal imaging (e.g. MRI or CT), which should then be repeated at 1- to 3-yr intervals.

• Screening should possibly commence in early childhood because the disease has developed in some individuals by the age of 5 yr, and it should be repeated throughout life because the disease may not manifest in some individuals until the eighth decade.

• Screening history and physical examination should be directed toward eliciting symptoms and signs of hypercalcemia, nephrolithiasis, peptic ulcer disease, neuroglycopenia, hypopituitarism, galactorrhea and amenorrhea in women, acromegaly, Cushing's disease, and visual field loss and the presence of sc lipomas, angiofibromas, and collagenomas.

• Biochemical screening should include estimations of serum calcium, PTH, gastrointestinal hormones (e.g. gastrin, insulin with a fasting glucose, glucagon, VIP, and pancreatic polypeptide), chromogranin A, prolactin, and IGF-I in all individuals, and more specific endocrine-function tests should be undertaken in individuals who exhibit symptoms or signs suggestive of a clinical syndrome.

• Radiological screening should include an MRI (or CT scanning) of the pancreas, adrenal glands, and pituitary, initially as a baseline and then every 1 to 3 yr, as well as imaging for thymic and bronchial carcinoids using CT or MRI every 1–2 yr.

Screening for MEN1

MEN1 manifestation	Screen Starting at Age	Clinical Screening	Annual Biochemical Tests	Imaging
Insulinoma	5 yrs	Syncope, light-headedness, documented hypoglycemia, ⇑growth	Fasting Glucose & Insulin	None
Pituitary neuroendocrine tumor	5 yrs [+]	Headaches, visual changes, galactorrhea, ⇑growth	Prolactin, IGF-1	Brain MRI (q3 years)
Parathyroid Adenoma/ 1º HyperPTH	8 yrs	Back pain, bone pain, weakness, fatigue, psychiatric changes, kidney stones, nausea, vomiting, constipation. Multiple or pathologic fractures.	Calcium [++]	None
Pancreatic NET	10 yrs	Generally not identified symptomatically. VIPoma can cause profuse diarrhea. Glucagonoma assoc. with hyperglycemia, nausea, polyuria, thirst.	(Chromogranin A, glucagon, proinsulin, pancreatic polypeptide, VIP) [+++]	Abdominal MRI (annually)
Adrenal adenoma	10 yrs	None	None	MRI (contemporaneous with pancreatic imaging)
Gastrointestinal, Bronchial and Thymic NETs	20 yrs	Frequently asymptomatic, but h/o flushing, diarrhea, wheezing, edema or abdominal pain should arouse suspicion		CT/MRI Chest and Abdomen (q1–2 years)
Gastrinoma (duodenal and pancreatic)	**20 yrs**	Abdominal pain, gastric ulcers. Proton-pump inhibitor usage.	Fasting Gastrin	None

[+] MRI surveillance is to begin once patient is able to tolerate a non-sedated MRI. In the authors' experience, this is generally at about the age of 5 years, but may be deferred on an individualized basis.

[++] Hypercalcemia on screening should prompt assessment with contemporaneous serum calcium and iPTH to establish a diagnosis of PHPT.

[+++] Pancreatic tumours may be non-secretory, therefore the added sensitivity contributed by biochemical screening has not been demonstrated.

(Data from Thakker et al. Ref: (5))

MEN1 monitoring in mutation carrying children

Patients carrying the mutation should be monitored annually, starting from childhood (**before 5 years of age**) in order to identify specific clinical manifestations :

1. Monitoring the annual growth rate and dosing of ionic calcium and PTH.
2. If the growth rate is high (gigantism) or low (prolactinoma, Cushing syndrome), specific tests are needed to diagnose these pathologies.
3. If there is a weight gain, a possible Cushing syndrome should be considered, specific tests being required to establish with certainty the respective pathology.
4. Routine screening for pituitary tumours is not required in the absence of symptoms until after the age of **10-15 years.**
5. Routine dosing of gastrin, peptide C, plasma insulin, PRL as well as imaging of the pituitary and abdomen are not required until **adolescence**.

Monitoring the patient with MEN2

• Patients should be monitored throughout their lives for early detection of recurrences. Checks are performed at 6 months or 12 months if the patient is asymptomatic.

• The evaluation includes :

1. clinical exam

2. plasma and urinary catecholamines
3. carcino-embryo antigen (CEA)
4. calcitonin
5. ionic calcium and PTH (in case of hypercalcaemia)

• The RET codon mutations (stratified into three levels of risk from MTC) can predict not only the MEN2 syndromic variant and the age of of onset of MTC but also the aggressiveness of MTC.

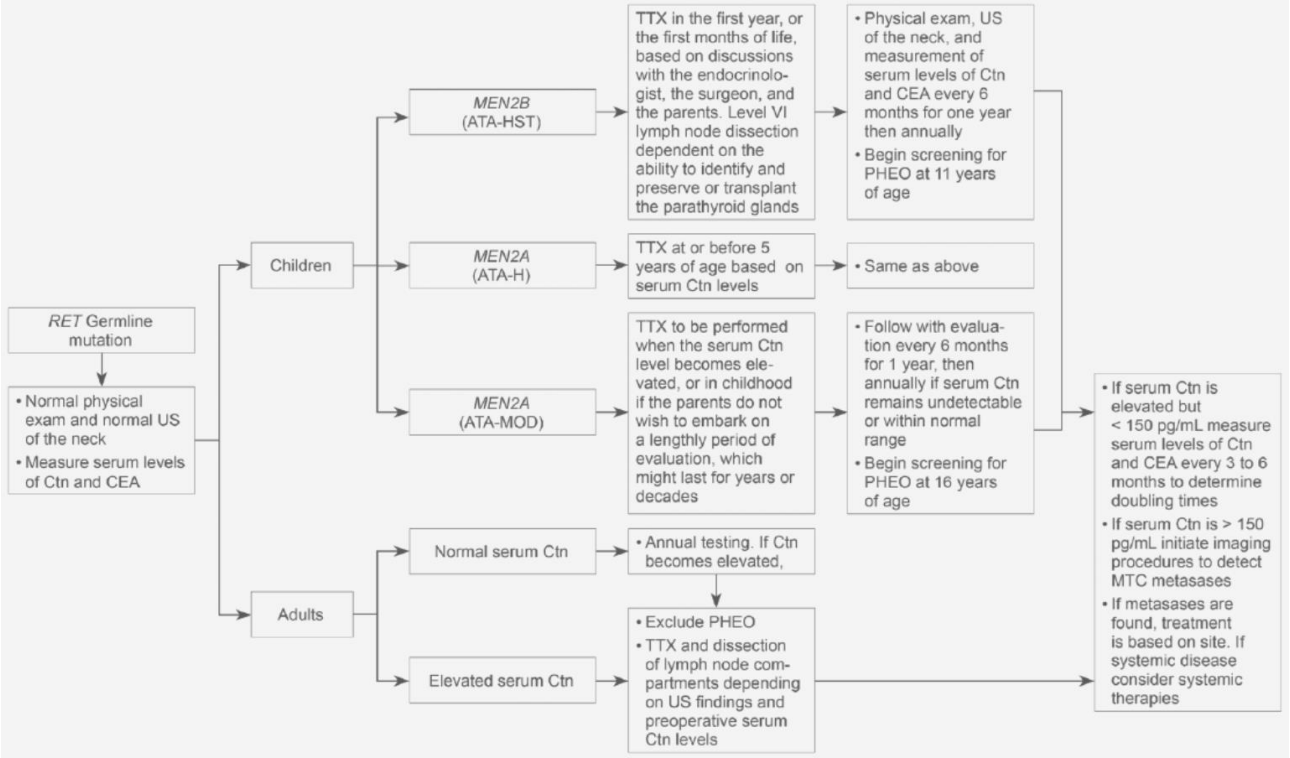
• Detailed recommendations about monitoring are derived from knowledge about the specific RET codon mutated and/or from a clear familial pattern.

Monitoring thyroid medullary carcinoma in MEN2

Follow-up is performed throughout the life, starting from 3 months post-operatively and then at longer intervals when there are no signs of relapse in the first post-operative year.

