NATIONAL REPORT ON SITUATION/STRATEGIES/SUPPORT INSTITUTIONS AND SPECIALISTS/BEST PRACTICES OF THE GENETIC CONSULTANCY/ ONCOGENETICS IN ROMANIA

Report created in the framework of the project 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, Reference number 2018-1-RO01-KA202-049189, Strategic Partnerships for vocational education and training Erasmus+ programme

Introduction

Romania faces a challenging epidemiological situation that imposes to initiate measures for the monitoring and implementation of prevention strategies in oncological pathology. Over the last decade, there is increased attention focused on hereditary cancer problems. There is a growing body of research projects at national or international level addressing this issue, as well as increasing number of heath care centers that provide molecular testing services for patients where hereditary factor involvement is predicted. The interest for oncogenetical issues is racing across the teams of researchers and clinicians as the principal strategy for monitoring and management of cancer genetic risks patients and their families to face the complex challenges imposed by hereditary cancer.

The present report examines the background and representing an analysis of current situation cancer genetic testing and management sketching out the main elements that oncogenetical management of patients should ideally include. From this basis, a summary of necessary components of oncogenetical programs is presented. Contextual factors (scientific, economic, epidemiologic, human resources, and technological) in Romania was examined in order to outline the factors that condition oncogenetical centers development at a national level.

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1. The current epidemiological situation on cancers, in general, and on hereditary cancers (breast, ovarian, colorectal cancer and other) in Romania

1.1. Incidence

Analysing the estimated incidence values for the main categories of cancers developed by Romanian women in 2008, one can see a change in the 2nd and 3rd positions, the colorectal neoplasia climbed one step in hierarchy, and the cervical cancer recorded a slight decline. For the 5th and 6th positions, ovarian cancer increased (although as numerical values, the situation has not significantly changed), and the gastric cancer decreased. (Fig. 1) [GLOBOCAN 2008]

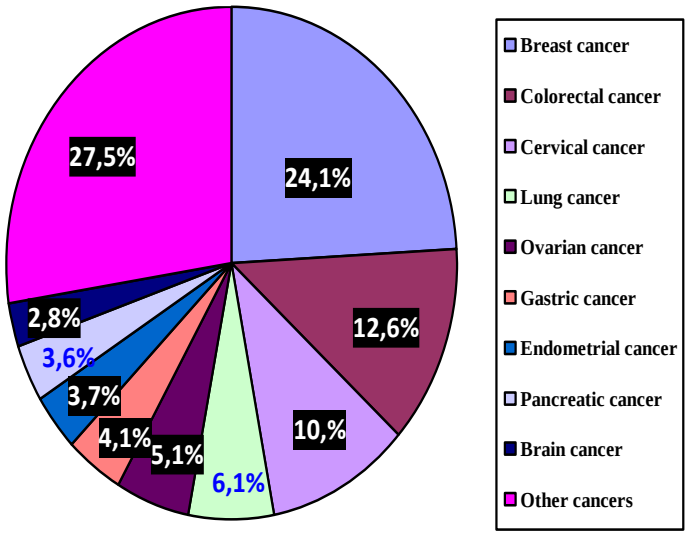

Fig. 1. Incidence (standardized by age) for the main types of cancers developed by women, in Romania, 2008

In 2008, among women in the North-East Region of Romania, ovarian cancer had an incidence of 12.17 / 100.000 women (the 6th position) and a frequency of 5.02% of all female cancers. In the same year, in Iasi County, ovarian cancer had the 5th place (with 12,17 cases per 100,000 women), after breast cancer (50,11 cases per 100,000 women), colorectal (33,4 cases in 100,000 women), cervical (25.05 cases in 100.000 women) and lung cancer (18.37 cases per 100.000 women). According to the same source, the incidence of ovarian cancer in Iasi County in 2008 was 12,17 cases per 100,000 inhabitants, placing this county on the 4th place when we compare the incidence of ovarian cancer among the counties of the North-East region of Romania (Table I) [Gheorghiu, 2011].

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Nr.crt	County	Number of new cases of ovarian cancer	Female population at 1 July 2008	Incidence (per 100.000 inhabitants)
1.	Suceava	49	357,220	13.71
2.	Botosani	31	229,260	13.52
3.	Neamt	36	286,173	12.57
4.	Iasi	51	419,113	12.17
5.	Vaslui	27	226,689	11.91
6.	Bacau	35	362,822	9.64
	North-East Region	229	1,881,277	12.17

Table I The incidence of ovarian cancer in counties within the North-East Region of Romania, in 2008

Regarding to age, the same report states that the most affected women in the North-East Region of Romania were those in the 55-59 age group (17.03% of the new ovarian cancer), 75-79 years (13,10%), 70-74 years (11,35%) and 65-69 years (10,04%). 68% of the cases were diagnosed among women aged over 55 years [Gheorghiu, 2011].

In 2008, in Romania, ovarian cancer was ranked 5th among women's neoplasms with an incidence value (standardized by age) of 9.4 cases / 100,000 women.

According to the International Agency for Research on Cancer (IARC), in Romania, in 2018, the estimated total number of new cases of cancer for both sexes at all ages was 4,313,123 (GLOBOCAN 2018). Case distribution by location indicates that breast cancer was the most common (12.3%), followed by colorectal cancer (11.8%), lung cancer (11.2%), prostate cancer (10.6%), bladder cancer (4.7%), skin melanoma (3.4%), renal cancer (3.2%) and other cancers (42.8%) (Fig. 2). Among the other cancers that were recorded, we mention uterine cancer (2.9%) and ovarian cancer (1.6%) [GLOBOCAN 2018].

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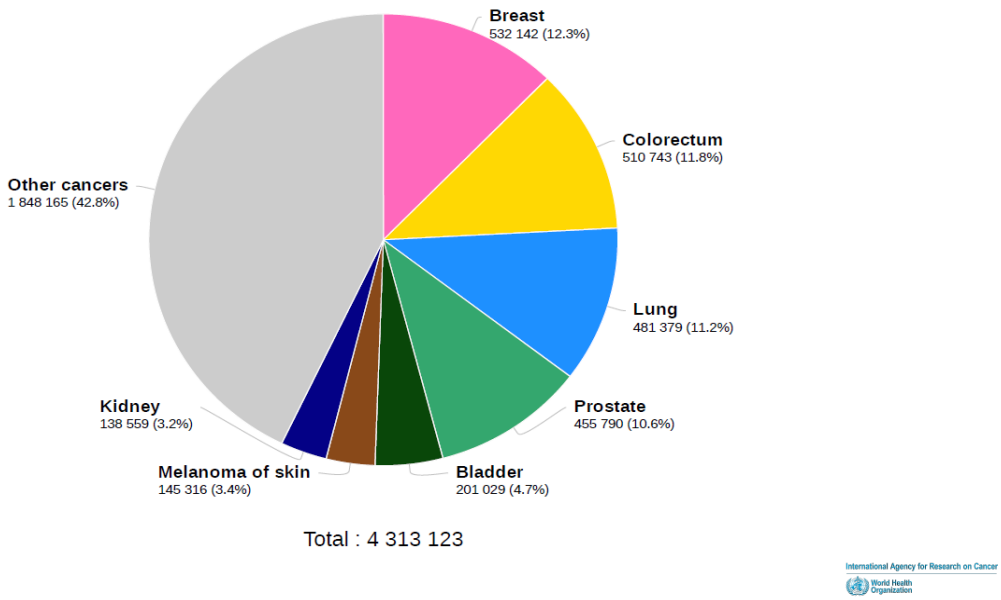

Fig. 2. The estimated number of new cases of cancer, both sexes, all ages, in Romania

(*Source*: *** International Agency for Research on Cancer. GLOBOCAN 2018. Globocan cancer observatory. Available from: http://gco.iarc.fr.)

Comparing these data with those published by Straja ND et al. in 2015 one can observe the upward trend in the number of new cases for all types of cancer. For breast cancer, the number of new cases has risen from 8,981 estimated for both sexes in 2012 to 532,214 in 2018. Colorectal cancer has evolved from 10,256 new cases in 2012 to 510,743 in 2018. Uterine cancer has varied from 1,539 new cases in 2012 to 124,048 in 2018 and ovarian from 1,850 new cases in 2012 to 69,611 in 2018 (Table II) [GLOBOCAN 2018; Straja et al, 2015].

Types of cancer	Years	TOTAL		WOMEN		MEN	
		No.	%	No.	%	No.	%
Breast	2018	532,142	12.33	532,142	26.33	-	-
	2012	8,981	11.40	8,981	25.22	-	-
Colorectal	2018	510,743	11.84	232,643	11.51	278,100	12.13
	2012	10,256	13.02	4,496	12.63	5,760	13.35
Ovarian	2018	69,611	1.61	69,611	3.44	-	-
	2012	1,850	2.35	1,850	5.20	-	-
Uterine	2018	124,048	2.87	124,048	6.13	-	-
	2012	1,539	1.95	1,539	4.32	-	-

Table II Variation of new cases of breast, colorectal, ovarian and uterine cancer in Romania, 2018 versus 2012 (adapted from [GLOBOCAN 2018; Straja et al, 2015])

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1.2. Prevalence

The 2008 Annual Report of the North-East Regional Registry of Cancer shows a prevalence of cancers in Iasi county of 15.08 per 100,000 inhabitants, which is below the average of the North-East Region (16 / 100,000 inhabitants) and a specific cancer mortality of 207.73 per 100,000 inhabitants, well above the average of the North-East Region (188.38 / 100,000 inhabitants) [Gheorghiu, 2011].

