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QUARTERLY

Germline mutations in the *BRCA1* gene predisposing to breast and ovarian cancers in Upper Silesia population★✪

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Germline mutations in the *BRCA1* or *BRCA2* genes predispose their carriers to breast or/and ovary cancers during their lifetime. The most frequent mutations: 5382insC, 185delAG, C61G and 4153delA in *BRCA1,* and 6174delT and 9631delC in *BRCA2* were studied in a group of 148 probands admitted for genetic counseling, using allele-specific amplification (ASA) PCR test.

Fifteen carriers of three different mutations: 5382insC, 185delAG and C61G in *BRCA1* were found. Two families carried the 185delAG mutation and additional two C61G in *BRCA1*. Nobody carried the mutation 4153delA in *BRCA1* nor 6174delT or 9631delC in *BRCA2*. Most of the carriers of a germline mutation were observed among the patients who developed bilateral breast cancer (17%). The lowest frequency of the germline mutations was found in the healthy persons who had two or more relatives affected with breast or ovarian cancer.

Breast cancer is the most prevalent malignancy affecting women in the Western world. The disease is usually sporadic, but in some cases it occurs in the presence of germline mutations in the predisposing genes. Germline alterations of the *BRCA1*(MIM 113705) or

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Abbreviations: ASA, allele-specific amplification; HA, heteroduplex analysis; HB(O)C syndrome, human breast (ovary) cancer syndrome; SSCP, single-strand conformation polymorphism.

BRCA2(MIM 600185) genes result in susceptibility to breast and ovarian cancer. Positive linkage of hereditary breast cancer to chromosome 17q21 was observed in 1990 and to chromosome 13q12-q13 in 1994 (Miki *et al.,* 1994). Later, these chromosome regions were shown to carry breast cancer susceptibility genes, termed *BRCA1* and *BRCA2* (Tavtigian *et al.,* 1996; Wooster *et al.,* 1995; Wooster *et al.,* 1994). A large number of germline mutations in both genes has been reported. Most of these mutations are small insertions or deletions that result in frameshift and truncated protein (Ford *et al.,* 1998).

The lifetime risk of breast and ovarian cancer is about 85% and 60%, respectively, in women carrying a mutation in the *BRCA1* gene. For the carriers of germline mutations in the *BRCA2* gene it is 85% and 30%. Male carriers of constitutive mutations in either of these genes are also at a higher risk of developing malignancy than the average population but it is much lower than for women, being 6–8% (Couch *et al.,* 1997).

The *BRCA1* gene contains 24 exons encoding a large protein of 1863 amino acids. The *BRCA2* gene consists of 27 exons encoding a protein of 3418 amino acids. Hundreds of mutations, most of which are unique, have been identified throughout the entire coding sequences of both the *BRCA1* and *BRCA2* genes in different European and American populations (Narod *et al.,* 1995; Martin & Weber, 2000; Easton *et al.,* 1993; Zheng *et al.,* 2000). We found out that in Polish population one particular mutation in the *BRCA1* gene — 5382insC — accounts for about 80% of mutations found in the *BRCA1* and *BRCA2* genes (Górski *et al.,* 2000; Grzybowska *et al.,* 2000).

This enabled us to develop an allele-specific amplification (ASA) PCR test for the screening of families with HB(O)C syndrome. In our earlier papers we identified by direct sequencing six most frequent mutations in the Polish population: 5382insC, 185delAG and C61G, and 4153delA in the *BRCA1* gene, and 6174delT (not published) and 9631delC in *BRCA2* gene.

The aim of this study was to identify these mutations in HB(O)C families without a strong history of cancer cases.

MATERIALS AND METHODS

Tests were done for persons admitted for genetic counseling who have met the following criteria: (1) at least two cases of breast or ovary cancer in the family, or (2) persons who developed breast or ovary cancer before 35 years of age, or (3) persons who developed bilateral breast cancer or breast and ovarian cancer without a family history.

We screened for the six mutations 148 persons of which 65 were healthy with at least two cases of breast or ovarian cancer in the family, 41 with bilateral breast cancer, 27 with unilateral breast cancer, nine with ovary cancer, six with other types of cancer.

Ten ml of blood was drawn from each person. DNA was isolated from leukocytes according to the phenol:chloroform extraction (Grzybowska *et al.,* 1993). Carriers of germline mutations were asked to give a second blood sample to confirm the results of the test.

ASA PCR tests. The primers used for PCR have been described in the Breast Cancer Information Core (BIC) database and in the paper by Chan (Chan *et al.,* 1999). Applied Biosystems reagents were used for the amplification of *BRCA1* and *BRCA2* gene fragments. The C61G substitution mutation was studied using the RFLP method. Exon 5 was amplified and further digested with *Ava* II restriction enzyme. The C61G mutation created a restriction site for this enzyme. For heterozygotes having one mutated allele, in electrophoresis three bands were observed instead of one for wild-type homozygotes.

ASA PCR was used to analyze frameshift mutations. Three primers were used for the amplification. Two served to amplify the wild-type allele and the third one was designed specifically for the mutated allele. In electro-

phoresis there were two bands for heterozygotes and one band for the wild-type homozygote.