• If calcitonin is undetectable, it is measured every 6 months in the first year after surgery and then annually.

• Calcitonin may no longer be measured after a variable period of years - during which time the values remained undetectable.

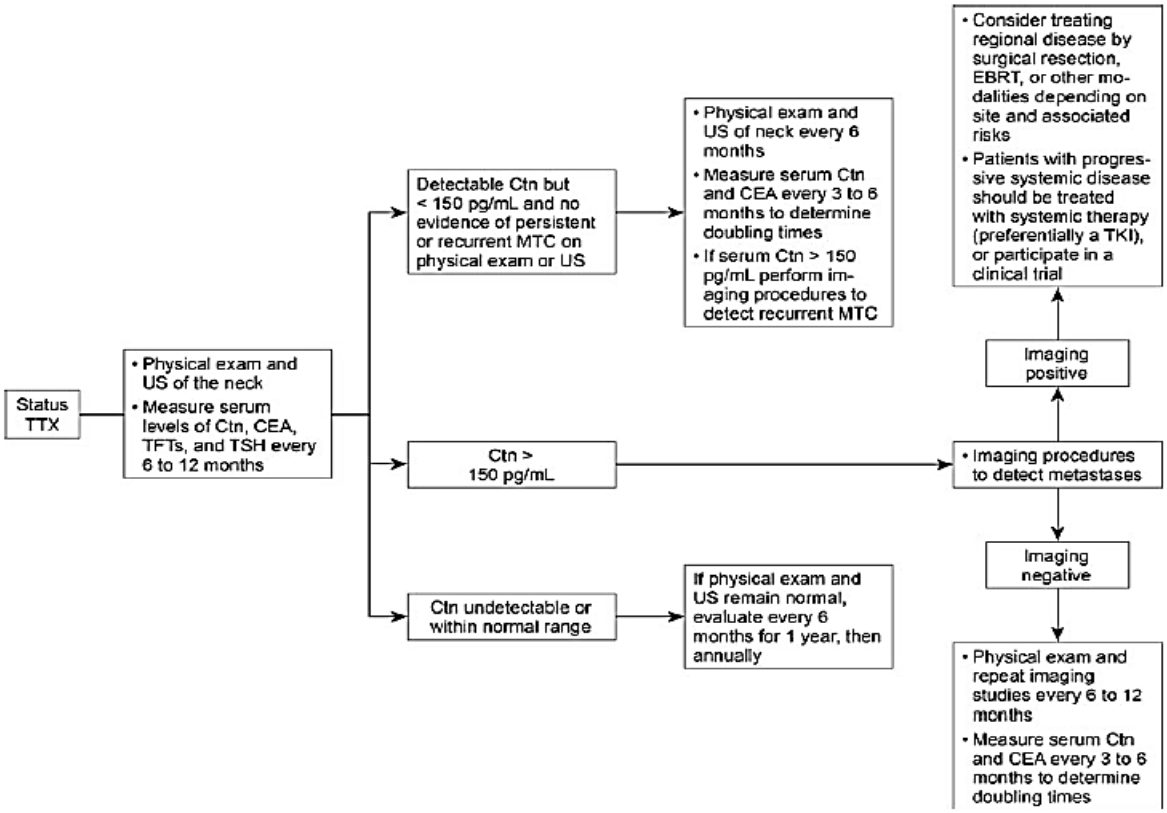


Management of patients with an RET germline mutation detected on genetic screening. ATA, American Thyroid Association risk categories for aggressive medullary thyroid carcinoma (MTC) (HST, highest risk, H, high risk, MOD, moderate risk); Ctn, calcitonin; CEA, carcinoembryonic antigen; HPTH, hyperparathyroidism; PHEO, pheochromocytoma; RET, REarranged during Transfection; TTX, total thyroidectomy; US, ultrasound. From Wells *et al.* (2015).

Monitoring post-op CMT in MEN2

• Patients with elevated postoperative calcitonin values but below 150 pg / mL will be examined by cervical ultrasonography. If the result is negative, patients will be monitored clinically, biologically (calcitonin and CEA) and imaging (cervical ultrasonography) every 6 months.

• If post-operative calcitonin exceeds 150 pg / mL, the patient should be imaged (cervical ultrasonography, neck and chest CT, abdomen, bone scintigraphy, axial skeletal and pelvic MRI).



Management of patients following thyroidectomy for persistent or recurrent medullary thyroid carcinoma.

Ctn, calcitonin; CEA, carcinoembryonic antigen; EBRT, external beam radiotherapy; MTC, medullary thyroid carcinoma; TFTs, thyroid function tests; TSH, thyrotropin; TKI, tyrosine kinase inhibitor; TTX, total thyroidectomy; US, ultrasound. From Wells *et al.* (2015).

Pheochromocytoma MEN2 monitoring

• The risk of developing pheochromocytoma is also variable depending upon genotype. Due to screening programs, pheochromocytomas may be diagnosed at a young age and before symptoms are present.

• For children in the high-risk categories, annual screening for pheochromocytoma should begin by age 11 years.

• For children in the moderate-risk category, annual screening starts at 16 years of age.

• Screening tests include plasma fractionated metanephrines or 24-hour urinary metanephrines and normetanephrine.

• If biochemical results are positive, the next step is adrenal imaging with CT or MRI. If initial imaging is unable to identify the unilateral versus bilateral disease, adrenal venous sampling can be done.

• After surgical treatment in unilateral pheochromocytomas it is extremely important to monitor the remaining adrenal, because in the majority of patients a controlateral pheochromocytoma will develop in about 10 years after the surgery.

• Post-operative monitoring in pheochromocytoma is vital in the early detection of any cortical-adrenal insufficiency that must be treated promptly and correctly.

Patients who have developed cortico-adrenal insufficiency following surgical removal of pheochromocytoma will receive substitution treatment (glucocorticoid and mineralocorticoid) and will be warned of the life-threatening if they discontinue treatment.

• Annual biochemical screening starts at the age of 11 years for high-risk patients and by the age of 16 for moderate risk patients.

• The test of choice for screening is serum calcium corrected for albumin levels. If elevated, serum parathyroid hormone (PTH) is measured, and the diagnosis is established with high or inappropriately high levels of serum PTH in the presence of hypercalcemia.

Take Home Message

• The monitoring of the patient with hereditary endocrine tumors differs depending on the type of tumor, the syndromic character, the physiological state (pregnancy) and the age of the patient.

• The monitoring team is multidisciplinary (neo-natologist, endocrinologist, surgeon, oncologist, radiologist) pediatrics and adult medicine in order to monitor the patients who have the disease and belong to the same family.

• Each type of tumor has a specific monitoring, which must be adapted to each patient individually.

• In most hereditary endocrine tumors, monitoring is permanent, the patient being followed throughout his life.

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II.13. International regulations applicable in oncogenetic department

Learning objectives

After completing the course students should be able to:

• Identify the **ethical issues related to genetic testing** that are covered by international regulations

• Describe how to deal with the relevant ethical issues surrounding genetic testing in oncogenetics, according to the **international legislation**

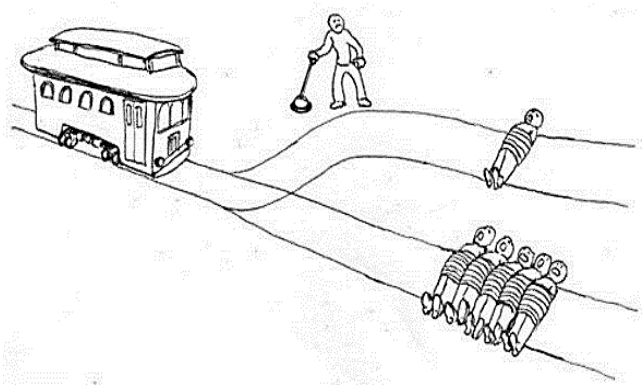
Introduction

- Case studies – revision of the first part of the course
- List of international regulation with coverage for testing in oncogenetics4
- The main ethical issues related to genetic testing in oncogenetics – covered by international legislation Non discrimination and non stigmatisation
- Genetic services
- Information, genetic counselling and consent
- Testing the persons not able to consent
- Test for the benefit of the familly members
- Private life and the right to information
- Genetic screening programmes for health purposes

Case study 1 – Deontology, utilitarianism, confidentiality

Doctor X is considering whether or not to break a confidence. His patient has presented with an STD which he wishes to have treated confidentially. His wife is also your patient. What do you do?