In 2018, IARC published for Romania an estimated number of prevalent cases (5 years) of cancer for both sexes at all ages of 12,334,517 (GLOBOCAN 2018). Case distribution by location indicates that breast cancer was the most common (16.9%), followed by prostate cancer (12.7%), colorectal cancer (11.6%), bladder cancer (5.2%), lung cancer (4.1%), skin melanoma (4%), uterine cancer (3.7%) and other cancers (41.6%) (Fig. 3). Among the other cancers that were recorded we mention: ovarian cancer with 1.6% [GLOBOCAN 2018]

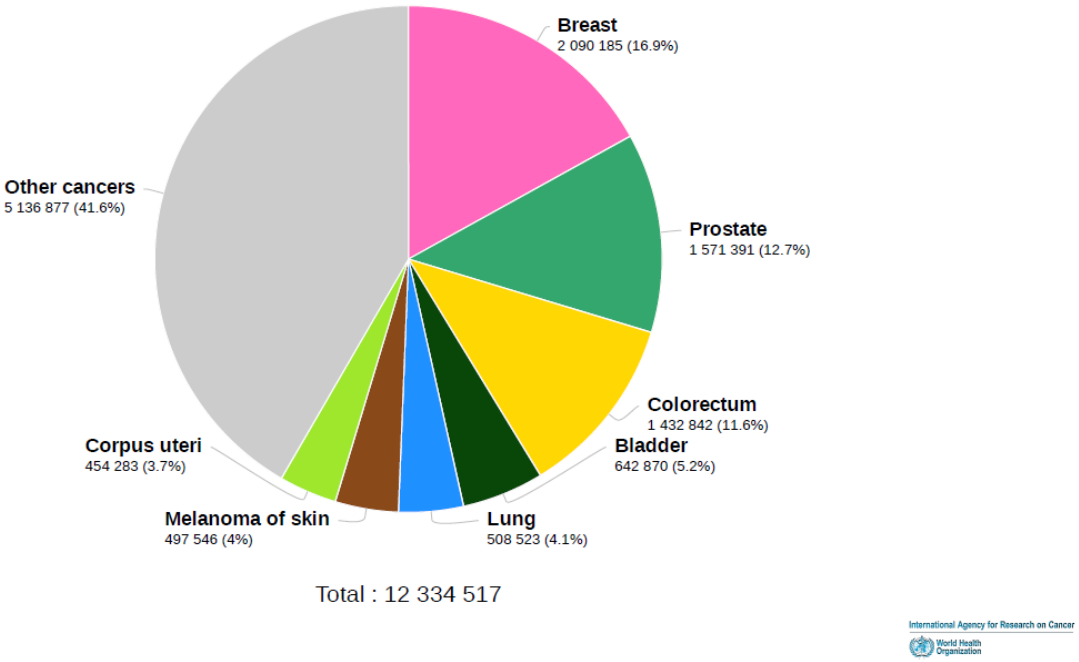

Fig. 3. Estimated number of prevalent cases (5 years) of cancer, both sexes, all ages, in Romania

(Source: *** International Agency for Research on Cancer. GLOBOCAN 2018. Globocan cancer observatory. Available from: http://gco.iarc.fr)

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1.3 Risk factors

A study by Matei M et al. in 2009, regarding the risk factors for genital neoplasia (ovarian cancer, uterine cancer and cervical cancer) among women of the North-East region of Romania, identified the pathological history as associated with an increased risk (with statistical significance) as well as fertilization treatment or BMI over 25 Kg / m2 (without statistical significance) [Matei et al, 2009]. In another study developed in the same region of the country, the authors identified the following factors associated with an increased risk of developing ovarian cancer: age (OR = 12.44; p <0.01x10-5) (58.63% of cases were diagnosed at ages between 50 and 69 years, which means during postmenopausal period); origin in urban area (OR = 3.45; p = 0.04x10-3); ovulatory period over 30 years (OR = 10.84; p <0.01x10-5) and age at diagnosis (OR = 2.01; p=0,016x10^{-3}). In contrast to most research founded in the literature, the authors did not identify the early age at first menstruation, late menopause, obesity, smoking, and high socioeconomic status as factors that increase the risk for ovarian malignancies, although the results of the current study were in agreement with some conclusions published by other authors [Matei et al, 2010].

1.4. Mortality

In terms of mortality, in Romania, in 2008, ovarian cancer ranked the 7th position, with a rate of 5.2 deaths / 100,000 women (Fig. 4) [GLOBOCAN 2008]. A rate of 8.23 deaths per 100,000 women (which far exceeds the country average) recorded in North-East Region. [Gheorghiu, 2011].

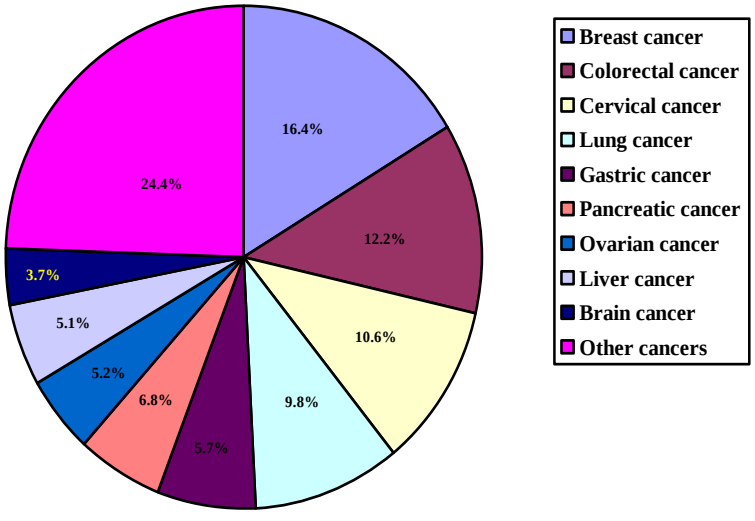

Fig. 4. Mortality (standardized by age) for the main types of cancers developed by women in Romania, 2008

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According to the International Agency for Research on Cancer (IARC), in Romania, in 2018, the estimated total number of cancer deaths for both sexes at all ages was 1,994,380 [GLOBOCAN 2018]. Deaths distribution by location indicates that lung cancer was on the first place (20%), followed by colorectal cancer (12.5%), breast cancer (7.1%), pancreatic cancer (6.6%), prostate cancer (5.5%), gastric cancer (5.3%), liver cancer (4%) and other cancers (39.1%) (Fig. 5). Among the other cancers that were recorded we mention: ovarian cancer (2.3%) and uterine cancer (1.5%) [GLOBOCAN 2018].

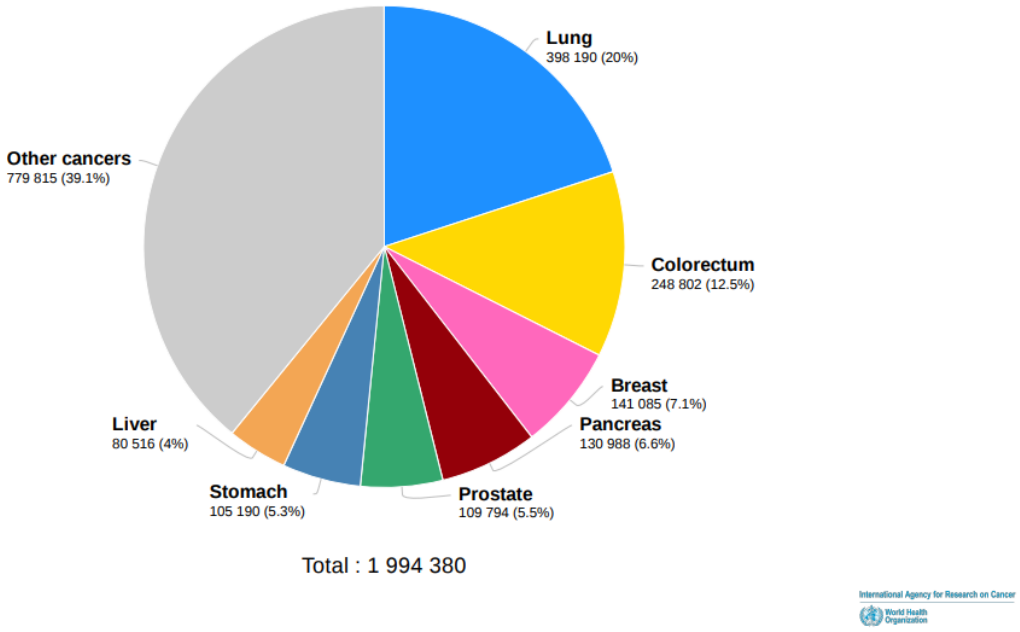

Fig. 5. Estimated number of cancer deaths, both sexes, all ages, in Romania

(Source: *** International Agency for Research on Cancer. GLOBOCAN 2018. Globocan cancer observatory. Available from: http://gco.iarc.fr.)

Comparing these data with those published by Straja ND et al. in 2015 one can observe the upward trend in the number of deaths for all types of cancer. For breast cancer, the number of deaths has risen from 3,244 estimated for both sexes in 2012 to 141,085 in 2018. Colorectal cancer has evolved from 5,675 deaths in 2012 to 248,802 in 2018. Uterine cancer has varied from 359 deaths in 2012 to 30,148 in 2018 and ovarian from 1,020 deaths in 2012 to 45,664 in 2018 (Table IV) [GLOBOCAN 2018; Straja et al, 2015].

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Types of cancer	Year	TOTAL		WOMEN		MEN	
		No.	%	No.	%	No.	%
Breast	2018	141,085	7.07	116,144	16.36	-	-
	2012	3,244	6.72	3,244	16.74	-	-
Colorectal	2018	248,802	12.47	86,529	12.19	111,569	11.62
	2012	5,675	11.76	2,446	12.62	3,229	11.18
Ovarian	2018	45,664	2.28	40,075	5.64	-	-
	2012	1,020	2.11	1,020	5.36	-	-
Uterine	2018	30,148	1.51	25,807	3.56	-	-
	2012	359	0.74	359	1.85	-	-

Table IV Variation of deaths caused by breast, colorectal, ovarian and uterine cancer in Romania, 2018 versus 2012 (adapted from [GLOBOCAN 2018; Straja et al, 2015])

2. The current situation on the National Strategies about the models used in genetic risk assessment for hereditary cancers

According to *Cancer Screening in the European Union Report on the implementation of the Council Recommendation on cancer screening*, by 2015, 20 out of the 28 member states had piloting, ongoing or rollout complete breast cancer screening population-based programmes; in addition, three member states (Estonia, Germany and Luxembourg) were planning to start population -based programme in 2016. Three member states (Germany, Greece and Latvia) had only non-population-based programme, although Germany was planning to introduce a population-based programme in 2016, for which the legal framework was adopted in 2013. No programme had been initiated in the remaining three member states (Bulgaria, Romania and Slovak Republic).

The population-based colorectal cancer screening programmes in the EU countries were mostly introduced after the Council recommendation was issued. In Romania there is just one regional registry that covers less than 15% of the country, and this data is used to extrapolate figures for GLOBOCAN. It must be a priority to found a way to stimulate the doctors to complete the database in order to obtain more correct and actual data.

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There are significant differences between Romania and Western European countries regarding the detection, treatment and survival rate in cancer diseases. Of all the risk factors for cancer, hereditary predisposition is the only risk factor that reaches positive or negative predictive value, justifying a medical monitoring and Oncogenetics. Oncogenetic monitoring of HBOC patients and the recommendation for a BRCA molecular genetic testing are made based on general inclusion criteria, such as: 3 or more cancer patients in the same family line, regardless of gender. Exceptions to this rule may be accepted if there are cancer cases diagnosed at early onset (before the age of 40), multiple cancers, and medullary or triple-negative breast cancers – for its high association with germline BRCA mutations. HNPCC patients monitoring is mainly based on the reunion of 3 criteria defined in Amsterdam in 1991 and reviewed in 1999: (1) at least three affected family members diagnosed and with reduced Lynch syndrome histologically proved (gastric, small bowel, ovarian, urothelial, pancreatic, or bile duct) cancers; (2) at least 2 first degree affected relatives in 2 different generations; (3) at least one of 5 affected individuals receiving such a diagnosis at age <50 years.