RESULTS

We analyzed six germline mutations 5382insC, 185delAG, C61G and 4153delA in the *BRCA1* gene, and 6174delT and 9631delC in the *BRCA2* gene in 148 probands. Altogether we found 15 families carrying germline mutation in the *BRCA1* gene. We found three different mutations: 5382insC, 185delAG and C61G in the *BRCA1* gene. Nobody carried the mutation 4153delA in the *BRCA1* gene nor 6174 del T or 9631del C in the *BRCA2* gene. The mutation 5382 insC in the *BRCA1* gene was the most frequent germline mutation (11 out of 15–73%). Two families carried 185delAG and additional two C61G in the *BRCA1* gene. We analyzed germline mutations in different groups of patients who were admitted for genetic counseling. We had healthy persons and persons who developed malignancy. Most of the carriers of a germline mutation were found among the persons who developed bilateral breast cancer (17%). The carriers of a mutation affected with bilateral breast cancer developed the malignancy in the second breast 0 to 22 years after the onset of the cancer in the first breast (Table 1).

Table 1. Germline mutations detected in *BRCA1* gene and description of associated cancers.

Family No.	Mutation in *BRCA1* gene	Diagnosis of proband	History of cancers in the family
90	185delAG	Breast cancer	None
98	185delAG	Bilateral breast cancer 51	None
107	5382insC	Bilateral breast cancer 66,67	None
109	5382insC	Bilateral breast cancer 40	None
118	C61G	Bilateral breast cancer 43,47	Pancreas cancer
128	5382insC	Bilateral breast cancer 40,45	Breast cancer, hepatocarcinoma, stomach cancer, lung cancer, 2 × colon cancer
131	5382insC	Bilateral breast cancer 38,47	Nasopharyngeal cancer
146	5382insC	Bilateral breast cancer 49,71	None
148	5382insC	healthy	Colon cancer, 2 × breast cancer
154	C61G	Bilateral breast cancer 54,61	Stomach cancer
172	5382insC	Breast cancer48	Breast cancer, osteosarcoma
204	5382insC	healthy	Breast cancer, lung cancer, stomach cancer
213	5382insC	Breast cancer 40	4 × breast cancer, skin cancer, ovary cancer, 2× stomach cancer, lung cancer,
221	5382insC	healthy	Ovary cancer, endometrium cancer, 4 × breast cancer
238	5382insC	healthy	Peritoneum cancer, hepatocarcinoma

The lowest frequency of the germline mutations was found in the healthy persons who had two or more relatives affected with breast or ovarian cancer. We did not find any mutation in the group of patients who were diagnosed with ovary cancer, most likely because this group comprised only 9 persons.

DISCUSSION

In our earlier papers (Górski *et al.,* 2000; Grzybowska *et al.*, 2000) we analyzed mutations in the *BRCA1*, *BRCA2* , and *TP53* genes in 66 and 47 families, respectively. We applied strict Amsterdam criteria to diagnose hereditary predisposition to breast and ovary cancer using pedigree analysis. All the probands were diagnosed with breast and/or ovary cancer.

We found a few recurrent mutations which accounted for more than 80% of all germ-line mutations described in the Polish population. The most important finding in both papers was a very high frequency of single *BRCA1* 5382insC mutation. Mutations 185delAG and C61G were less frequent. A recurrent mutation was earlier described also in the *BRCA2* gene–9631delC (Grzybowska *et al.,* 2000). It is likely that this particular mutation is limited to the Southern part of Poland because all families carrying this mutation lived in Silesia and came from Silesia or a neighboring district. In this group of patients we did not find any family with this mutation. This paper confirms the distribution of recurrent mutations in the Polish population and it is not different from the rest of Poland. Earlier, a high frequency of recurrent mutations was ascribed mainly to Ashkenazi Jews population (Roa *et al.,* 1996; Struewing *et al.,* 1997; Thorlacius *et al.,* 1996; Abeliovich *et al.,* 1997).

Single-strand conformation polymorphism (SSCP) and heteroduplex analysis (SSCP-HA) and direct sequencing were used to find these mutations. These methods are expensive and time consuming but their mutation detection rate is very high (80% for SSCP-HA and almost 100% for automatic sequencing).

In this paper using data obtained from SSCP-HA and direct sequencing we analyzed only the already known mutations but in a much larger group of probands — 148. The simplicity of detection mutations using ASA PCR allowed us to study a larger group than before and it was possible to use less stringent criteria to qualify persons for the genetic tests. Using only ASA PCR it was possible to find carriers of known mutations but according to the earlier papers unique mutations are rare in the Polish population (Sobczak *et al.,* 1997). This approach gives the opportunity to study the real proportion of germline mutations connected with breast and ovary cancers also in the families where the mutation is passed on by men.

Most of the carriers of a germline mutation who developed bilateral breast cancer did not have a history of cancer cases in the family and 17% of them carried a germline mutation which must have been inherited from one of their parents. This finding corroborates the study (Hemminki *et al.,* 2000) done on a family cancer database where an excess of breast and ovary cancers was noted in the daughters of women affected with bilateral breast cancer, even though bilateral breast cancer alone without a family history did not meet Amsterdam criteria.

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