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Role of 5382 insC Mutation in BRCA 1 Gene in the Development of Hereditary and Multiple Primary Tumors (Clinical Case)

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Abstract. Case report of the development of multiple primary tumors, namely hereditary ovarian cancer and breast cancer in proband was described. According to the results of complex examination and molecular-genetic counseling proband's disease was associated with 5382 insC mutation in BRCA1 gene. This indicated the role of molecular genetic examination in detection of multiple primary malignant tumors of ovaries and breast in persons with the mutations in BRCA1/2 genes.

Keywords: genealogic tree; proband; hereditary predisposition; mutations in BRCA1/2 genes; ovarian cancer; breast cancer.

Introduction

The role of genetic factors in the development of hereditary forms of cancers of organs of female reproductive system (COFRS) was established with molecular-genetic studies in many countries. This is determined by germinal mutations in *BRCA1* and *BRCA2* genes [1-2] that under normal conditions act as genes-suppressors of tumor growth. Numerous clinical-genealogic and genetic studies demonstrated that mutations in *BRCA1* and *BRCA2* genes were associated with cancer development under the age of 50; bilateral breast cancer (BC); BC and ovarian cancer (OC) in patient or in her family members, two or more cases of cancer in one member of the family – carrier of these genes mutations, breast cancer in males; patient's ethnicity (Ashkenazi Jewish). Therefore, clinical-genealogical examinations of families and molecular-genetic studies of DNA of family members do not lose relevance and are advantageous not only for database entrants concerning association of mutations in *BRCA1* and *BRCA2* genes, but also for determination of separate steps of molecular pathogenesis of COFRS and development of preventive measures of BC or OC development in families that carry germinal mutations in indicated genes [2].

Over the last 3 years we have conducted clinical, clinical-genealogical and molecular-genetic examinations in patients with BC and OC that receive treatment in CE "Cherkassy Regional Oncologic Dispensary" of ChRC. Molecular-genetic methods involve identification of mutations *5382insC* and *185delAG* in *BRCA-1* gene, 6174delT in *BRCA2* gene in peripheral blood and surgical material by multiplex polymerase chain reaction (PCR). We consider one clinical case to deserve special attention due to determined hereditary ovarian cancer (OC) in proband's family and metachronous tumor development, namely, breast cancer, and cancer association with *5382insC* mutation in *BRCA1* gene.

Clinical case No.107. Proband was patient M.N.M., born 1965, resident of district centre of Cherkassy oblast, Ukrainian, dairymaid by profession. Obstetric history: childbirths – 2 (at the age of 19 and 24), abortions – 2, miscarriages – 0, menarche from the age of 14, menstruations lasted 8-10 days in 30 days, menstrual cycle was regular, lactation in 12 months, IUD was absent. At the age of 43 the patient had UAE (uterine artery embolization) due to symptomatic uterine fibromyoma and polymenorrhagia, after which menstruation period reduced to 4-5 days, reduction of uterine fibromyoma dimensions from 7-8 to 5-6 weeks of pregnancy was observed. At the same age (43 years) diffuse breast fibroadenomatosis was diagnosed, for which the patient received conservative drug therapy. The patient also received treatment for seasonal aggravations of chronic inflammation of uterine appendages.

At the age of 48 due to secondary amenorrhea during 3 months the patient visited district obstetrician-gynecologist, therefore ultrasound examination of small pelvis organs was conducted and bilateral ovarian cysts were detected. After peripheral blood examination for tumor marker CA-125 its increased level up to 115 U/mL was determined, and the patient was referred for surgical treatment to oncogynecologist to Cherkassy ROD. After complex clinical examination and combined treatment, the patient was diagnosed with OC stage 3C (pT3CN0M0G3). The patient received optimal cytoreductive surgery: panhysterectomy, omentectomy, microirrigator was placed into Douglas pouch for polychemotherapy (PCT). The patient received 6 courses of PCT in adjuvant regimen by the scheme "CC" – cisplatine + cyclophophane (1-st course in combination of i/v and intraperitoneal administration) without dose reduction. Then the patient was taken to the dispensary registration.