Before 2010, there was no information on BRCA mutations, sequence variants, or polymorphisms in the population of Romania. The first characterization of this population started with a cumulative effort of Gr. T. Popa University of Medicine and Pharmacy in Iasi, Alexandru Ioan Cuza University of Iasi, Auvergne University and the Jean Perrin Centre in Clermont-Ferrand (France). Thus, the first data on BRCA status in the population of North-Eastern Romania were published in 2010 [Negura, 2010a]. Molecular analysis performed for a small number of predisposing families was possible both because of the technological partnership with the French team, especially because of the local development of rapid methods of mutational pre-screening. These preliminary data justify the interest in understanding the genetic variations responsible for the hereditary predisposition to cancer in Romania.

There is no National Oncogenetic Healthcare System in Romania. And consequently there is no National Strategies for Diagnosis and oncogenetic management of hereditary cancer patients. Diagnosis of hereditary risk for breast / ovarian cancer through BRCA genes has been performed on couple of hundreds at risk patients and only in a context of scientific research, although the genetic counselling is a vital need for monitoring cases with this pathology. The screening of hereditary gene predisposition genes is currently a standardized practice in Western

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European countries, a practice that allows tailor-made monitoring and genetic counseling for those at risk in these families.

There are several priorities identified in the field of Oncogenetics:

1. The necessity to implement and develop medical oncogenetics in Romania, through the training of specialists in the field of oncogenetic consultations; the need to acquire know-how regarding criteria for the identification of cancers with hereditary predisposition, patient recruitment and oncogenetic monitoring. The long-term goal is to generate an oncogenetic expertise that will connect Romania to similar European structures, designed to monitor patients with hereditary cancer risk.

2. The need to identify and recruit patients with hereditary risk for monitoring: Hereditary Ovary and Cancer (HBOC), Hereditary Colorectal Cancer (HNPCC), Familial Colonial Polyposis (PAF). Patient identification will generate a consistent database and a representative biobanks, according to European norms and models, including clinical, epidemiological, morphopathological, molecular and genetic family history data. The information will contribute to complete the National Cancer Registry in Romania.

3. The need for molecular testing of genetic risk factors (BRCA1, BRCA2, MMR, APC). The molecular study will need to include a pre-screening - dedicated and rapid techniques - of known, specific, recurrent or frequent mutations in the population and, in particular, a full screening of mutations by complete gene sequencing.

4. The need to interpret the results obtained, which will be at the basis of an epidemiological assessment of the risk factors for cancer in the Romanian population [Matei et al, 2018].

Need feedback's genetic information to oncogenetician (thus the patient) and the development of monitoring Oncogenetics risk patients and their families through surveillance programs Oncogenetics Custom (PSOP) proposed that a first improvement of public health activities and Oncology in Romania.

There are also no National approved risk assessment models available in Romania. There was a prospective study conducted at IOCN Cluj that evaluated the performance of the Myriad risk assessment model for 250 high-risk breast cancer pts tested for BRCA1/2 mutations between February 2015 and December 2016 at IOCN. Inclusion criteria selected pts diagnosed with triple negative breast cancer under the age of 50, or having conventional family history criteria. Myriad

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web-based interface was utilized in order to assess the score for all 250 patients. BRCA testing was performed using an AmpliSeq-based sequencing analysis, on the Ion Torrent Personal Genome Machine at RCFG. Pathogenic mutations were validated using Sanger technology. MLPA was performed for all pts The Myriad genetic risk model can be an acceptable risk assessment tool for determining the risk of carrying BRCA mutations in Romanian population if the score is between 10-20%. The inaccuracy in carrier prediction using Myriad model represents a challenge worthy of additional investigation and comparison with other genetic models. Genetic counselors should recognize this limitation when using Myriad model and recommending genetic testing for Romanian high-risk breast cancer pts.

In 2016, partial or total mastectomies were most commonly performed in Belgium, the only EU Member State to record in excess of 200 of these procedures per 100 000 inhabitants, while the next highest rates were around 150 per 100 000 inhabitants in Italy, Croatia and Denmark. Poland, Cyprus and Romania recorded the lowest frequency for these procedures, with less than 50 partial or total mastectomies per 100 000 inhabitants in 2016. For partial or total mastectomies, more than half of the EU Member States for which data are available reported increases in the frequency of these operations between 2011 and 2016: the largest increases were reported by Cyprus (more than doubling between 2010 and 2016; note the break in series) and Romania (up 86).

Between 2010 and 2015, most EU Member States reported increases in the frequency with which these operations and procedures were performed. The most rapid increases were normally reported for colonoscopies, with increases between 30 % and 110 % in Croatia (2012-2015), Lithuania, Romania, Finland, Denmark, Malta (2012-2015) and the United Kingdom; only Slovenia and Italy reported falls in frequency (relative to population size). For colectomies, the largest increase in frequency of operations and procedures was reported by Cyprus and increases between 10 % and 30 % were observed in Romania, Malta (2012-2015), Lithuania, Finland, Denmark and Spain, whereas around half of the EU Member States with data available recorded decreases in the frequency of these operations, most notably in Luxembourg (2012-2015).

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3. The current practical situation on Genetic Testing Availability for HBOC and CCR in Romania.

According to "Cancer Screening in the European Union Report on the implementation of the Council Recommendation on cancer screening" In Romania, access to genetic testing is provided by a pharmaceutical company. The evaluation of current situation on cancer genetic testing and counselling availability was made by "ROHEALTH - Clusterul pentru sanatate" association. They conducted a survey of Genetic testing provider entities using they own network for identify the potential responders. To target our study to providers who could possible made genetic testing for breast/ovarian, colorectal or any other type of cancer, they elaborate a questionnaire and asked for information about availability of genetic testing for hereditary cancer diagnosis. The analysis of the answers received from 19 providers that agree to complete the questionnaire, revealed at this moment in Romania a very limited availability of cancer genetic testing and hereditary cancer genetic risk evaluation. Moreover, the health care entities who are interested to provide genetic testing services for population have little or no information about cancer genetic diagnosis and hereditary cancer risk molecular evaluation. Only 2 responders: Genetic Center (private entity) and Pius Brinzeu Emergency County Clinical Hospital (governmental entity) both from Timisoara reported availability of hereditary cancer genetic testing for diagnosys. Other few health care entities like Synevo Laboratories, Bioclinica, Medlife, Regina Maria and only one governmental institution - Cluj Oncologic Institute provide genetic testing for breast/ovarian and colorectal cancer.

The possible reasons for this pore situation could be: Sheer distance to travel can be an issue, and in Romania it was highlighted that the road network was of very poor quality making travel difficult. An insufficient workforce is also a major issue. Whilst respondents in some countries felt there were enough gynaecologic oncologists and medical/clinical oncologists to meet the needs of women with ovarian cancer, others said the situation was very different. For example, in Romania there are very few centers to treat women and there are no formal training programs for gynaecologic oncologists.

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4. The current situation on Research programs on mutation screening and founder mutation for HBOC and CRC.

Genetic testing has become an increasingly important tool in oncology. Patients with a personal or family history of breast or ovarian cancer are currently the most common users of such tests. Traditional genetic testing historically included the sequential analysis of single and generally well-described genes involved in heritable cancers, such as *BRCA1* or *BRCA2*. Single-gene testing is typically conducted when a family or personal history of disease is highly suggestive of involvement with a single or a small set of genes. An advantage of single-gene testing is that because the target gene is usually well described, test results are typically easily interpreted and there are often established clinical practice guidelines or standards of care associated with a pathogenic variant. Patients also typically receive pretest and post-test genetic counseling to help them understand the potential implications of their test results. The identification of *BRCA1* and *BRCA2* mutation carriers and individualized risk assessment is an important procedure growing in clinical importance, since management protocols for mutation carriers become well established and proven life-saving, risk-reducing preventive medical interventions exist [Janavicius R, 2010]. While the training of specialists in oncogenetics and the development of multidisciplinary biomedical networks to take charge of women at risk is becoming the standard of care in Western Europe, such services are only now becoming available in Romania. Over one thousand small sequence variation shave been reported in the Breast Cancer Information Core (BIC) database. More than half of these mutations cause the loss of function by premature protein synthesis termination, and around 60% are unique to a family. Not all variants can be considered pathological, notably missense alterations and intronic variants with unknown disease relevance. To date, 43.5% of *BRCA* variants are of uncertain clinical significance. The distribution of *BRCA* mutations has been studied in many populations, though only recently so in Romania. Different ethnic and geographical regions have different *BRCA1* and *BRCA2* mutation spectrum and prevalence. The knowledge of the genetic structure of particular populations is important for developing effective screening protocols and may provide more efficient approach for the individualization of genetic testing. Elucidating of founder effect in *BRCA1/2* genes can have an impact on the management of hereditary cancer families on a national and international healthcare system level, making genetic testing more

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affordable and cost effective. Eastern Europe is mostly characterized by few recurrent mutations responsible for the majority of HBOC families, while vastly diverse unique mutations can be found in most outbred western populations Although in the Eastern European countries like Poland, Upper Silesia, and Pomerania, BRCA mutations were found in more than half of HBOC families, and the large majority carried recurrent mutations like BRCA1 5382insC mutation predominated in Russian families; three known founder mutations common in Ashkenazi Jews and elsewhere (185delAG and 5382insC in BRCA1, and 6174delT in BRCA2) frequent in western Poland, Belarus, and Slovak HBOC families [Janavicius, 2010], until 2010 the distribution of BRCA mutations has not been studied in Romania.

The first study conducted by Negura L. et al in 2010 at Iasi Gr.T Popa University of Medicine, aimed to characterize BRCA mutations in relation to the predisposition to breast and ovarian cancer in Eastern Romania. These first results open the door to studies targeting the most common or ‘‘local’’ mutations, with the aim of comparing groups within Eastern Europe or with western populations. They observed a variety of BRCA mutations, which may place the North-Eastern Romanian population somewhere between western populations (few recurrent, many rare or unique mutations) and eastern ones (recurrent mutations responsible for the majority of predisposed families, very few novel or unique mutations). The overall mutation frequency was 41% (7/17; 5 BRCA1 and 2 BRCA2). About 40% of the familial breast cancer cases carried a BRCA mutation, regardless of age at diagnosis, familial aggregation, or bilateral cancer. Fifty percent of ovarian cancer cases and all patients with multiple cancers carried a mutation. Pedigrees that were extremely suggestive of a BRCA mutation, using for example the INSERM scores used in France [Eisinger 2004], more frequently carried mutations than those that were less suggestive. Exceptions were observed, however, with one very likely pedigree without a mutation, and one very modest pedigree bearing the recurrent BRCA1 c.181T>G mutation. One healthy member of a high-risk family presented a recurrent BRCA1 mutation.

Of the two unclassified variants observed, BRCA1 c.427G>C occurred in a family with a deleterious BRCA2 mutation, and in silico analysis suggests it is unlikely to cause disease. BRCA2 c.4589A>G was the only variant observed in breast/ovarian cancer family, and *in silico* analysis was suggestive of a deleterious mutation. Analysis of additional family members may clarify its role in oncogenesis.