Case study 2: The trolley dilemma - Deontology and Utilitarianism



• You find yourself at a lever.

• A runway trolley approaches five people whoe are tied to set of tracks

• Pulling the lever will divers the trolley to a different set of tracksm where only one person is tied down.

• Do you pull the lever?

Case Study 3: Pre-implantation genetic testing

• Ion and Maria both suffer from deafness.

• Maria is also infertile, so the couple resort to IVF.

• Once into this procedure, they were offered the possibility of pre-implantation genetic diagnosis (PGD), based on the presumption that they did not want a child with deafness.

• However, they requested that out of the 9 embryos obtained, the one with congenital deafness be implanted first (along with any other, unaffected). The rest – will be frozen.

• What do you do in this case?

Case Study 4: Pre-natal fetal genetic testing

• A young acondroplastic dwarf couple want to have a child, which is why they seek genetic advice. The young mother is pregnant (gestation age = 6 weeks).

• They are calling for a genetic testing of the fetus, saying they will abort a fetus that does not contain the mutant gene.

• Would you agree to do this prenatal diagnosis knowing that a normal and healthy fetus could be aborted?

Case study 5: Genetic testing for Huntington disease - implication of the results

• A middle-aged patient is on the waiting list for a heart transplant. The medical team has just learned that the patient is at approximately 20% risk of developing Huntington's disease and as a result they require genetic testing to obtain prognostic data.

• Would you perform the testing????

Case study 6: Genetic testing for colon cancer and confidentiality

• A person undergoes a genetic test.

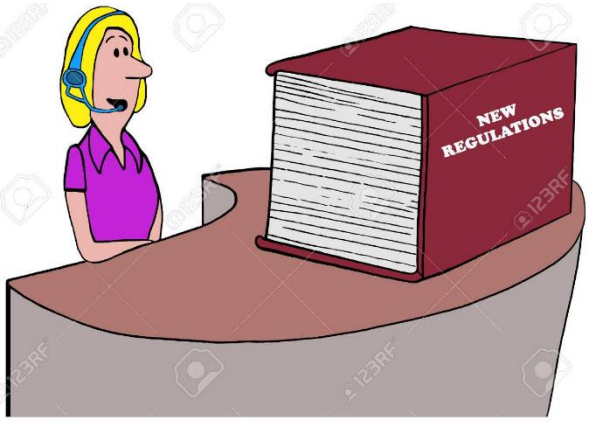
• She signs the consent and the first blood sample is taken.

• The person dies (colon cancer at 32 years old).

• We do NOT know if the relatives know about this test (in his/her consent they specify that the test result should be transmitted only to the husband).

• The husband does not want to know about genetic testing, although they have 2 boys.

Can we contact the relatives?



"Give me a couple years, and I can answer your simple question about the new regulations."

International regulations

1. Documents with a global covering

a. Guidance documents

• World Medical Association

• Declaration of Helsinki: Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects

• Declaration of Lisbon on the Rights of the Patient

• WHO: Quality and safety in genetic testing

CIOMS: International Ethical Guidelines for Biomedical Research Involving Human Subjects

b. International law documents

• UNESCO Universal Declaration on the Human Genome and Human Rights

• UNESCO Universal Declaration on Human Genetic Data (2003)

• UNESCO. Universal Declaration on Bioethics and Human Rights (2005)

2. Documents with European covering

a. European Comission Directives

• Directive **2004/23/EC** on **setting standards of quality and safety for the donation, procurement, testing, processing, preservation**, storage and distribution of human tissues and cells

• Commission Directives 2006/17/EC - and 2006/86/EC - established specific technical requirements for the human tissue and cell procurement and preparation processes.

b. Council of Europe Documents

• **Oviedo** - Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine:

• Additional Protocol to the Convention on Human Rights and Biomedicine, concerning *Genetic Testing for Health Purposes (2008)*

c. European guidance documents

EGE Opinion n°18 - 28/07/2003 - Ethical aspects of genetic testing in the workplace

The main ethical issues related to genetic testing in oncogenetics – covered by Additional Protocol to the Oviedo Convention on Human Rights and Biomedicine, concerning *Genetic Testing for Health Purposes*

First international legally binding instrument in this field

Non-discrimination and non-stigmatisation

• Any form of discrimination against a person, on grounds of his or her genetic heritage is prohibited.

• Appropriate measures shall be taken in order to prevent stigmatisation of persons/groups in relation to genetic characteristics.

Genetic services
Quality of genetic services

Genetic services. Clinical utility of genetic testing

Clinical utility of a genetic test shall be an essential criterion for deciding to offer this test to a person or a group of persons.

Genetic services. Quality of genetic services

• genetic tests meet the criteria of scientific validity and clinical validity;

• quality assurance programme in each laboratory

• persons providing genetic services have appropriate qualifications

Genetic services. Individualised supervision of genetic testing

A genetic test for health purposes may only be performed under individualised medical supervision.

• only Slovenia, Norway and recently the Czech Republic have agreed to be bound by it.

• However, to date, none of these countries have put into force provisions rendering health-related genetic testing performed without medical supervision illegal.

• Austria, France, Hungary, Italy, Germany, Austria, Lithuania, the Netherlands, Portugal and Spain prescribe mandatory medical supervision and restrictions in the way some genetic tests are performed.

France

tests can only be performed for healthcare purposes, with a medical prescription and realized by an authorized laboratory (Code Civil 2006, LOI n° 2011–814 2011)

the professional prescribing genetic tests can be either a geneticist or a non-geneticist, as long as he/she is familiar with the medical situation of the patient and he/she works in close relationship with a reference centre

penalization of the users (i.e. consumers ordering a test outside the clinical setting).

More specifically, the infringement of this provision is punishable under the criminal code by a fine of 3.750 euro

Source: Borry P. et al (2012) Legislation on direct-to-consumer genetic testing in seven European countries. Eur J Hum Genet 20(7):715–721.

Germany

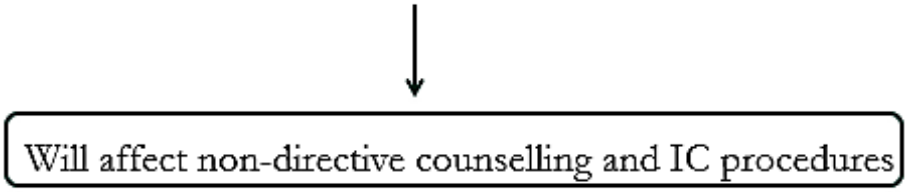
• **diagnostic genetic examinations** may be performed by any physician licensed to practice medicine, whereas

• **predictive genetic examinations** may only be performed by physicians specialized in human genetics or by other specialized physicians qualified during their medical training to perform them in their specialist area of practice.

A genetic test

2 main features:

- **Clinical validity** (accuracy with which a test predicts a particular clinical outcome)
- Availability of an **effective treatment** for the clinical situation or the risk identified by testing



Information, genetic counselling and consent

• The person concerned in the genetic testing shall be provided with prior appropriate information in particular on the purpose and the nature of the test, as well as the implications of its results: for the individual in case as well as for the whole family.

• For predictive genetic tests, appropriate **genetic counselling** shall be available for the person concerned. The tests concerned are:

- tests predictive of a monogenic disease,
- tests serving to detect a genetic predisposition or genetic susceptibility to a disease,
- tests serving to identify the subject as a healthy carrier of a gene responsible for a disease.

• Genetic counselling shall be given in a non-directive manner.

• Special consideration must be given to whether a personal responsibility to disclose genetic information to family extends to young children.

• Feelings of guilt and stress in the relationship can determine whether, when and how a parent tells his or her children about a genetic risk.

• The decision involves factors like:

- age and ability to comprehend
- severity of the disease and availability of prophylactic measures

• There are no clear rules on how and when to inform children of genetic risk, parents are being advised to delay involvement of children in the genetic counselling process.