At the age of 50 (in 2 years after finishing of combined treatment for OC) during regular dispensary examination tumor of upper outer quadrant of the left breast was detected at mammography screening – BC, stage 1 (pT1N0M0G1) was diagnosed. The patient received complex treatment – organ-saving surgery (left-sided sector resection and axillary lymph node dissection), post-surgical course of radiotherapy in total dose of 40 Gy and 6 courses of PCT according to the scheme "CC" without dose reduction. The patient is under dispensary observation, currently the patient has remission.

Histopathological conclusion decision: 1) Ovarian tumor – serous-papillary ovarian cancer, low differentiation grade (G3), metastases into contra-lateral ovary, greater omentum. Cancer papillary structures are covered with atypical epithelium of tubular-ovarian type with pronounced polymorphism of tumor cells nuclei; 2) Left breast tumor – infiltrative ductal cancer with tubular and trabecular structures, moderate number of mitoses, differentiation grade G1, molecular subtype – luminal A (ER+, PR+, HER2-) (analysis of expression of hormonal receptors and HER2 by the scale Histochemical score by McCarthy).

Clinical-genealogical analysis of the family tree (Fig.1) determined the following: OC in proband's mother (57 years), fibroadenoma of the left breast at the age of 42 in proband's sister (surgery in the extent of breast sector resection). Basing on the obtained results the multiple primary, character of tumors and the role of BRCA genes in the development of BC and OC was suggested.