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The research conducted at UMF Iasi continues. After the first description of genetic factors in Romanian population [Negura et al, 2010a] within a lot of HBOC families and implemented oncogenetic follow-up in North-Eastern Romania [Negura et al, 2010b]. A variety of *BRCA* mutations was observed, which may place the North-Eastern Romanian population somewhere between Western and Eastern populations. When performing a systematic BRCA test in larger populations, pre-screening PCR-based methods for the rapid and cheap identification of most common mutations can be particularly useful by limiting systematic BRCA gene sequencing, therefore reducing the time and costs of the analysis. Herein they describe a technique developed *in-house* for screening of an apparently common *BRCA2* mutation. Oncogenetic testing of *BRCA* status is becoming a powerful therapeutically predictive tool. It is now obvious that in a near future the uptake and demand for rapid *BRCA1/2* mutations testing will increase and more flexible genetic counselling strategies will be needed. Currently, most laboratories performing diagnostic analysis of the *BRCA* genes use PCR of exons and intron-exon boundaries coupled to a pre-screening step to identify anomalous amplicons. However, a full *BRCA1* and *BRCA2* gene screening still remains a labour and time consuming challenge due to large genes size, diverse mutations or variants of unknown significance (VUS) and complexity of large genomic rearrangements (LGRs), requiring special technical approach. This procedure still remains too complex and expensive to cover a broader target (e.g. all breast or ovarian cancer patients and their first degree relatives) and cannot be routinely applied in less privileged countries. We identified by sequencing a novel *BRCA2* mutation in exon 21, c.8680C>T. As full sequencing of the *BRCA* genes is complex, time-consuming and expensive, developing rapid and cheap pre-screening methods for targeting common mutations offers the advantage of quick and less expensive diagnosis. This strategy can be applied to populations with strong founder effects, where a limited number of mutations are to be screened. The PCR-RFLP assay presented here represents one such method for such a large-scale study. They firstly identified the *BRCA2* c.8680C>T mutation in a breast cancer family with five early onset cancer cases. This mutation is novel and not yet stored in the BIC database. While screening by the in-house PCRRFLP technique a sporadic lot of 50 breast and 25 ovarian cancer cases, unselected for familial history, the *BRCA2* c.8680C>T mutation was identify in a ovarian cancer sporadic case. This could open the hypothesis of a Romanian mutation, therefore strengthening the necessity of screening in a larger lot of patients. This data represent one early contribution in understanding the Romanian

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population genetic characteristic concerning *BRCA* genes and predisposition to breast and ovarian cancer. Research should be further developed in order to elucidate a mutation spectrum at a population level. But this first result open the way to targeted studies on most common or "local" mutations. This study also opens a way to investigate *BRCA* status in Eastern populations more in detail, with the aim

Due to the big size of both BRCA genes analysed for HBOC cases, as well as to the uniform distribution of mutations along the genes, the complete Sanger sequencing, which is the only accepted method in diagnosis, imply huge costs and time. Therefore, a relevant image of the mutation profile for each population is particularly useful for adapting screening and pre-screening strategies. Moreover, neither mutation hotspot nor neutral genic regions had ever been detected for those genes, so full sequencing is absolutely necessary. The only aspect, which could limit sequencing costs, is the non-uniform distribution of mutation in different populations, with founder or recurrent mutations opening the way for pre-screening methodology. Since the frequency of BRCA mutations in general population is low (prevalence of BRCA1/2 mutation carriers is estimated at 0.2% (1/500)), a general screening for mutation carriers in general population is not possible neither suitable. Unfortunately, the majority of models used for probability calculations often underestimate the probability of finding a mutation. Moreover, familial history is also absent or unknown in at least half of all mutation positive families and mutation detection methods vary between most centers. One possible strategy of more rapidly and efficiently detecting BRCA mutations is a pre-screening of the most common or recurrent mutations in middle- or big-size populations groups. In certain countries and ethnic communities, especially in geographically, culturally or religiously isolated populations the BRCA1/2 mutation spectrum is limited to a few founder mutations while in outbred populations, especially in the western world, mutation spectrum is significantly large. This leads to distinct mutation detection approach strategies applying for molecular diagnosis. Full-gene sequencing is required in oncogenetic diagnosis in the majority of western countries, while adapted pre-screening approach may be useful for founder/recurrent mutations, or even to identify anomalous amplicons prior to sequence. The need of pre-screening techniques is justified by the costs and time-consuming of full sequencing. However, a good knowledge of the target population is essential, comprising mutation diversity and frequency, founder and recurrent effect, geographic

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and ethnical distribution of haplotypes, briefly a large understanding of population oncogenetics [Negura, 2010].

By optimizing an allele-specific multi-plex-PCR method, 2010 Negura et al searched for recurrent 5382insC BRCA1 mutation in 26 recruited patients from 19 HBOC families. The BRCA1 5382insC mutation was found in two different HBOC families, with different breast/ovarian cancer familial history and without any apparent degree of relatedness. Sanger sequencing confirmed the presence of the mutation. They demonstrated the feasibility of a rapid screening in Romanian population for known recurrent BRCA1 mutations, by simple PCR-based techniques. This outcome, the first one in Romania, could open the way for a population study to determine the frequency of 5382insC in the Romanian population. This could also develop the oncogenetic approach and follow-up of BRCA mutations bearers in Romania

Aggregation of breast cancer within the family is the most important factor that increases the probability to be a carrier of BRCA mutations. Different guides were published to increase the cost-efficiency of genetic tests for BRCA mutations [Eccles, 2000, Burcos, 2013]. However, increasing the stringency of selection criteria reduces the chance to identify new BRCA mutations, especially in sporadic forms of cancers. Screening for common mutations in BRCA1 and BRCA2 has become a routine part of the investigation and management of familial breast and ovarian cancer. There are limited data regarding BRCA gene mutations or polymorphisms in Romania because these genetic tests are not currently provided by Romanian hospitals [Negura L 2010]. In a study conducted at Carol Davila University by Burcos T. et al 2013, the presence of nine previously reported as recurrent mutations was tested in consecutive women with breast cancer presented in a single surgical clinic from Bucharest and in control subjects. Among these, only 5382insC mutation in exon 20 of BRCA1 was found in three patients with breast cancer. The classical protocol, the detection kit and SSCP confirm the presence of this mutation in the blood and tumor tissue in heterozygous status. Thus, the BRCA1 5382insC germinal mutation has a high frequency in patients with breast cancer and absent in controls. The frequency of BRCA1 5382insC is much higher in breast cancer patients. Thus, the frequency of this mutation was higher than in unselected breast cancer patients of Ashkenazi Jewish (0.75%), German (1.0%) [Backe, 1999] and Hungarian (1.4%) [Van Der Looij, 2000] origins. The distribution of BRCA1 5382insC in breast and ovarian cancer high-risk families presents significant differences between populations. Thus, it was not identified in families with breast cancer from Sweden,

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Norway and Chile or it was found to be relatively common in families from Germany, Canada, Hungary and Romania [Burcos, 2013]. BRCA1 5382insC is well represented in this study authors detect it in 1 of 14 cases with family history of breast cancer. Based on the analysis of BRCA1 5382insC frequency and its geographical distribution this mutation was considered to have originated in the Baltic area during the medieval period and it have been spread through migration. This may explain its prevalence in Central and Eastern Europe and its decreasing prevalence from the East to the West regions of Europe [Burcos 2013].

In contrast to the promising results obtained at Carol Davila University related to possible founder effect of BRCA15382insC mutation and despite the fact that`s the second most recurrent mutation in Eastern European populations, having been already identified in several Romanian HBOC patients - no mutation carrier of BRCA15382insC was identified among breast or ovarian cancer patients investigated at the UMF Gr.T Popa Iasi by L Negura and colab. 2015. They made a complete screening of consecutive series of breast and ovarian cancer patients for the presence of BRCA15382insC using allele specific multiplex PCR on genomic DNA extracted from peripheral blood in consecutive series of breast (120 patients) and ovarian (50 patients) cancer patients. The selection of the cases was consecutive and independent of any familial cancer aggregation, familial oncologic history, or clinical/ histopathological criteria. The patients were identified and recruited at the Regional Oncology Institute of Iassy, Romania, between 2012 and 2013.

Previous research on Romanian population [Negura, 2010a; Negura, 2010b] highlighted about 50% of novel/familial mutations, but also 50% recurrent ones, no founder effect having been done yet as a little number of HBOC families already analysed. Some of the recurrent BRCA mutations in our population proved to be common with neighbouring countries, while some founder eastern mutations did not appear at all in our population. In the specific case of BRCA15382insC, according to National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) the average risk by the age of 70 years is 67% for breast cancer and 30% for ovarian cancer. The 5382insC (c.5266dupC) mutation in BRCA1exon 20 is the second most frequently reported mutation in the BIC database, being very prevalent in Central and Eastern Europe. Morover, Burcos and colab. from Carol Davida University revealed the presence of 5382insC in two different HBOC families, with distinct cancer phenotypes, out of a group of 20 families, which strongly suggested a recurrent effect of the mutation in Romanian

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population . Surprisingly, not one carrier of the mutation was identified out of 120 breast and 50 ovarian cancer patients in Iasi study. The results somehow interfere with previous works and seriously decrease the probability for 5382insC to be the most important recurrent mutation in Romania. This directly influence the pre-screening strategy for mutations detection, proving the higher importance of complete sequencing screening, more than in neighbouring populations anyway

More recently, technological advances have led to the development of multigene panels and next-generation sequencing (NGS), allowing for the analysis of multiple genes at one time. Although there are considerable advantages to this approach, there are also many challenges.

A major limitation of single-gene testing is that it only provides information about 1 gene, and thus, misses any other potential deleterious gene variants. Multigene panels identify pathogenic mutations in about 9% of patients with or at risk of a hereditary cancer. A study of 10,030 consecutive hereditary cancer NGS tests identified at least 1 pathogenic or likely pathogenic variant in 9% of cases; nearly half of these variants were in genes with moderate or unknown cancer risk. Similarly, pathogenic mutations have been identified in approximately 9% to 10% of patients at risk of developing hereditary breast or ovarian cancer. Similarly, several studies have identified pathogenic mutations harboured by patients who were negative for *BRCA1/2* mutations [Susswein, 2018].

Multigene testing comes with several challenges. Numerous laboratories now conduct multigene panels and NGS, many with their own unique panels. Plichta JK 2016 As a result, selecting a laboratory with an appropriate panel that is also captured by a patient's insurance coverage can be challenging. Because approximately half of the pathogenic mutations that are identified by multigene panels are of moderate to unknown risk, the clinical utility of the information provided by multigene analysis is unclear. Plichta JK 2016 Not all mutations warrant a change in clinical management, and inexperience or uneducated interpretation of test results can lead to inappropriate treatment decisions. An increasing number of referrals for prophylactic surgery — many as a result of low- to moderate-penetrance pathogenic mutations — have been reported. Many of the referrals were attributed to a misunderstanding of test results [Yu, 2015].