Information, genetic counselling and consent

Currently, there are 16 countries requiring genetic counselling for some types of genetic tests: Austria, Cyprus, Czech Republic, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Norway, Slovakia, Slovenia and Spain.

Denmark, Estonia, Finland, Latvia, Lithuania, Slovakia and Romania → Despite having signed and ratified the Oviedo Convention, do not stipulate explicitly in their national legislation that genetic counselling should be mandatory for the provision of health-related genetic testing

• Countries that have only signed but not ratified the Oviedo Convention (so although they acknowledge the principles underlined by the Convention, they have no obligation to introduce its principles into their national law) are Italy, Luxembourg, the Netherlands, Poland and Sweden.

• Austria, Belgium, Germany, Ireland and the UK have neither signed nor ratified the Oviedo Convention.

• Despite this fact, Austrian and German laws provide a detailed framework for genetic counselling in the context of genetic testing.

• A genetic test may only be carried out after the person concerned has given free and informed consent to it.

• The person concerned may freely withdraw consent at any time.

Austria, Czech Republic, France, Germany, Hungary, Ireland, Italy, Norway, Portugal, Slovakia, Slovenia, Spain, Sweden, UK include specific provisions regarding informed consent in the context of genetic testing.

E.g. Spain:

(1) the **purpose** of the genetic analysis;

(2) the **place** where the analysis shall take place and the way in which the biological sample will be treated at the end of the analysis, whether it is the **disassociation of the identifying data** from the sample, its destruction or other treatments;

(3) **the persons who will have access** to the results of the analysis when samples will not undergo a process of disassociation or anonymisation;

(4) a warning about the possibility of **unexpected findings** and the possible implications for him or her, as well as the patient's right not to know;

(5) a warning about **potential implications of this information** for his or her family members, and their interest, where appropriate, in having that information conveyed to them;

(6) an agreement to provide **genetic counselling**, once the results of the analysis are obtained and evaluated.

Persons not able to consent

• a genetic test on a person who does not have the capacity to consent may only be carried out for his or her direct benefit.

• Where, according to law, **a minor** does not have the capacity to consent, **a genetic test on this person shall be deferred until attainment of such capacity** unless that delay would be detrimental to his or her health or well-being.

• E.g.: even if colonoscopy may be performed at 10-12 years old and thus, we may rationally think that a test must be offered in minors, we need to carefully assess whether this is an urgent decision with a direct benefit before age of 18.

• **the legal representative or authority** shall be provided with prior appropriate information in particular with regard to the purpose and the nature of the test, as well as the implications of its results.

• Appropriate prior information shall also be provided **to the person not able to consent** in respect of whom the test is envisaged, to the extent of his or her capacity to understand.

• A qualified person shall be available to **answer possible questions**

Tests for the benefit of family members: Tests on persons NOT able to consent

Exceptionally, if the following conditions are met:

1. the purpose of the test is:

to allow the family member(s) concerned to obtain a **preventive, diagnostic or therapeutic benefit** that has been independently evaluated as important for their health, or

to allow them to make an **informed choice with respect to procreation**;

2. the **benefit envisaged cannot be obtained without carrying out this test;**

3. **the risk and burden of the intervention are minimal** for the person who is undergoing the test;

4. the expected **benefit** has been independently evaluated as **substantially outweighing the risk for private life** that may arise from the collection, processing or communication of the results of

the test

5. the **authorisation of the representative** of the person not able to consent, has been given;

6. **the person not able to consent** shall, in proportion to his or her capacity to understand and degree of maturity, **take part in the authorisation procedure**.

The test shall not be carried out if this person objects to it.

Tests for the benefit of family members. Tests on biological materials - when it is not possible to contact the person concerned

• When it is not possible, with reasonable efforts, to contact a person for a genetic test for the benefit of his or her family member(s) on his or her **biological material previously removed for another purpose,** the test will be carried out in accordance with the **principle of proportionality:** where the expected benefit cannot be otherwise obtained and where the test cannot be deferred

Tests for the benefit of family members. Tests on deceased persons

A genetic test for the benefit of other family members may be carried out on biological samples:

• removed from the body of a deceased person, or

• removed, when he/she was alive, from a person **now deceased, only if the consent** or authorisation required by law has been obtained.

Oviedo Convention, Additional Protocol to the Convention on Human Rights and Biomedicine, concerning Genetic Testing for Health Purposes

Private life and right to information

= the right to protection of his or her personal data derived from a genetic test.

The right to know any information collected about his/her health derived from this test.

The conclusions drawn from the test shall be accessible to the person concerned in a comprehensible form.

The wish of a person not to be informed shall be respected.

= the right to protection of his or her personal data derived from a genetic test.

• **Biological samples** → shall only be used and stored in such conditions as to ensure their security and the confidentiality of the information

• Where the results of a genetic test undertaken on a person can be relevant to the health of other family members, the person tested shall be informed

General Data Protection Regulation (EU) 2016/679 (GDPR)

regulates data protection and privacy
in the European Union (EU) and the European Economic Area (EEA)

+

addresses the transfer of personal data outside the EU and EEA

Genetic screening programmes for health purposes

Only if approved by the competent body → ethical acceptability and fulfilment of the following specific conditions:

the programme is **recognised for its health relevance** for the whole population or section of population concerned;

the scientific **validity and effectiveness of the programme** have been established;

appropriate preventive or treatment measures in respect of the tested disease are available

equitable access to the persons concerned is ensured

the population is adequately informed about the existence, purposes and means of accessing the screening programme as well as the voluntary nature of participation in it.

Take home message

The main ethical issues surrounding the genetic testing in oncogenetics, covered by law are:

• The right to information and informed consent
• Protection of people unable to consent for genetic testing
• Respect for confidentiality of personal data
• Quality of genetic tests and genetic services

Genetic testing in oncogenetics:

• Risks for private life of:
- person concerned
- members of his/her family
• Difficulty to understand the implications of the test

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II.14. Psychological counseling in oncogenetic monitoring

Learning objectives

- knowledge of general aspects of oncogenetic counseling
- understanding oncogenetic counseling from the individual and family cognitive-behavioral perspective
- knowledge of the psycho-social aspects of genetic counseling in the context of genetic risk of cancer

Introduction

The multidisciplinary approach in oncogenetics aims to facilitate the understanding of the risk of genetic predisposition as well as of the possibilities of medical management of this risk, without provoking dysfunctional emotional reactions.

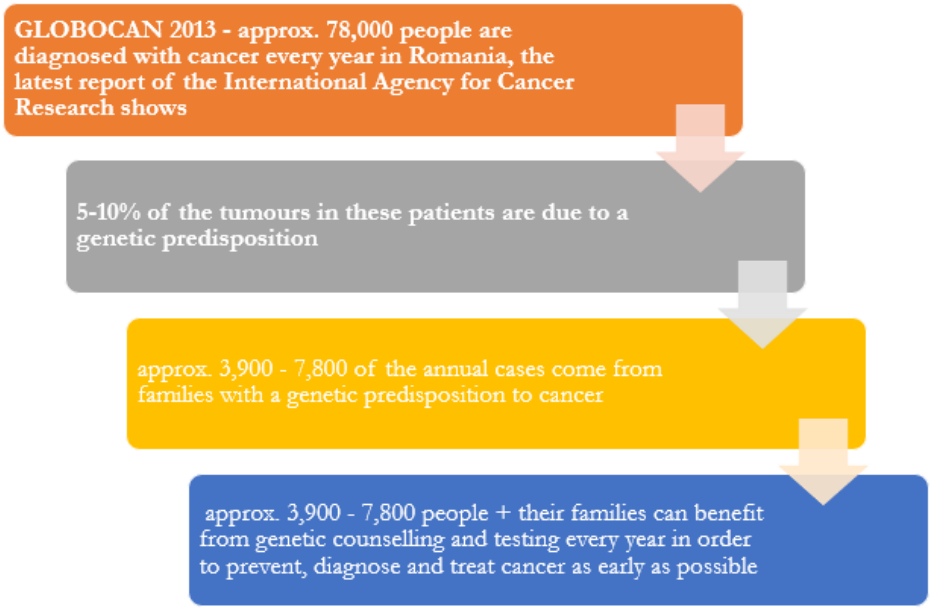
Although genetic testing can be regarded as a "simple" blood collection and gene analysis, the psychosocial implications for patients and their families are substantial. The assessment of the counselor's need to consult a psychologist (psychosocial assistant) must be done during the entire genetic testing process, including the monitoring period after the test results are provided.

