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Molecular diagnosis methods of BRCA mutations in breast cancer

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Breast cancer is the most common malignancy among females in the world. It results from genetic and environmental factors leading to accumulation of mutations in essential genes. Approximately 5%–10% of all breast cancers are associated with hereditary susceptibility due to mutations in autosomal dominant genes, such as BRCA1 and BRCA2, p53, PTEN, and STK11/LKB1 in women. The molecular analysis of breast cancer relies on several technical approaches, which allow genetic and physical mapping, characterization of the gene structure, expression studies, and identification of disease-causing mutations. The most common gene changes in breast cancer are those of the BRCA1 and BRCA2 genes. Women who know they carry the mutated gene may use this information to make more informed decisions about their health care, including whether to use tamoxifen and/or prophylactic surgery to delay or prevent the onset of cancer. Mutation screening methods vary in their sensitivity for BRCA1 and BRCA2. Nearly 2,000 distinct mutations and sequence variations in BRCA1 and BRCA2 have already been described. Methods widely used in research laboratories miss nearly a third of the mutations that are detected by DNA sequencing. In addition, large genomic rearrangements are missed by most of the techniques, including direct DNA sequencing, currently used for clinical testing. Such rearrangements are believed to be responsible for 10% to 15% of BRCA1 inactivating mutations. There is no single perfect method to screen for unknown mutations; combinations of these methods may be necessary for accurate genetic diagnosis. This overview will consider the nature of breast-cancer susceptibility genes, theirs function in breast cancer and comparison of novel molecular diagnosis methods for mutation detection in these genes. It covers the methods used for detection of unknown and known BRCA mutations, namely the Two-Dimensional Gene Scanning (TDGS), single-strand conformation polymorphism (SSCP), heteroduplex analysis (HDA), Denaturing High-Performance liquid chromatography (DHPLC), DNA microarray technology, Real time PCR, Mass spectrometry, Allele-specific PCR, Multiplex mutagenically separated PCR, and the Protein truncation test. The methods such as allele-specific amplification (ASA) and multiplex mutagenically separated PCR can detect one mutant allele in a background of 104–106 wild-type alleles but they are not amenable to automated, high-throughput, and multiplexed applications.

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BRCA1 gene mutation in exon 20 (5382insC) is frequent in patients from breast and/or ovarian cancer families from Eastern part of Poland

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Mutations in BRCA1 gene predispose a carrier to breast and/or ovarian cancer development. It is known, that mutations in this gene very rare occur de novo and have founder character. This peculiarity makes the identification of patients with mutations and consequently increased risk of breast and ovarian cancer development much easier. For Polish population there were discovered 5 the most frequent mutations in BRCA1 gene: 5382insC (exon 20), C61G (exon 5), 4153delA (11p region of exon 11), 185delAG (exon 2), and 3819del5 (11o region of exon 11). Still there is no enough information about founder mutations in BRCA1 gene for population from Eastern part of Poland.

The aim of the study: To seek three - 5382insC, C61G, 4153delA - polish recurrent mutations of BRCA1 gene in patients from Eastern region of Poland.

Material and methods: The studied group (168 patients from 143 families) consisted of breast cancer patients and healthy persons with breast and/or ovarian cancer aggregation in family history, who live in Eastern part of Poland.

DNA of patients was isolated from peripheral blood cells, and then was subjected to analysis with the help of MULTIPLEX method (patent: PL 185957) based on simultaneous amplification of three examined BRCA1 regions. Amplification of exon 20 and exon 11p was mutation specific and was yielded only in presence of the mutations; while PCR products of exon 5 was obtained in all probes and subjected to digestion with enzyme AvaII in order to reveal the mutation.

Results and conclusions: We detected 5 (3.5%) families with mutation in BRCA1 gene and all mutations were 5382insC (exon 20), that let us to conclude this BRCA1 mutation the most frequent in patients from Eastern part of Poland.

Among mutation carriers' families there were families with the only breast cancer (3 families) and the only ovarian cancer (1 family) as well as both cancers (1 family) presence in family history. In these families except breast and ovarian cancers also malignancies of lung, uterus and liver were found.

The lack of histopathological data of all breast cancer patients with mutation in BRCA1 gene did not allow to perform statistical analysis of dependence of histological breast cancer origin upon the presence of 5382insC mutation. Nevertheless, it is worth to note, that in our study patients with BRCA1 gene mutation developed breast cancer of ductal and lobular origin.