A prospective study aimed to evaluate the germline BRCA1/BRCA2 mutations in 250 high risk -triple negative under the age of 50, or having conventional family history criteria breast cancer patients tested between 02.2015-12.2016 at IOCN using an AmpliSeq-based

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sequencing analysis, on the Ion Torrent Personal Genome Machine at RCFG. Pathogenic mutations were validated using Sanger technology. MLPA was performed for all pts.

Of the 250 pts with breast cancer, 44 (17.6%) carried pathogenic mutations, 29 pts (11.6%) in B1 and 15 (6%) in B2, while 18 patients (7.2%) carried a Variant of Uncertain Significance (VUS). Patient features analysis confirmed the prevalence of younger age, higher grade, hormone receptor negative and Her2 negative status among mutated patients (data not shown). Out of the 16 distinct deleterious mutations identified, 7 (43.75%) occurred in B1 and 9 (56.25%) in B2. The founder mutations identified in B1 gene were: c.5329_5330insC (c.5266dupC) 11 pts (37.93%), c.3607C>T 9 pts (31.03%) and c.181T>G 4 pts (13.79%). Other B1 mutations where c.1687C>T 2 pts (6.89%), and c.4218delG (3.44%), c.212þ1G>T (3.44%), c.68_69delAG (3.44%) in one patient respectively. For B2 gene, c.9371A>T (46.66%) was identified as founder mutation (7 pts, 46.66%). Other mutations were found each in one patient (6.66%): c.1528G>T, c.4022C>G, c.7007G>A, c.8695C>T, c.9253delA, c.8680C>T, c.8755-1G>A, c.8695C>T. Of the founder mutations identified, two (c.3607C>T and c.9371A>T) have not been previously identified as founder mutations in any Eastern European country. This prospective study presents the first extensive results of germline BRCA1/BRCA2 mutations in Romanian high-risk breast cancer patients. The results indicate that at least 4 recurrent BRCA1/BRCA2 mutations qualify as founder mutations; two being newly identified as possible carrying a founder effect.

In the last few years multigene panel testing for Hereditary Breast and Ovarian Cancer (HBOC) using next generation sequencing is becoming more common in medical care.

Godescu et al , Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania in collaboration with IMOGEN Research Center Institute, Cluj-Napoca, Romania report their results on deleterious mutations screening of high and moderate-risk breast cancer genes BRCA1/2, TP53, STK11, CDH1, PTEN, PALB2, CHEK2, ATM. Genetic testing was performed in 130 consecutive cases with breast cancer referred for surgical evaluation and who met the 2016 National Comprehensive Cancer Network (NCCN) criteria for genetic testing.

The most frequent BRCA1 variant was c.3607C>T (7 cases) followed by c.5266dupC and c.4035delA (each in 4 cases). BRCA-2 mutations c.9371A>T and c.8755-1G>A was identified in 6 cases and VUS mutations in 3 cases.

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The complete list of detected mutations was:

BRCA1 c.3607C> T variant was diagnosed in 7 patients and was not reported until now in Romanian population, being the most frequent in the study group, all the patients originating from North-Western counties of Romania [Godescu, 2016].

In 6 cases the association with triple negative breast cancer (TNBC) was detected *BRCA1 c.5266dupC (p.Gln1756Profs*74), exon 20* - the most prevalent mutation in the north-eastern Romania population, in the only two studies conducted so far in the *BRCA1* mutation carriers from this region [Negura, 2010]. but an increased incidence of this variant is also found in countries bordering with Romania, like Hungary [Van der Looij, 2000], Serbia and Montenegro [Papp,1999].

BRCA1 c.4035delA was identified in 4 patients, of which 3 were relatives (mother and 2 daughters). All patients had as immunohistochemical characteristic the overexpression of HER2, being known that most *BRCA1* mutations are triple negative.

BRCA1 c.181T>G (p.Cys61Gly), exon 5 –, 2 patients were diagnosed with breast cancer and *BRCA1 c.181T> G* variant both of Hungarian ethnicity, mother and daughter.

For the rest of *BRCA1* mutations found in one case each in our study (*c.3726 C>T, c.2241dupC, c.135- 2A>G, c.1789G>A, c.737delT, c.3700_3704delGTAAA, c.843_846delCTCA, c5329_5330insC*) there is limited data available.

11 BRCA 2 variants, eight pathogenic and likely pathogenic (*c.9371A> T* (3 cases), *c.8755-1G> A* (3 cases), *c.1528G> T* (1 case) and *c.8695 C>T*(1 case)) and 3 VUS mutations. Only *BRCA 2 c.8695C>T* (p.Gln2899Ter) – was mentioned in Romanian population in a recent study [Eniu, 2017].

The two patients with *TP53* mutation had both *c.469G>T* variants.

PALB2 mutations: *c.93dupA* (2 cases), *c.3549C>G, c.509_510delGA, c.79G>T*.163

10 CHEK2 mutations, 8 pathogenic (*c.470T>C* (5 cases), *c.1232G>A* (2 cases), *1283C>T* (1 case), and 2 VUS cases.

ATM c.7630-2A>C ;c.2250G>A –c.1564_1565delGA –

In conclusion, this study has successfully identified 2 mutations in the *BRCA1* gene that are less common in the Romanian population, *c.3607C>T* and *c.4035delA*. Furthermore, the two pathogenic variants were associated with different histological and molecular phenotypes. The *c.3607C> T* variant was associated with triple negative breast cancer (negative ER, PR and HER2), respecting the general phenotype of patients with *BRCA1* mutations while variant

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c.4035delA was associated with HER positive, hormone-negative forms, an atypical element for patients with *BRCA1* mutations.

For the rest of the genes, the information could be useful for establishing specific variants of these mutations in the Romanian population and thus for their targeted identification and thus to a decrease of costs and a reduced time for obtaining results. The absence of VUS mutations in patients in the *BRCA1* group is most likely due to the fact that mutations in the *BRCA1* gene were more extensively studied compared to those of other genes. This could be also the explanation for the numerous VUS mutations identified in the group of moderate penetrance and intermediate risk genes, where larger studies are missing.

The same team conducted in 2017 a study provides information regarding the main deleterious variants with high and moderate penetrance involved in the pathogenesis of breast cancer in the population of North Western Romania. It also provides information regarding the immunohistochemical features of the breast tumors in the mutation carriers, demonstrating that the association of some immunohistochemical features with certain mutations may entitle us to a genetic testing in some situations.

The results showed an association of BRCA1mutations with a low percentage of ER, PR, an increased Ki67 index and a HER2 negative status when compared to the negative mutation group Also, patients with pathogenic BRCA1mutations associated poorly differentiated tumours (13 cases) or moderately differentiated tumours (6 cases), the results being similar to those in the literature [Goidescu I, 17]. Within the BRCA1group in this study, the BRCA1c.3607C>T mutation was identify in 7 cases, BRCA1c.5266dupC mutation in 4 cases and BRCA1c.181T>G mutation in 2. The BRCA1 c.5266dupC and c.181T>G mutations were reported to be present in the North-Eastern Romanian population in the only two studies conducted so far in our country [Negura, 2010]. The BRCA1 c.3607C>T mutation was the most frequent variant reported, and has not been reported to date in the Romanian population. Furthermore, this mutation was associated with TNBC in 6 cases and all patients were from cities located in north-western Romania. This sequence change creates a premature translational stop signal at codon 1203 (p. Arg1203*) resulting in an absent or disrupted protein product.

BRCA2 positive breast tumours behave as sporadic breast carcinoma variants, usually expressing ER and PR, unlike positive BRCA1 cancers as demonstrated in our study, where only one patient was diagnosed with TNBC (BRCA2 c.9371A>T). BRCA2 group - 7 mutation of

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which 3 cases had c.8755-1G>A mutation, 3 cases had c.9371A>T and c.1528G>T in one case. BRCA2 c.8755-1G>A mutation was never reported in Romanian population.

Conclusions : this research revealed a new BRCA 1 mutation which has not been reported to date in the North-Western Romanian population, BRCA1c.3607C>T, as the most frequent in this population. To date, the only studies conducted in this population argue that c.5266dupC is the most common variant in this region. The BRCA1c.3607C> T variant associates TNBC type, which is consistent with the general immunohistochemical profile of patients with breast cancer and positive BRCA1mutation.Regarding BRCA2mutations both c.8755-1G>A and c.9371A>T variants were never described in Romanian population and the data from the Eastern European countries for these two mutations are confined, the immunohistochemical pattern being similar to the sporadic forms of breast cancer. For the rest of the mutations our results regarding their correlation with immunohistochemical features were similar to the data reported by other studies, but the small number of cases makes it difficult to establish some pertinent conclusions. Subsequent studies on a larger number of patients will be able to clarify whether CHEK2 mutations are associated with multifocal or bilateral forms, or whether ATM and TP53 mutations have the overexpression of HER2 as an immunohistochemical feature. The main limitation of our study is that it is a retrospective study addressing only the North-Western region of Romania. Maybe a future multicenter study addressing more regions in Romania will gather more pathogenic variants that could create the premises for stronger conclusions.

Hereditary colorectal cancer

Hereditary cancer syndromes are classically characterized by markedly increased lifetime risks of multiple cancers, typically at young ages. Identifying individuals with inherited predispositions to cancer thus greatly impacts risk counselling for affected patients and their families, including the type and timing of cancer surveillance and potential recommendations for prophylactic surgery [Yurgelun et al, 2015]. Lynch syndrome is an autosomal dominant genetic condition that confers a high risk of colon cancer as well as other cancers including endometrial cancer and cancers of the ovary, stomach, small intestine, hepatobiliary tract, upper urinary tract, brain, and skin [Jasperson, et al, 2010]. Lynch syndrome is among the most common hereditary cancer syndromes, and estimates suggest that as many as 1 in every 300 people may be carriers

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of an alteration in a gene associated with Lynch syndrome and it may account for as much as 3% of all colon and endometrial cancers [Cohen and Leininger, 2014]. Although 30% of individuals diagnosed with CRC report a family history of the disease, only a small fraction carry germline mutations in genes associated with known hereditary cancer syndromes (Stoffel and Kastrinos, 2014). Lynch syndrome is caused by germline mutations in one of the DNA mismatch repair (MMR) genes (MSH2, MLH1, MSH6, PMS2, EPCAM) and is transmitted in an autosomal dominant fashion. The two most commonly mutated genes, MSH2 and MLH1, account for approximately 90% of mutations found in Lynch cases in most populations tested (Stoffel and Kastrinos, 2014). Mutation of MSH6 and PMS2 are identified in < 10% of Lynch cases, with EPCAM accounting for the rest. Next-generation sequencing assays are rapidly being incorporated into clinical laboratory practices and have diagnostic applications for hereditary cancer syndromes. Whole exome sequencing (WES) uses next-generation sequencing technology to provide information on nearly all functional, protein-coding regions in an individual's genome (Hitch et al., 2014). Sequencing can provide valuable information regarding pathogenic germline variants in individuals with suspected Lynch syndrome.