In order for the full benefit of genetic testing to be achieved, patients must ultimately comply with screening and cancer prevention recommendations.

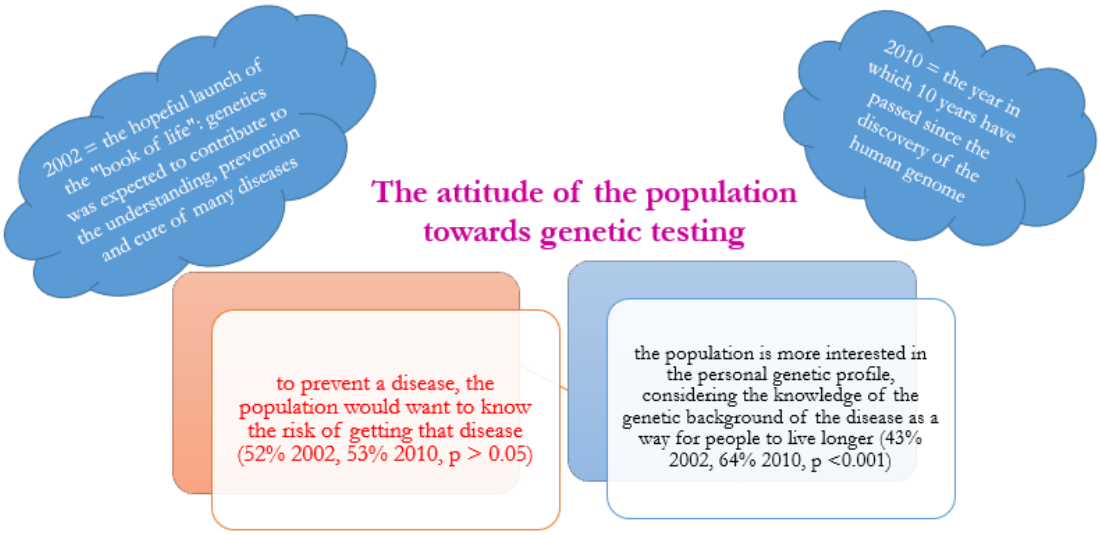
I. Oncogenetic counseling - general aspects

Both people at higher risk than the general population generally develop cancer and the general population have the right to psychosocial and supportive care needed to manage concerns and fears about the risk of cancer or those related to screening, such as and to receive information to assist them in pursuing primary, secondary or tertiary prevention programs, in changing behavior and lifestyle towards sanogenesis and any perceived challenges related to cancer, prevention, screening.

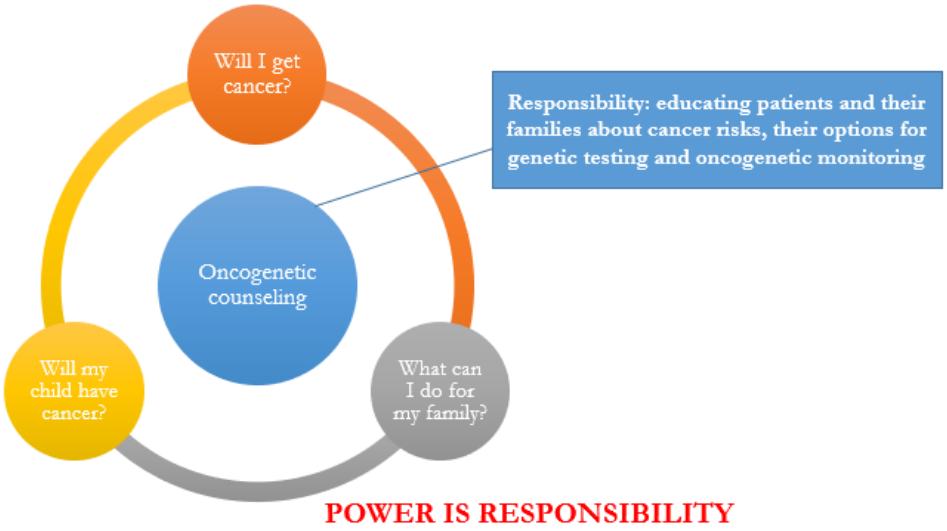
According to the standards of psycho-social assistance in oncology (www.ipos-society.org, www.capo.ca), people with hereditary risk have the right to receive genetic counseling and genetic testing that fully integrates psycho-social and supportive care to facilitate informed decision-making about risk reduction options (e.g. prophylactic surgery, preventive chemotherapy).



Source: Fitzmaurice et al., 2015.



Source: Henneman et al., 2013.



• According to the definition given by the American Society of Human Genetics, **genetic counselling** is "**a communication process that deals with human problems associated with the occurrence or risk of a genetic disorder in a family**."

• In 2006, the National Society of Genetic Counselors (Resta et al., 2006) refined the definition of **genetic counselling** to include **the process of helping people understand and adapt to the medical, psychological, family implications of the contributions of genetic changes** in disease onset/recurrence, including the integration of the following aspects: interpretation of family and medical history to evaluate the risk of disease onset or recurrence; education regarding genetic inheritance, testing, management, prevention, resources, current state of knowledge; counselling for the purpose of making informed decisions and adapting to the risk condition.

The principles that guide genetic counselling are (Baker et al., 1998):
voluntary use of genetic counselling services;
equal access to genetic counselling services;
education of the person;
complete transparency towards the person advised;
non-directive counselling;
considering the psychosocial aspects;
ensuring confidentiality (David et.al, 2007)

• Soerjomataram et al. (2007) argue that, at present, at least one third of all cancers can be prevented, another third can be diagnosed early and treated effectively

• Studies conducted to date support **the existence of numerous emotional, cognitive and behavioural factors involved in the attitude of individuals regarding the assessment of their own genetic risk of cancer**, the follow-up of cancer screening programs, the decision on genetic testing and the application of the recommendations received following information on the results. genetic testing

The oncogenetics approach should highlight a multidisciplinary process that will associate geneticists, genetic counselors, oncologists, specialists in various medical areas and psycho-oncologists, in order to better respond to the 3 main dimensions:

• education (need for information),

• help in making a decision

• psychological support (helping the adaptation)

Regular exchanges of information between professionals allow the gathering of information and perceptions into a common whole for a better global understanding of expectations, values, choices of counselors and their possible psychological difficulties.

• Studies have shown that, in general, **people have little or no knowledge of their own risk of developing cancer**. Even people who have already become victims of this disease are not aware of the increased risk of recurrence compared to the general population

• Therefore, **genetic counselling specialists need to know how families perceive and assess their own cancer risk and the family's preventive attitudes and behaviours**.

• It is important to understand how these individuals are aware of their own cancer risk, regardless of whether this risk is perceived differently from their family history of cancer, and what are the implications of these perceptions regarding adherence to surveillance programs, including performing targeted tests preventive.

• Regarding the **satisfaction of individuals with genetic counselling**, several studies have shown **that most people who have benefited from genetic counselling have been satisfied with this experience**.

• A study of 61 women who participated in a genetic testing program for BRCA1/2, however, shows that **beneficiaries' satisfaction with genetic testing is influenced by psychological variables** such as optimism, family functioning, general stress and specific stress related to cancer.

• It has been shown that **the satisfaction of the beneficiaries of oncogenetic counselling depends mainly on the quality and quantity of the information provided by the consultant**, and the evaluation of their adaptation strategies was essential to minimize the emotional consequences of genetic testing.

II. Genetic counselling and testing from an individual and family cognitive-behavioural perspective

• Marteau & Weinman (2006) believe that individual representations for different diseases vary to the extent that these diseases include causal genetic factors. Health threats from genetic factors are often perceived as more difficult to control, compared to threats that are caused by behavioural factors/causes (smoking, unhealthy diet, lack of exercise) or the environment (toxic environmental exposure).