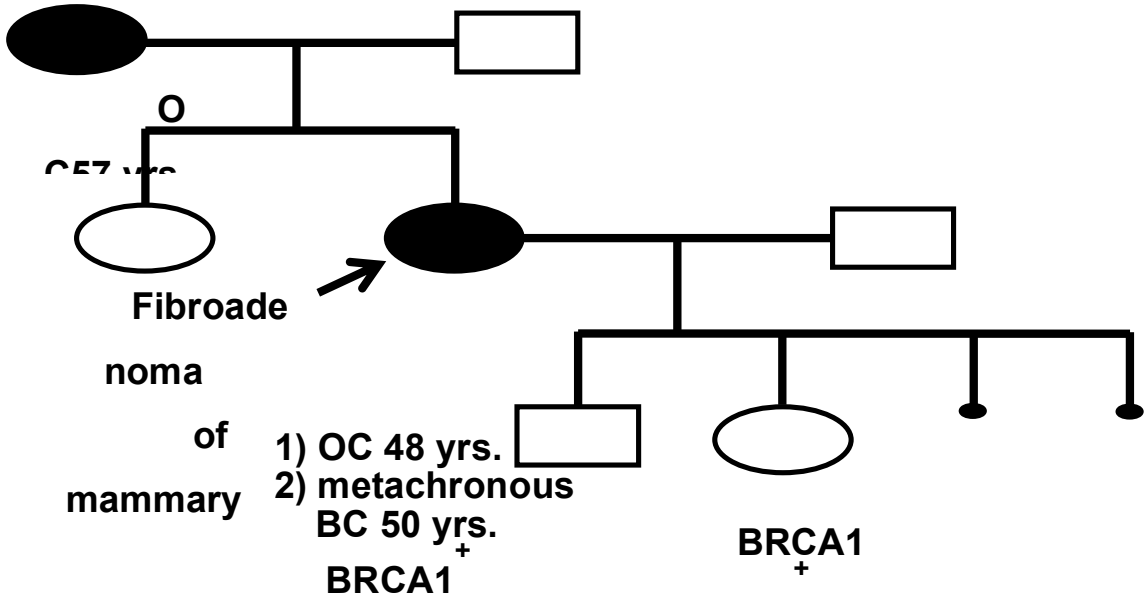


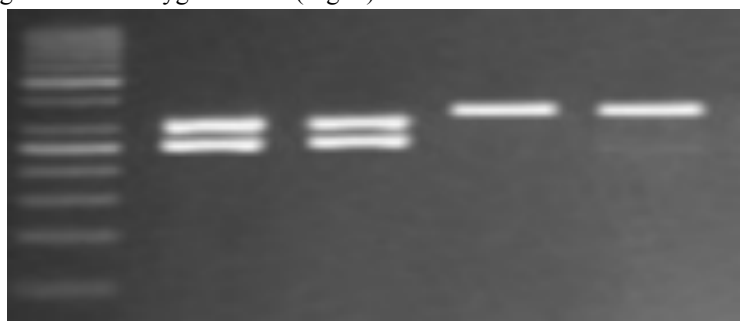
Fig.1. Family tree of proband (indicated by the arrow) with aggregation of cancer of female reproductive system organs in two generations: hereditary OC (proband's mother), multiple primary tumor in the proband: BC and OC (case No.107). Proband's sister has breast fibroadenoma, also proband's daughter is a carrier of mutation in BRCA1 gene. Abbreviations: BC – breast cancer, OC – ovarian cancer.

For confirmation of OC hereditary character we conducted molecular-genetic examination of peripheral blood of the patient (in 2013 after detection of the first tumor – OC). Molecular genetic studies conducted in the SE "Reference center for molecular diagnostics of the MPH of Ukraine".

For determination of mutations *185delAG* and *5382insC* in *BRCA1* gene, *617delT* in *BRCA2* gene we used modified protocols with oligonucleotide primers, applying the method of allele-specific polymerase chain reaction (PCR). The studied genes areas were amplified with specific primers ("Metabion", Germany). Amplification fragments' status was analyzed in 2.5% agarose gel (agarose of the company "ThermoScientific", USA), with the addition of ethidium bromide, molecular weight marker GeneRuler 50 bpDNALadder ("ThermoScientific", USA) and further visualization with computer program Vitran.

Molecular-genetic examination of proband's peripheral blood (patient with OC and BC) demonstrated the presence of mutation *5382 insC* in *BRCA1* gene in heterozygous state and absence of mutations *6174delT* in *BRCA2* gene and *185 delAC* in *BRCA1* gene (Fig. 2).

Taking into account the obtained results complex examination of proband's daughter (M.T.M., born 1984, resident of district centre of Cherkassy oblast) was decided to be conducted. From obstetric history – menarche from the age of 14, menstruations by 4-5 days in 30 days, regular, childbirth – 1 (at the age of 26), miscarriages – 0, abortion – 0, lactation - 12 months. At the age of 29 she was diagnosed with postpartum cervical erosion, for this case laser cervical destruction was performed. Since 2013 she has undergoes dispensary observation once a year with ultrasound control of small pelvis organs, breasts, and thyroid gland, with determination of the levels of CA-125, HE4, Roma index in dynamics, currently she receives preconception preparation before 2-nd pregnancy planning. Molecular-genetic analysis of peripheral blood of proband's daughter revealed the mutation analogous to mother's mutation - *5382 insC* deletion in *BRCA1* gene in heterozygous state (Fig. 2).



M **1** **2** 3 4

Fig. 2. Electrophoregram of *BRCA1 5382 insC***.** The results of amplification products distribution, according to which in peripheral blood of proband and her daughter the deletion in heterozygous state was detected (slots 1,2), slots 3 and 4 – homozygous norm by *BRCA1 185 del AG*. Note: M – molecular weight marker.

Thus, hereditary OC was determined in mother and in her daughter (proband) and multiple primary tumors in proband associated with mutation *5382 insC* in *BRCA1* gene. Proband's daughter was also determined to be a carrier of analogous mutation that was proved by the results of molecular-genetic study of peripheral blood DNA. Received data indicated the role of mutation *5382 insC* in *BRCA1* gene in development of hereditary and multiple primary tumors in this family. BC of stage I was diagnosed due to well-timed mammography screening. Considering the fact that mutations in genes *BRCA1* and *BRCA2* are associated also with development of pancreatic cancer and stomach cancer [3], the patients with oncogynecologic pathology need more extended dispensary observation and preventive measures with endoscopic examination of gastro-intestinal tract according to oncoprogram in cancer prevention not only in female reproductive system organs.

References

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