No information was available on the presence of high - risk CRC mutations in the Romanian population until 2018 when the results of a large study conducted at Carol Davila University in collaboration with deCODE genetics, Inc. Reykjavík, Iceland performed whole genome sequencing of 61 Romanian CRC cases with a family history of cancer and / or early onset of disease, focusing the analysis on candidate variants in the LS and FAP genes (Iordache et al 2018). The sequencing revealed 11 rare coding variants in CRC genes: six variants in MLH1 , one variant in MSH6 , one variant in PMS2 , and three variants in APC. We examined literature and clinical trial submission data collected by ClinVar with respect to pathogenicity, six out of the 11 variants have been previously reported by ClinVar. All six previously reported variants have a frequency lower than 1% in the ExAC database.

Novel variants :

MLH1 : c.251_255delAACTG is a frameshift variant with predicted amino acid change Lys84ThrfsTer4, and consequently assessed as a high impact variant.

MLH1 :c.1755dupT is a frameshift variant with predicted amino acid change Ala586CysfsTer7, and consequently annotated as a high impact variant.

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MLH1 :c.2104 - 6T > C is a splice region variant, and consequently annotated as having moderate impact.

The frameshift variant PMS2 :c.630dupA results in the predicted protein change Arg211ThrfsTer38, and consequently annotated as having high impact. The patient was diagnosed at age 44 and had extensive family history of gastrointestinal tract cancers.

The missense variant APC :c.5116T>A results in a protein change of Ser1706Thr, and is consequently annotated as having moderate impact. It has not been previously reported either by ClinVar or other studies. we consider this variant to be likely benign,

APC variant of uncertain significance (VUS),c.2780C>G (Ala927Gly)

Previously documented variants

MSH6 :c.3202C>T (RS63749843), is a stop -gained variant, assessed as a high impact variant with a protein change of Arg1068Ter. This variant was previously reported as pathogenic in ClinVar by 10 different submitters

MLH1:c.1148T>C (RS141344760) is a missense variant, and consequently of moderate predicted impact, resulting in a protein change of Met383Thr. The variant was previously reported as being of uncertain significance in ClinVar by five different submitters

MLH1 :c.1559-1G>C is a splice acceptor variant, annotated as high impact. This was the only candidate mutation found in two RC cases. The tumour samples from both carriers had lost MLH1 protein and both had documented family history or CRC. The variant has been reported previously by two different submitters as likely pathogenic for LS.

MLH1 :c.2041G > A (rs63750217) is a missense with a predicted protein change of Ala681Thr, and consequently of moderate impact; was previously reported as pathogenic in ClinVar by nine different submitters from clinical testing and research. In addition, OMIM has classified the variant as pathogenic for LS II.

APC:c.2780C>G (rs587781500) results in the amino acid change Ala927Gly and consequently of moderate impact. It was previously reported as a VUS in ClinVar by four different submitters

APC:c.5116T>A (Ser1706Thr).

APC:c.3682C>T is a stop - gained variant resulting in the protein change Gln1228Ter, and consequently of high impact. It was reported in ClinVar by a single submitter as pathogenic

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for familial multiple polyposis syndrome and was found in a recent study investigating somatic APC mutations and loss of heterozygosity status for 630 patients with sporadic CRC.

The frequencies of all candidate variants were assessed in a cohort of 688 CRC cases and 4567 controls.

This study is the first assessment of rare variants underlying LS in CRC patients in Romanians. It identify new variants specific to the Romanian population and show that some variants previously reported to be pathogenic in other populations also occur in Romania. Three novel pathogenic variants and 3 previously published pathogenic variants were identify.
In order to determine the prevalence of these variants in Romania, the frequencies of 11 variants were assessed in the full ROMCAN cohort. None of the mutations were found in more than 1 CRC patients except for MLH1 :c.1559 1G>C.

Identification of LS variants in the Romanian population is important in order to reduce the incidence and mortality of this multicancer disorder. This study is the largest effort, to our knowledge, to examine the genetic profile of this pathology in Eastern Europe. Due to study limitations, they were not able to extrapolate any other clinical observations, and we emphasize the need for future follow -up studies in the Romanian population. This study is the first step towards improving our understanding of the genetic particularities of this pathology in Romania and provides new insights for the scientific community studying the genetic epidemiology of LS.

A few other papers reporting results of screening of some previously reported polymorphisms associated to CRC genetic rick in Romanian population One of them was published in 2017 Colorectal Cancer Carcinogenesis: a Multivariate Genetic Model in a Cohort of Romanian Population by Lucia M. Procopciuc, Gelu Osian, Mihaela Iancu Babes-Bolyai University

The aim of the study was to examine whether the MTHFRC677T, MTHFR- A1298C, TS-2rpt/3rpt, TS-1494del6bp, NAT2*5C-C481T, NAT2*5A-T341C, NAT2*6B-G590A, NAT2*7B-G857A, NAT2*18-A845C, GSTM1-null, XRCC1-Arg399Gln, XRCC3-Thr241Met, XPD-Lys751Gln genetic variations are associated with CRC prognosis, in the presence of environmental and demographic factors. They genotyped 150 patients diagnosed with sporadic CRC using PCR-RFLP and sequencing methods. The performance of the final model was quantified using Nagelkerke's coefficient, the Hosmer-Lemeshow test, C statistics, and Somers' (D) index, capable of describing the model's goodness-of-fit and discrimination Multiple logistic

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regression analysis established a significant independent association of NAT2*18-A845C, MTHFR-C677T, XRCC3-Thr241Met, NAT2*7B-G857A, XPD-Lys751Gln, XRCC1-Arg399Gln and NAT2*6BG590A with an increased prevalence of sporadic CRC, regardless of the presence/absence of colonic tumors. After an adjustment for other polymorphisms and environmental risk factors, the risk to develop sporadic CRC was 2.25 ($p = 0.011$) and 2.31 ($p = 0.01$) in association with the NAT2*18-A845C and MTHFR-C677T genetic variants, respectively. The risk increased to 3.22 ($p = 0.0005$) and 3.69 ($p = 0.0009$) in association with the XRCC3Thr241Met and NAT2*7B-G857A polymorphisms. Also, patients carrying the XPD-Lys751Gln, XRCC1Arg399Gln, and NAT2*6B-G590A polymorphisms had a 4.16 ($p < 0.0001$), 5.16 ($p < 0.0001$), and 5.46-fold ($p < 0.0001$) increased risk for sporadic CRC, under the dominant genetic comparison model. In addition, an interaction between gender and alcohol, the effect of alcohol consumption on the risk of developing sporadic CRC being different in female and male patients was found.

This study confirmed the predictive role of some polymorphisms associated with DNA methylation and procarcinogen transformation into carcinogenic compounds in sporadic CRC risk and, also, the influence of environmental risk factors such as diet, smoking, and alcohol consumption on this association.

The other preliminary study -MTRR polymorphism and the risk for colorectal and breast cancer in Romanian patients conducted by Burcos T, et colab also at Carol Davila University evaluate if A66G MTRR (rs1801394) polymorphism is involved in predisposition for colorectal and breast carcinogenesis in Romanian patients. 300 individuals divide in four groups: sporadic CRC patients (n = 120), control CRC (n = 60), BC patients (n = 60) and control BC (n = 60), were genotyped by PCR-RFLP method.Frequency of genotype AA was 11.7% in CRC control and 5% respectively in BC control. For cancer groups the frequency of genotype AA was 9.2% in CRC and 0% in BC. Study results do not demonstrate an association between A66G MTRR polymorphism and CRC or BC in Romanian patients.

Until now, it have been described some polymorphisms of DCC gene that were associated with CRC. Thus, polymorphism at codon 201 of the DCC gene was associated with type of carcinoma, stage of disease and prognosis of CRC. For another SNP (rs2298606) the CC genotype was associated with the tumors located to the left colon (Burcos et al, 2010).

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Recently, Mates and colab conducted a study regarding several single nucleotide polymorphism (SNP) markers described in the literature as having site and/or stage specificity, including rs10795668, rs3802842, rs6983267, and rs4939827. Replication of initial findings in different ethnic groups by independent studies is required to unravel the population-specific differences in risk. They examined whether inherited risk variants at rs10795668, rs3802842, rs6983267, and rs4939827 exerted a differential effect on colon and rectal cancers in a Romanian hospital based series of 153 CRC cases and 182 non-affected control subjects prospectively recruited between 2007 and 2010. Rectal tumours were significantly associated with rs4939827 (OR = 4.85, P = 0.002) and rs6983267 (OR = 3.00, P = 0.036), suggesting that carriers of risk alleles at these loci had increased susceptibility to development of rectal cancer rather than colon cancer. Carrying the C allele at rs3802842 appeared to be associated with a lower risk for rectal tumours in our dataset. They found no association between genotypes and tumour aggressiveness as reflected by TNM staging. The associations between SNPs, and tumour site and staging remain to be further clarified. These results should be considered cautiously, but may be taken into account in future, larger epidemiological studies. (Mates 2012)

There was no information regarding the type of mutations in APC gene or their frequency for Romanian population until 2008 when Toma M and a team from Institute of Genetic of Bucharest conducted a first research on this aspect. The examined samples were constituted from 16 patients (10 men and 6 women) with CRC and 21 first and second degree relatives without clinical signs of CRC for occurrence of somatic and germinal mutations in the APC gene. The frequencies and the specific types of mutations in APC gene were investigated. In a number of studies, somatic mutations are reported to be present in 34 to 70% of sporadic CRC. The majority of mutations that determine a truncated and an inactivated APC protein are nonsense point mutations and frameshift mutations. Depending on the phenotype of patients examined and the methods used for mutation analysis, germline mutations have been detected in 30-85% of FAP families. Despite the small cohort of patients this study reveal mutations in eight region of APC gene that were tested by specific methods. This represents the initiation of implementation of new approaches searching for APC mutations detection in patients with CRC in Romanian population. At the same time, it assesses the possibility of using molecular analyses to improve populational epidemiologic screening in order to offer a real and productive genetic counselling and diagnosis for those persons being at risk for CRC. Authors aim to develop a project to

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increase the size of studied lots and optimizing new testing protocols for mutations in APC gene in order to enhance the sensitivity of the detection and to have possibility to apply statistical methods in order to avoid false positive results. The low penetrance variants of the genes that can contribute to the risk for colorectal cancer in Romanian population must also to be taken into consideration [Toma, 2008].