• Findings in molecular genetics offer individuals the opportunity to test their susceptibility to developing certain types of cancer due to genetic mutations, while also generating different attitudes about knowing the risk of developing the disease.

• People do not work in isolation, sharing their health beliefs and beliefs with family members and the social environment they belong to, when their health is threatened. In addition to beliefs, family interactions also influence the mechanisms of psychological adjustment and adaptation to illness or to the threat to health.

• Hereditary cancer, being a family problem, can directly or indirectly affect all its members in terms of individual representations about the disease, strategies of adaptation and psychological adjustment to the disease or to the threat to their own health.

• Considering family communication as an important factor in its functioning, Vangelisti (2012) recommends that, when evaluating each family in the context of genetic risk and the impact of this risk, consider their own communication model, as what is functional from the point of view of communication in one family, it may be dysfunctional for other families.

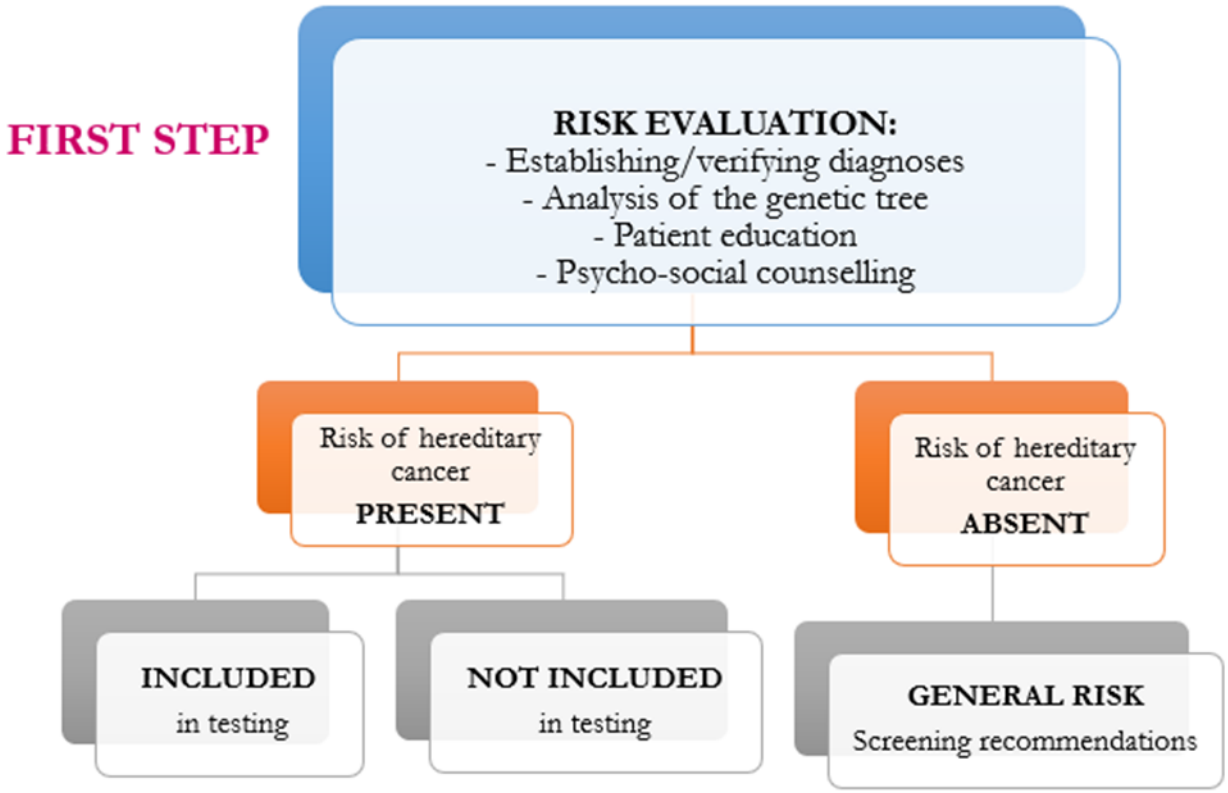
• The variability of penetrance or probability of developing the disease due to genetic mutation (from high to moderate probability, to low probability) can affect family members in terms of their level of uncertainty and anticipation of the challenges they will face.

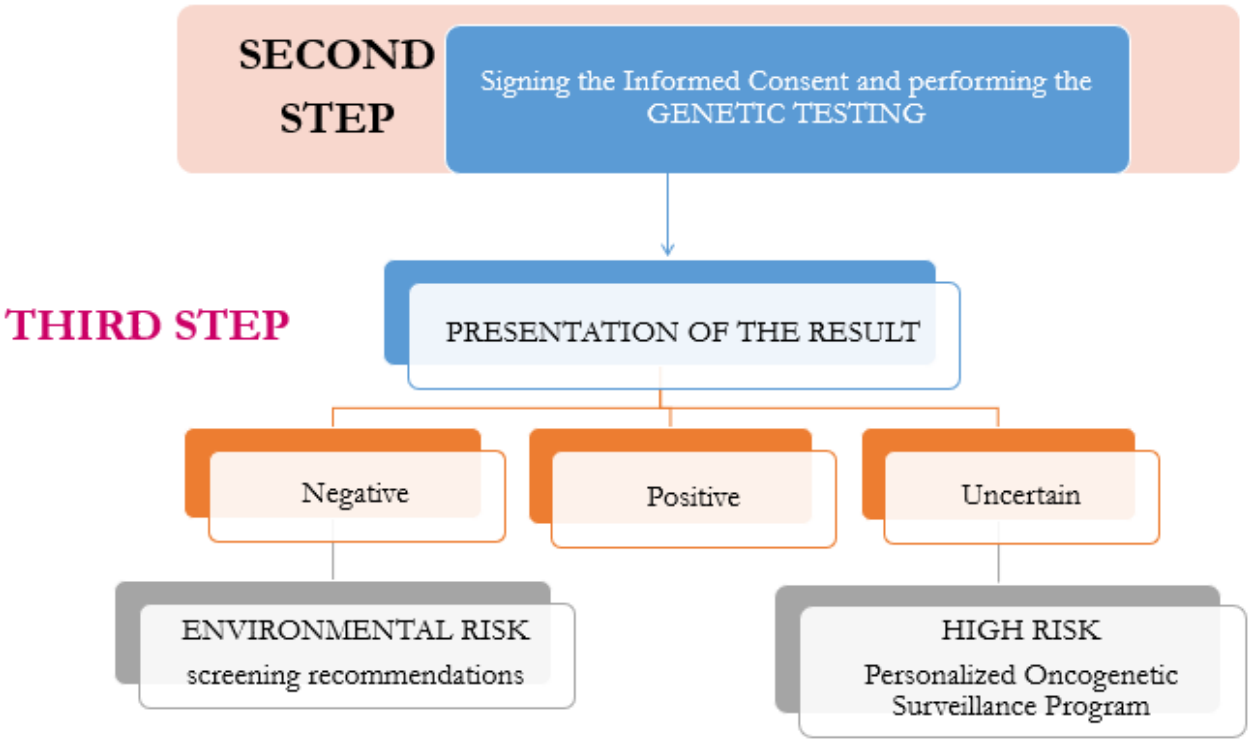
III. Psycho-social aspects of genetic counselling in the context of genetic risk of cancer

III.1. The objectives of the counsellor

- Provide patients with accurate and useful information
- Identify patients and families at increased risk of developing hereditary cancer
- To assist patients in carrying out genetic tests
- To help patients, their relatives and implicitly their health, through people who are active in the field of health, to understand the implications of genetic test results
- Provide psycho-emotional support to patients
- To facilitate the directing of patients to certain health departments that deal with the identification, detection and monitoring of oncological disorders.

Structure of oncogenetic counselling





III. 2. Relationship patient – counsellor

• It is based on the values of care and respect for the autonomy of the counsellor, individuality, well-being and freedom. The primary concern of genetic counsellors is the interests of counsellors

• The important moral traits for a genetic counsellor, according to Schneider (2011), are: compassion, conscientiousness, discerning, fidelity, integrity, courtesy, respect, credibility, veracity, wisdom.