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Family syndromes of cancer of female reproductive organs in Chernivtsi region

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The features of pathogenesis of cancer of female reproductive organs are defined by the endogenous factors – endocrine metabolic impairments and genetic factors. The study of the

role of genetic factor is a perspective direction of oncogynecology. The clinical-genealogical analysis of thousands of genealogies of patients with cancer of female reproductive organs, allowed H.Linch et al to unify the clinical-genealogical criteria which allow to distinguish between inherited and sporadic tumors. The study goal was establishment of features and patterns of distribution of tumors in families of patients with ovarian and endometrial cancer. The clinical-genealogical analysis of 520 patients with ovarian cancer and 482 patients with endometrial cancer in Chernivtsi region was conducted. The analysis revealed that the most common malignancies in families of these patients were tumors of female reproductive organs (ovarian, endometrial and breast cancer), gut (stomach, colon), lungs, prostate etc. Among 520 genealogies of patients with ovarian cancer and 482 genealogies of patients with endometrial cancer we selected 103 genealogies in which malignant tumors of female reproductive sphere were twice more common, which is a clinical-genealogical criterion of family cancer of female reproductive sphere. We analyzed the spectrum of family aggregation of cancer of different anatomic localizations in these 103 genealogies. The features of spectrum of aggregation of cancer in these families were a base of classification of family cancer of female reproductive organs. The analysis revealed 6 typical family situations. 2 of 6 syndromes are organ-specific (they show up as a cancer of the same localization, and 4 syndromes are variants of "general family cancer syndrome" and are characterized with wide spectrum of tumors mainly of the female reproductive organs.

Classification of variants of accumulation of cancer in Chernivtsi region:

1. Family ovarian cancer
2. Family endometrial cancer
3. Family ovarian and breast cancer
4. Family ovarian/endometrial and breast cancer
5. Family ovarian/endometrial/breast/colon cancer (Lynch II)
6. Family endometrial/gut cancer.

Conclusions: The results of the study could be a base for development and planning of early diagnostics and prevention of tumors in families of patients, such as the use of tumor markers, determination of genes of disposition, which is effective from the economic and social point of view.

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Generation of Coronavirus-based multigene RNA vectors

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Coronavirus-based vectors are currently considered a promising means to genetically deliver multiple heterologous genes to specific target cells. They are positive-stranded RNA viruses replicating in the cytoplasm without a DNA intermediary, making insertion of viral derived sequences into the host cell genome unlikely. Coronaviruses have the largest known RNA genome, therefore a cloning capacity of more than 6 kb is expected. They posses a unique transcription strategy resulting in the synthesis of 6-8 subgenomic mRNAs encoding mainly structural genes encoded at the 3' third of the genome, these genes can be replaced by multiple heterologous genes such as tumor antigens and cytokines. These vectors are attenuated since 2-3 structural genes are replaced resulting in replication competent but propagation deficient vectors. An important consideration for viral vaccine vectors is the potential for efficient delivery of their genetic material to specific target cells. Targeting of viral vaccine vectors to dendritic cells (DCs) is highly desirable in order to optimise vaccine efficacy. The mouse hepatitis virus (MHV) and the human coronavirus (HcoV) 229E have their receptors (CEACAM-1 and hAPN or CD13, respectively) expressed on DCs and macrophages. This indicates that MHV and HCoV 229E based vectors could be used to deliver genetic cargo efficiently to DCs via receptor-mediated transduction. Since MHV is able to infect DCs, recombinant MHV vectors in the context of a murine model can serve as a paradigm for the development and evaluation of coronavirus vaccine vectors suitable for in vitro and in vivo transduction of human DCs.The anticipated results in the murine animal model will guide the development of coronavirus-based vaccines in general and will pave the way for the generation of HCoV 229E-based vaccines in humans.

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Immune surveillance-related genes are significantly over expressed in the breast epithelium of postmenopausal parous women

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Endocrine and reproductive influences significantly affect play major roles in the lifetime risk steadily increasing incidence of breast cancer development in women. Nulliparity is one of the most firmly established risk factors for breast cancer, whereas early full term pregnancy and parity confer a significant protection. In order to elucidate the molecular pathways through which pregnancy exerts a protective effect, we have analyzed the genomic profile of lobules type 1 (Lob 1) present in reduction mammoplasty specimens obtained from 5 parous and 2 nulliparous postmenopausal women. Total RNA was obtained from epithelial cells of Lob 1 that were dissected using laser capture microdisection. RNAs were individually amplified employing oligo-DT T7 primer and in vitro transcription reaction. We hybridize cDNA microarrays containing 40,000 genes and after normalization of the data using Lowess method, we performed the confidence analysis of 99% level using GeneSight software. We found an interesting cluster of genes up-modulated (>2.0 log, $p<0.01$) that were related to the immune system. In the present work we performed real time RT-PCR using a microfluidic card platform. The TaqMan® Low Density Immune Profiling Array contained 90 genes related to the immune system, and 18S rRNA as an internal control (Applied Biosystems 7900HT TaqMan® Low Density Array Upgrade, ABI). Epithelial cells from parous women significantly over-expressed 20 genes related to immune surveillance when compared with the RNA from nulliparous women. Those genes included: BCL2-associated X protein (BAX), complement component 3 (C3), chemokine (C-C motif), receptor 4 (CXCR4), CD34 antigen, Collagen IV (COL4A5), glucuronidase beta (GUSB), interleukin 1 b (IL-1b), interleukin 6 (IL6), major histocompatibility complex class II, DR alpha (HLA-DRA),