Another study conducted at University of Bucharest in 2015 by Alexiu et al have analyzed the association between four polymorphisms of APC gene (rs41116, rs465899, rs2229992 and rs2019720) and colorectal cancer in Romanian population. Blood samples were obtained from individuals with CRC (M:F=95:85) and from healthy persons (M:F=27:33). Genomic DNA was extracted from peripheral blood samples and the APC gene polymorphisms were assessed by PCR-RFLP. The promoter polymorphism rs2019720 was associated with CRC. Thus, the CC genotype (OR 2.307) and allele C (OR 1.843) increased the disease risk, while the AA genotype (OR 0.453) and allele A (OR 0.543) decreased the CRC risk. We found that the APC polymorphism rs2019720 was associated with CRC in Romanian population.

Research programs on mutation screening in HBOC and CRC patients

A. ROMCAN

Project consortium:

University of Medicine and Pharmacy "Carol Davila" (http://www.umf.ro/)

National Institute of Public Health (NIPH) (http://www.insp.gov.ro/)

deCODE Genetics (http://www.decode.com/)

Reykjavik University RU (http://www.en.ru.is/**)**

Genetic epidemiology of Cancer in Romania

Project financed through the SEE Mechanism

The ROMCAN Project ("Genetic Epidemiology of Cancer in Romania") proposes a systematic evaluation of genetic risk factors associated with breast cancer in female (BrCa), colon and rectum (CRC), prostate (PrCa) and lung (LuCa) cancers, representing almost half of the overall burden of cancer in the country, in the Romanian population, aiming to define high risk groups for whom specific preventive measures can be implemented. We also aim to examine

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if there is any effect modification of the genetic risk by ethnicity, focusing on the Roma ethnic group. The specific objectives of the project are the following:

- To establish a bio bank with biological samples, lifestyle-, demographic-and clinical data for a total of up to 4,300 cancer cases and 1,450 controls from the Romanian population;
- To catalogue the genetic variation in the biological samples collected in objective a), sing a high-content genotyping chip and imputation of un-genotyped variants;
- To assess the association between genetic variants and LuCa, BrCa, CRC and PrCa risk and disease phenotype;
- To identify high-risk mutations underlying BrCa and CRC by whole-exome (or whole genome) sequencing of 100 unrelated cancer cases with family history of the disease (50 BrCa, 50 CRC).
- To assess whether environmental/lifestyle risk factors and ethnicity modify genetic susceptibility to LuCa, BrCa, CRC or PrCa;

In Romania, there is a lack of quantitative data associating the cancer risk with lifestyle factors and even less information regarding the genetic characteristics. The ROMCAN project performed an evaluation of genetic risk factors associated with four major cancer types and examined if there is any effect modification by ethnicity, focusing on the Roma group, and created a valuable resource for genetic epidemiology studies, starting with a database containing lifestyle-, demographic- and clinical data. The major outcome is the information gained on the population frequencies of common genetic variation and the identification of high-risk mutations in breast and colorectal cancer genes. All genetic data was transferred to the Romanian partners who have through the course of the project developed the skills to analyze them. Valuable information on the genetic makeup of familial colorectal cancers, breast cancers of the Roma group would be provided. The first results show that both Roma and Romanian individuals can be seen as an admixture of other major European populations and are genetically indistinguishable from one another. ROMCAN project expanded the collection of data and samples over a total of 6,000 participants (4,290 cases and 1,730 controls), including a nested sample set from individuals of Roma ethnicity. These samples were genotyped with a chip of high content creating a valuable resource for genetic research on cancer in Romania. Furthermore, ROMCAN started to unravel the diversity of high-penetrance mutations in breast cancer and colorectal cancer in the Romanian population.

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The partners had previously enjoyed a very successful collaboration in a research project funded by the EU (Promark). With ROMCAN, the Romanian scientists were able to start building the necessary infrastructures for transferring the genetic data and to start the analysis. deCODE funded a large part of the cost of genotyping and sequencing done in the project and had a major involvement in the processing of biological samples, genotyping and analysis of genetic data. deCODE is among the world leaders in human genetic research and their expertise and technical capabilities were instrumental to the work conducted. Reykjavik University had a major contribution in setting up the training program for the Romanian scientists. ROMCAN has thus had tremendous effect on the bilateral relations.

The People program of the EU Marie Curie can be explored. In particular, the PhD student involved in ROMCAN apply for a Standard European Fellowship (EF-ST) with a proposal built on the results of ROMCAN with continued collaboration with the Icelandic partners.

B. European Union FP7 Program (ProMark project 202059)

Genetic prostate cancer variants as biomarkers of disease progression

Coordinated by: ISLENSK ERFDAGREINING EHF

Partner- University of Medicine and Pharmacy "Carol Davila"

The first major objective of PROMARK was to test if inherited genetic variants can serve as biomarkers for prostate cancer prognosis or treatment selection. The second major objective was to perform genomic and functional analysis of predictive variants in order to shed light on the patho-physiology of disease progression. For implement this objective the establishment of comprehensive collections of biological specimens and clinical data for prostate cancer biomarker research in four European populations was made.

Hospital-based prostate cancer cases and controls from the Romanian population was established. A collection of samples and data from at least 1 000 Romanian prostate cancer cases and 1 000 controls in order to start dissecting the epidemiology and genetics of the disease in this population was initiated. Such a collection was the first of its kind in the country. They will have established the first biospecimen repository for prostate cancer in Romania and greatly added to three existing sample collections from other Northwestern European region. This

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resource will be used to assess the utility of inherited genetic variants as biomarkers of disease progression and outcomes. Importantly, we will have created a valuable resource for future studies on prostate cancer.

C. Clinical trial information: NCT02317120.

ESR-14-10102/17.10.2014 ASTRA ZENECA Ctr.ESR-14-10102/17.10.2014

Period: 2014-2016

Project name -BRCA1 and BRCA2 Mutation in Romanian Population: a Study of Genotype - Phenotype Correlation at Diagnosis With Prospective Disease Outcome and Survival

Project consortium :

Iuliu Hatieganu University of Medicine and Pharmacy

I Chiricuta Institute of Oncology

Responsible -Alexandru Eniu, Prof. Dr.

The aim of the project is to determine types and frequencies of BRCA 1 (B1) or BRCA2 (B2) mutations in high-risk Romanian breast cancer patients, as there is no data published in this population. This prospective study evaluates the germline *BRCA1/BRCA2* mutations in 200 Romanian high-risk breast cancer patients tested between February 2015-January 2017 at IOCN. Inclusion criteria selected patients diagnosed with breast cancer before 40 years, triple negative breast cancer under the age of 50, or having conventional family history criteria. *BRCA1/BRCA2* testing was performed using an AmpliSeq-based sequencing analysis, on the Ion Torrent Personal Genome Machine (Life Technologies) at RCFG. The pathogenic mutations were validated using Sanger technology. MLPA was performed for all 200 patients. 200 high-risk breast cancer patients was analyzed and detected 32 (16%) patients with pathogenic mutations, 23 (11.5%) patients with B1 and 9 (4.5%) patients with B2 mutations. The majority of patients (99.5%) presented normal MLPA results; only one sample (0.5%) presented a deletion at CHEK2-9(10). The frequency of class 5 mutations identified in B1 gene were c.1687C > T (1%), c.181T > G (2%), c.3607C > T (3.5%), c.4218delG (0.5%), c.5329_5330insC (c.5266dupC) (4.5%) and for the B2 gene c.1528G > T (0.5%), c.4022C > G (0.5%), c.7007G > A (0.5%), c.8695C > T (0.5%), c.9253 delA (0.5%), c.9371A > T (2%). Frequency of deleterious BRCA mutations in our cohort was 11.5% for *BRCA1* and 4.5% for *BRCA2*. This prospective study

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presents the first extensive results on frequency and types of germline *BRCA1/2* mutations in Romanian high-risk breast cancer patients.

D. Optimization and implementation of molecular biology technology in depistation of breast and ovarian cancer hereditary predisposition

Alexandru Ioan Cuza University of Iasi (http://www.uaic.ro/)

Project PN-II-ID-PCE-2008, code 1990/2008

Project director Conf. dr. Anca Negura, UAIC

Understanding human genetic variation is currently believed to reveal the causes of individual susceptibility to disease and the large variation observed in response to treatment. The BRCA1 and BRCA2 genes are responsible for a large percentage of hereditary breast and ovarian cancer (HBOC) families. Screening for mutations in these two genes is now standard practice for HBOC cases in western Europe and north America, and permits medical follow-up and genetic counseling adapted to the needs of individuals in such families. In Romania, little information is available concerning the proportion of breast and ovarian cases due to genetic hereditary factors. As our country strives to enter the world of modern molecular medicine, it is preferable that we take advantage of the experience of other countries in establishing the medical and scientific teams necessary to offer appropriate research and services. We propose the implementation at Iasi of modern methodology in molecular biology, in order to develop molecular diagnosis for BRCA mutations in HBOC families. The principal objectives of our project are HBOC families recruitment, multiplex-PCRr and SNP-genotyping analysis of three known specific BRCA mutations, screening of BRCA mutations by SurveyorTM heteroduplex analysis and DNA sequencing, as well as oncogenetic follow-up of BRCA mutations carriers. Foreign monitoring by our french research partners, the Auvergne university and the Jean Perrin molecular oncology center in Clermont-Ferrand, will allow us to draw up the first molecular model of hereditary breast and ovarian cancer risk characterization in north-eastern Romanian population.

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E. Comparative study of gene expression, in healthy and tumoral tissues, in carriers of mutations and polymorphisms of predisposition genes to breast and ovarian cancer

University of Medicine and Pharmacy Gr.T.Popa Iasi (http://www.umfiasi.ro)

Project CNCSIS-RU-PD 557/2009

Project director Conf. dr. Lucian Negura, UMF Iasi

Up to 10% of breast and ovarian cancer cases are attributable to hereditary factors. Germinal mutations in predisposition genes like BRCA1 or BRCA2 are responsible for up to 90% of cancer cases in hereditary breast and ovarian cancer (HBOC) families. For mutation carriers, the lifetime risk of developing cancer is significantly increased compared to risks in general population. Screening for BRCA mutations and polymorphisms is nowadays standard practice in the western world, allowing medical follow-up and genetic counselling of risk patients from HBOC families. Recently, we implemented in our laboratory molecular diagnosis for BRCA and oncogenetic follow-up, which permitted generation of the first data regarding genetic BRCA profile in Romanian population. The challenge in interpreting sequence variations within such genes derives from their ubiquitous presence as tumour suppressor / caretaker / gatekeeper genes, and from associated pathogenesis, mainly localized at the mammary and ovarian epithelium level. Although there are particular characteristics of phenotypes associated with BRCA neoplasias, little information is available concerning the direct consequences of different BRCA mutations and polymorphisms on the expression in the tumour of other cell cycle regulating genes as p53, p21, ATM, CHEK2, Rad50, Rad51. Also, little is known on correlations between regulation pathways involving these proteins, while gene expression represents the perfect instrument in understanding such correlations. We propose a comparative analysis, by multiple approaches of candidate genes expression quantification, in healthy and tumoral tissues, depending on the presence in germinal line or in somatic tumor of deleterious BRCA mutations or of polymorphisms with uncertain pathogenic role.