To be **an ethical genetic counsellor**, Schneider (2011) believes that he should have the ability to use the following strategies:

identification and solution for the benefit of the counsellor of ethical dilemmas
keeping accurate and complete records throughout the pre- and post-test counselling stages
permanent information on the latest advances in cancer genetics
assessing the client's autonomy
manifesting empathy towards the counselled person
respecting the confidentiality of the person advised
respecting the privacy of the counsellor
respecting the decisions of the counsellor
the transmission of the truth in an appropriate, comfortable way for the counselled person
addressing informed consent as a process and not as an act.

III.3. Pre–test counseling

• Genetic counseling is the process of interpreting and communicating information regarding the medical, psychological and family implications of genetic disease.

• Evaluation of personal and family history to determine if there is a predisposition to fundamental cancer.

• Finding and communicating this information helps patients and their doctors better understand an individual's cancer risk.

• Establish the best management of medical services for the counselor regarding cancer surveillance and/or risk reduction.

Objectives of the pre-test counseling

- Evaluation of some prior knowledge regarding genetic risk, genetic mutations, genetic testing
- Impact assessment of possible results of genetic testing
- Assessment of the need to consult a psychologist (psycho-social assistant)
- Advice on decisions.

III. 3. a. Collection and interpretation of family history of cancer

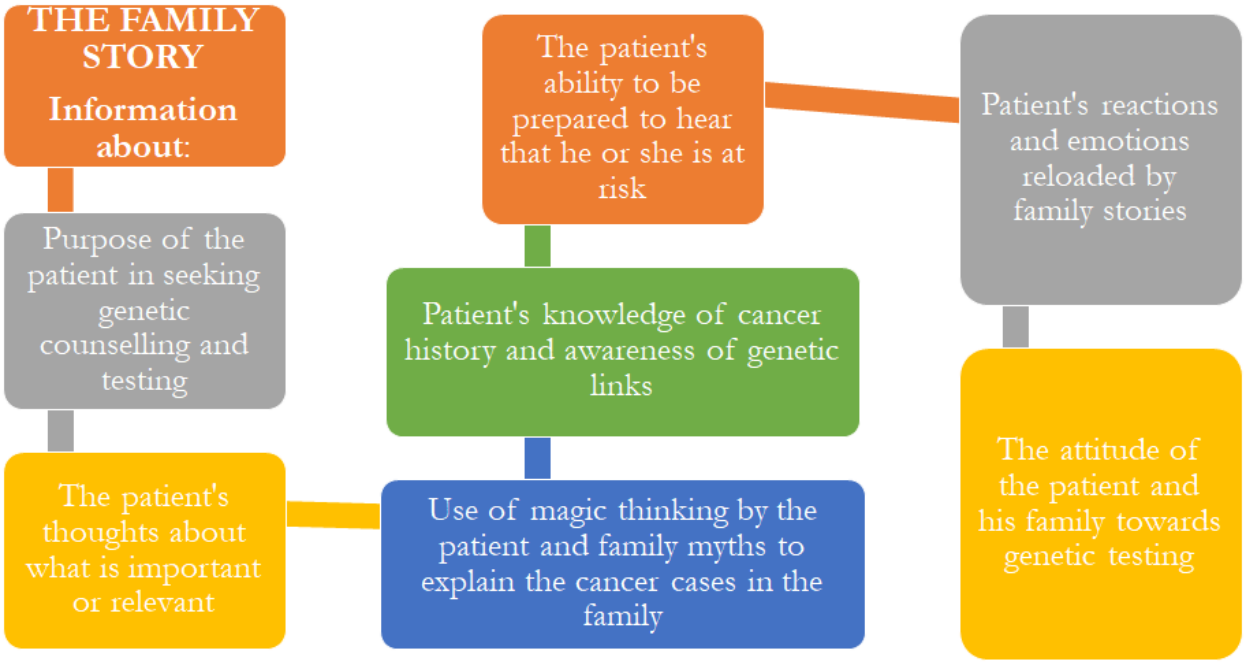
- Many factors can influence an individual's knowledge of their family history of illness (alienation, adoption, the patient has lost contact with family members). In many families, cancer is simply not discussed, so the information provided may be incorrect (e.g. malignant/benign tumor confusion, etc.).
- Genetic tree traits = clinician matrix - can assess risk, provide clinical recommendations and identify a diagnosis
- Careful collection and proper interpretation of a patient's personal and family history of cancer diseases will be the foundation of counselling sessions on hereditary risk for cancer.

Attention to family dynamics and support level!

Genetic tree - indicates information about important relationships, including the patient's relationship and degree of kinship, the number and sex of relatives in each generation, and whether they are alive or dead.

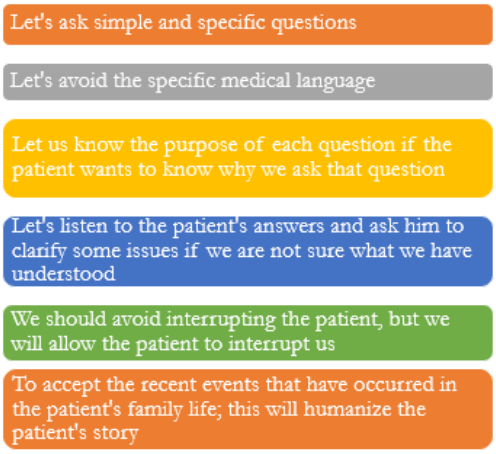
Psycho-social evaluation - informally by collecting the family history, either through the genogram or the eco-genetic coloured map (family dynamics, communication style, support, etc.).

LISTEN TO THE PATIENT'S POWERS !



Evaluation of the emotional state

Advices for collecting the family history



III. 3. b. Communicating the risk of hereditary predisposition

• The family history of cancer indicates the likelihood that the family has a hereditary predisposition to cancer

• The distinction between the "high risk" and the "low risk" families has an important impact on the counselling discussion, moving from the discussion about testing options to providing medical recommendations.

• Genetic counsellors assess, explain and discuss risks with their patients through a whole genetic counselling process

• Risk communication does not focus exclusively on the statistical probability of the patient having a genetic condition

• The communication also involves all the feelings, beliefs and reactions regarding the global concept of risk.

Risk	• the probability that a particular event will occur • implies some uncertainty about the result and at least an undesirable consequence
The main purpose of cancer risk communication	• provide the patient with sufficient information so that he or she can make decisions regarding genetic or medical testing
Perception of risk from the patient's point of view	• refers to how patients react to risk, understand it and assimilate it as personal risk • all patients filter risk information through their own lens of experiences and knowledge • they may see personal risks differently over time or as a result of an important event in their life • influences how patients will use risk data to make decisions • can predict whether or not patients will agree with genetic testing

Factors with impact on risk perception

Cognitive functioning = how to approach statistical, probabilistic concepts, abstract thinking, familiarity with medical terminology

Emotions and how to deal with the situation = they can affect the ability to understand and influence the final decisions regarding genetic or medical tests. In general, the greater the fear of a possible outcome, the less the person will be able to take the risk. Cancer - a feared outcome - some patients may insist on genetic testing even if there is a low probability that the outcome will be significant

Family interactions = how patients perceive risk is often strongly influenced by the attitudes of family members. Other factors: family communication style, value system, family support/support level.

Heuristic = e.g.: knowing a number of people with the same disease, having the feeling that the disease is "everywhere"; the patient has had multiple and/or negative experiences with the disease in the past; identifying with the sick relative and trying to feel the same feelings with it

Perceived load of the result = some patients have difficulty accepting that they may be at increased risk for diseases with high mortality and/or morbidity rates

Personal experiences = some patients who have had some personal experiences related to this disease tend to use them as reference frames in the process of interpreting their own risk. Eg: sometimes those whose relatives died due to this disease will view their own personal risk with greater concern than those whose relatives had the disease and survived. Those who have had some personal experiences with this disease may overestimate their own risk.