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F. Development of a multitechnological approach to evaluate the hereditary risk to colorectal cancer (MULTITECHLYNCH)

University of Medicine and Pharmacy Gr.T.Popa Iasi (http://www.umfiasi.ro)

Project CNCSIS-RU-PD 557/2009

Project director Conf. dr. Lucian Negura, UMF Iasi

Colorectal cancer incidence and mortality are continuously increasing in Romania. Moreover, there are important inequalities between Romania and other European countries regarding early detection, treatment and survival to oncologic disease. Oncogenetics is defined as medical and diagnostic follow-up of patients and families presenting a hereditary or familial risk to cancer. In Hereditary Non-Polyposis Colorectal Cancer (Lynch syndrome), cumulated lifetime risk is extremely elevated, due to mutations in mismatch repair MMR genes (MSH2, MLH1 and MSH6). Screening for germline mutations in these genes is part of oncogenetic molecular diagnostic for high risk families. As most of criteria used to identify hereditary predisposition largely underevaluate the proportion of mutation carriers, and mutations are found in less than 60% of analysed patients, a complete and efficient model of pre-screening is indispensible. We propose the development and implementation of a combined multitechnological approach in order to rapidly and accurately evaluate the hereditary risk to colorectal cancer. Our model will combine germinal and tumoral (somatic) analysis, by molecular mutational screening of MMR and BRAF genes, as well as microsatellite instability, promoter hypermethylation analysis and immunohistochemical investigations on MMR proteins. The benefit of the model will either concern patients, families and public health system.

G. CHRONEX-RD : Involvement of oncogenetics activities within the project "The East European Network of Excellence for Research and Development in Chronic Diseases CHRONEX-RD"

H. International research applications not granted

- Inherited prediSposiTiOn and genetic suscePtibility to Breast Cancer in ethnically diverse EUropean populations (STOP BC in EU). ERA-NET on Translational Cancer Research (TRANSCAN) Joint Transnational Call for Proposals 2012 (JTC 2012) on: "Translational research on primary and secondary prevention of cancer"

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- Development of oncogenetics in Romania (RONCOGEN). PN-II-ID-PCE JOINT PROPOSALS France-Romania 2012 (proiecte bilaterale România-Franţa tip Brâncuşi).
- Whole Genome sequencing in Greek and Romanian patients with hereditary breast cancer negative for mutations in BRCA1 & BRCA2 genes (SEQGENGRERO). PN-II-ID-PCE JOINT PROPOSALS Greece-Romania 2010 (proiecte bilaterale România-Grecia).
- Development of a set of tools enabling the interoperability of different systems (EHR, EDC, LIMS) from different centers. Demonstration on two medical cases : neurodegenerative diseases and inherited cancers (MedDecisionPIPE). Call FP7-ICT-2009-4 Small or medium-scale focused research project (STREP).

5. List of institutions, cancer centres etc. that provide genetic testing and counselling services in Romania

ROHEALTH survey also revealed a lack of information on availability of cancer genetic counselling centres. It could means a real lack of this kind of services provided in Romania at this moment. Only 2 of this kind of centres was mentioned by ROHEALTH survey responders; One is Oncogen – Centre for Gene and Cellular Therapies in the Treatment of Cancer is the first state-of-the-art research centre for gene therapies in Romania, dedicated to research in areas that are closely connected to clinical branches of the Pius Brinzeu Emergency County Clinical Hospital Timisoara. The second is Department of Oncogenetics at “Grigore T. Popa” University of Medicine and Pharmacy – Iasi.

All responders appreciated as useful the possibility of developing a network of specialized units to identify individuals at risk for hereditary cancer. These units would provide both molecular testing and further clinical management of identified cases. Respondents consider important to inform family members about a potential inherited cancer predisposition for they could take appropriate preventive measures.

The usefulness of these networks would result from a specialized surveillance of families with hereditary cancer risk and would significantly influence personalized therapy in existing cases. They considered being important to include the activities of the oncogenetic centers in a National Program financed by the Public Health Ministry so that the addressability, accessibility monitoring of the patients and their families could be achieved.

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6. The situation of Education Programs regarding Oncogenetics:

ROHEALTH evaluation also revealed a lack of training programs in Oncogenetics for medical staff and for the general population. Only one respondent mentioned that such programs were carried out at the initiative of a private medical unit (Genetic Center). Another postgraduate course for teaching stuff organized by University of Medicine and Pharmacy Carol Davila , Bucharest were mentioned. There is no much available information on research programs correlated with the educational ones in the field of Oncogenetics. The respondents mentioning the cross-border project Romania-Serbia (project coordinator - the Oncogenic Center) and several projects funded by the EU.

The educational programs conducted by Department of Oncogenetics at "Grigore T. Popa" University of Medicine and Pharmacy – Iasi.

For students

- Since 2014- Scientific Oncogenetic club for students
- 2014- Epidemiological evaluation of cancer patients within the Department of Oncogenetics -The 11th International Congress for Medical Students and Young Doctors (Congressis 2014), Iași România, 10-13 aprilie 2014
- 2015- Congressis 2015, Iași România, 1-5 aprilie 2014

For doctors

- 2012 – Oncogenetic postgraduate course organised by UMF Iași
- 2013 – Advanced School of Ontogenetics, Slănic-Moldova, România, 10-14 aprilie 2013
- 2014 - Advanced School of Ontogenetics, Gura Humorului, România, 30 oct.-1 nov 2014

Requests to Ministry of Health for inclusion of Oncogenetics in the National Cancer Program

- Justification note for including of Oncogenetic activities in National plan of Oncology - addressed to Public Health Ministry in December 2012;
- 2013 - Justification note for including of Oncogenetic activities in National plan of Oncology - addressed to Public Health Ministry in 20 March 2013

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- Justification note for including of Oncogenetic activities in National plan of Oncology - addressed to Public Health Ministry - 29 January 2014
- Justification note for including of Oncogenetic activities in National plan of Oncology - addressed to Public Health Ministry - 27 May 2015
- March 11, 2015 "Oncogenetics - Medicine of the Future in the present " organized by the Coalition of Romanian Chronic Diseases Patients Organizations and the Department of Oncogenetics from "Gr.T.Popa" University of Medicine and Pharmacy Iaşi, under the auspices of the Commission for Health from the Senate, Senate of Romania, Bucharest, with the participation of the Ministry of Health and the National Health Directorate

For general population

- Webpage of Department (www.oncogenetica.umfiasi.ro)
- Information for doctors and patients: Oncogenetic information brochure for doctors; Oncogenetic information leaflet for patients

7. The identification of the practical needs (infrastructure, human and financial resources) in order to establish and to develop an Oncogenetic Network

The Molecular Diagnostic Laboratory is organized in 3 main areas:

7.1. DNA extraction area (pre-PCR area), corresponding to the following activities:

1. Receiving and processing biological samples (peripheral blood / tissue)
2. DNA extraction by saline, non-enzymatic and non-toxic methods
3. DNA quantification and purity evaluation by spectrophotometry
4. DNA dilutions and aliquoting in a DNA biobank
5. DNA storage as a biobank

Required equipment:

- Laminar hood, HePa filter
- Cooling centrifuge for 50 ml tubes and 96-well plates
- Cooling centrifuge for 1,5 ml and 0,2 ml tubes
- Vortex mixer

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- Adjustable thermoblocks and Adjustable temperature waterbath
- UV-Vis spectrophotometer for small volumes measurement
- Adjustable micropipettes
- 4C fridges, -20C freezers

7.2. PCR amplification and DNA sequencing area (PCR area), for following activities:

- Dilution of all reagents
- PCR amplification mixes and reactions
- Migration of PCR products by agarose gel electrophoresis and visualization
- Amplicon purification by enzymatic non-toxic methods
- DNA sequencing mixes and reactions (dye terminator, Sanger method)
- DNA sequencing products purification by resin, non-toxic methods

Required equipment:

- Cooling centrifuge for 96-well plates
- Cooling centrifuge for 1,5 ml and 0,2 ml tubes
- Thermal cycler with dual block (2 x 96-well plates)
- Real-time thermal cycler
- Vortex mixer
- Adjustable thermoblocks
- Adjustable temperature waterbath
- Adjustable micropipettes, multi-channel micropipettes, adjustable volume dispensers
- 4C fridges, -20C freezers

7.3. Capillary electrophoresis / interpretation area (post-PCR area), for following activities:

- Preparing of 96-well plates for capillary electrophoresis
- Loading of 96-well plates on capillary sequencer (genetic analyzer)
- Capillary electrophoresis
- Data processing and export
- Data import and interpretation
- Elaboration of molecular diagnostic test results

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Required equipment:

- Cooling centrifuge for 96-well plates
- Capillary sequencer (genetic analyzer)
- Adjustable micropipettes, multi-channel micropipettes, adjustable volume dispensers
- 4C fridges, -20C freezers
- Desktop computer with appropriate software for data interpretation

Human resources requirement:

- Molecular biologist as diagnostic responsible (post-doc or higher) : 1
- Molecular biologist as workflow responsible (Masters degree or higher) : 1
- Biologist / biochemist for main workflow : 2
- Laboratory staff : 1
- Cleaning staff : 1

Typical diagnostic workflow:

1. DNA extraction by saline, non-enzymatic and non-toxic methods
2. DNA quantification and purity evaluation by spectrophotometry
3. DNA dilutions and aliquoting
4. Mutation pre-screening for recurrent mutations (PCR-based techniques)
5. PCR amplification
6. Migration of PCR products by agarose gel electrophoresis and visualization
7. Amplicon purification
8. DNA sequencing (dye terminator, Sanger method)
9. DNA sequencing products purification
10. Capillary electrophoresis
11. Data processing and export
12. Data import and interpretation
13. Elaboration of molecular diagnostic test results

Financial needs estimate:

- Equipment: EUR 150.000

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- Reagents: EUR 3000 /index case sample and EUR 100 / mutation verification in relatives sample

Conclusions

The cancer morbidity and mortality rates are significantly higher in Romania compared to other countries in the European Union. The threat that several more frequent hereditary cancers (colorectal cancer, breast cancer and ovarian cancer) represent to, both male and female population health is too serious to ignore. The efforts of recent years was focused on hereditary cancer risk assessment in order to address early genetic cancer diagnostic and prevention given the evolving roles of citizens, addressability of the patients and health professionals increased interest for new molecular methods for diagnosis. Principles of oncogenetic was promoted especially in Oncological Centers and Universities trough research dissemination at congresses and conferences, professional training programs , cornerstones of all medical progress hat must drive improvements in service delivery across these areas. Thus, the creation of national oncogenetical center network integrated into a similar European structure to fill a void in cooperation, collaboration and shared experiences among countries with similar needs in order to align national health policy with EU- strategies are needed more urgently now than ever.

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