Personality type = can influence the perception of risk

• Focused towards the threat of failure

• Optimistic about the pessimist

• The person who seeks additional information from the person who avoids any kind of information

• The person who takes the risk compared to the person who avoids the risk

III. 3.c. Decision making on genetic testing

• Assisting patients in making decisions about genetic testing - an important aspect of oncogenetic counselling

• Although some patients come to genetic counselling sessions with the preconceived thought of either doing the test or not, a number of patients are undecided and do not know how to proceed.

• The main goals of the patient's decision-making process:

✓ The patient's decision must be based on an adequate assessment of the options and consequences, which is compatible with his values.

✓ The patient must feel that he has made the best decision at that time

✓ The genetic counselling process should support and facilitate the implementation of the patient's decision.

The model of making a medical decision based on an information, shared between the clinician and the patient, responds to a situation of choice between several options:

• the choice of whether or not to perform a genetic test

• the choice whether or not to know the result of this test, whether or not to transmit the information received, immediately or later, to the family members

• choice that involves opting for simple surveillance or prophylactic surgical prevention in order to reduce the risk of developing cancer being one of the most complex decision-making situations

• Schneider (2012) considers that the reaction of a counselled person in the situation of a **high risk of genetic mutation** can vary greatly, depending on the personal and family history and the emotional well-being in general, some people considering that their fears regarding Cancer looks are real. At the same time, other people who have denied the possibility of an increased risk may show anger, mistrust and fear.

• In the case of a **low risk** that the person is carrying a genetic mutation, obtained from the evaluation of the individual and family history of the disease, when the genetic counsellor considers that the genetic testing is not indicated, the counsellors may react in different ways, some showing relief knowing that their risk it is similar to that of the general population, others showing anger, believing the susceptibility of the disease.

• Cochrane's systematic and up-to-date review (Hilgart et al., 2012) on the impact of oncogenetic counselling on breast cancer risk, including eight trials, concluded that assessing the genetic risk of cancer during counselling sessions contributes to reducing the psychological distress generated of risk.

• Anxiety, worries, intrusive thoughts, fear, anger, guilt, risk perception for oneself and for one's family, psycho-social stressors, the impact of the disease on the family are the psycho-social issues to be evaluated in the genetic counselling process and can be highlighted in the stage of collecting data on family history of illness.

III. 3. d. Strategies to assist patients in decision-making

• Presentation of all relevant factors
• Offering assistance and not recommendations
• Providing encouragement and support
• Supporting patients in the process of structuring the discussion so that it is useful and neutral
• Using the "best case/worst case scenario" type scenarios
• Exploring how patients cope with other situations related to certain medical circumstances and the decisions made in those cases
• Identifying other people (partner, relative, friend, attending physician) whose comments can help
• Encourage the patient to reflect and deliberate thoroughly before making a decision.

III. 3. e. Psycho-social evaluation

Emotional state

• the genetic testing process can exacerbate psychological problems
• questions regarding the patient's disposition, changes in the habits, eating habits or rest

Anticipated impact of the results

• discovering how patients anticipate certain outcomes and why they expect these results is helpful in providing the necessary support when presenting the test result

Resources to deal with the situation

• each person develops his or her own way of coping with difficult situations, although some strategies are less healthy than others (talking with friends, going to the gym, isolation, numbing feelings using alcohol or drugs)
• Useful: exploring with the patient how they can plan to cope with the results

Support network

• patients who appear to have no close friends or family members will need additional psychological support
• some feel the need to be isolated, others prefer not to talk during the counselling period with the people they can count on

Other major stressors

• cancer and all stressors related to this disease need to be addressed carefully - they can cause a much more intense reaction to finding the results of the genetic test
• stressors linked to noncancer should not be ignored - a positive result can add an additional, potentially overwhelming, stress factor

III. 4. Post – test counselling

III. 4.a. Disclosure of results

• Disclosure of the result may be the most difficult aspect of the testing process for both patient and counsellor

• Useful strategies for revealing a result in a professional and empathetic way:

• Regarding the impact of molecular testing when there is a genetic risk to the family, a genetic risk can affect the relationships between siblings, parents and descendants, partners may have difficulty adapting and have high levels of concern for the affected partner (Williams et al., 2000), life planning difficulties may arise.

• An issue with important psycho-emotional implications is generated by the situation in which the parent carrying the genetic mutation has to decide when and how to inform the adolescents or descendant adults.

• Other challenges arise around the responsibility of notifying the extended family, especially when they are dysfunctional or when there are different representations regarding genetic testing and adopting preventive behaviour.

• Psycho-social implications of genetic testing that should be examined with patients during oncogenetic counseling sessions

✓ If it has been proven that an individual has a genetic mutation, it can develop feelings of depression, anxiety and vulnerability

✓ Disclosing information about a positive outcome can affect family relationships

✓ Testing reveals a variant of uncertain significance, it can be disruptive for patients because it is not known whether this variant increases an individual's risk of developing cancer (increased feelings of suffering and insecurity).

✓ A "true negative" result may cause guilt to the individual, because he has not inherited the gene mutation as the other family members

✓ There is a possibility to find sensitive information about the family (non-paternity or unknown adoption)

✓ Genetic discrimination.

III. 4. b. Counseling within the personalized oncogenetic surveillance program (screening, prophylactic surgery)

- Risk reduction options for people at risk of hereditary cancer include screening or prophylactic surgical removal of risk organs (prophylactic bilateral mastectomy, prophylactic salpingo-ovariectomy, for example).

- The detailed discussion is provided by the respective specialists (surgeons, oncologists, gastroenterologists, gynecologists, etc.), but the genetic counselor has the unique opportunity to present these concepts to the counselors during the pre-testing sessions.

- If a mutation is identified and the subject must consider the different possibilities of surveillance or preventive surgery, the consultation with a psycho-oncologist - mandatory in the case of a decision to perform a prophylactic mastectomy and strongly recommended in the decision to practice a prophylactic ovariectomy.

- Psychological counseling in this context allows weighing the advantages and disadvantages of each option, verifying the subject's ability to anticipate his own reactions according to the different possible scenarios and evoking the psychological situation towards himself and his family.

- **Prophylactic bilateral mastectomy** is a radical alternative that has been shown to be effective in preventing breast cancer development

- There are important differences between countries regarding the use of prophylactic mastectomy, in France the acceptability among specialists and patients is low, while in England, Canada and the Netherlands the acceptability is quite high.

- At the psychological level, prophylactic mastectomy has shown a decrease in cancer development concerns, but could have negative consequences on self-respect, sexual relations and feelings related to femininity.

- Follow-up study of 14.5 years after prophylactic mastectomy (Frost et al., 2000) 70% of the women were satisfied with the procedure, 11% neutral, 19% dissatisfied. 74% reported a decreased level of emotional concern about breast cancer development. Most women did not report changes in emotional stability (68%/23%), stress level (58%/28%), self-esteem (69%/13%), sexual relations (73%/4%) , feelings of femininity (67%/8%).

- **The family at a whole** should also be taken into consideration. Who will inform other family members and who will motivate them to participate in this process? What is the way of communication or relationship within the family? What are the family beliefs and values regarding health and the medical, curative and preventive approach?

- Miller et al. (2005) point out that, as the biological implications of genetic information extend beyond individual risk, it is necessary to develop psycho-social approaches and techniques to support families in the process of adapting to genetic information, in the process of taking decisions and adopting healthy behaviors, taking into consideration the examination of the family development stage, the history of illness within the multigenerational family system, the family belief system, as well as the significance of the disease in the family.

Take home message

• *Human Genetics Commission* believes that health professionals need to recognize the value of family solidarity and altruism, by encouraging and facilitating the appropriate exchange of relevant information between family members directly involved in genetic testing.

• The most common reasons for genetic testing in Europe were the results of the test would lead to a healthier lifestyle and, consequently, to a longer life, contributes to a better knowledge of the health status and to the knowledge of the risk of getting a certain disease.

• Psychological care, as stated by Stiefel et al. (1997) and Decruyenaere et al. (2000), is well integrated into the standard of the genetic testing process to facilitate optimal adaptation and support of counseling individuals and their families to genetic risk of cancer.

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