The presence of hereditary *BRCA1* gene mutations in women with familial breast or ovarian cancer and the frequency of occurrence of these tumours in their relatives

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Summary

In 48 women with familial breast cancer as well as in 22 women with familial ovarian cancer, the presence of pathogenic mutations in *BRCA1* gene were found in 35.4% and 54.6% of patients, respectively. From the patients with mutations we created two groups: the CaM – probands with breast cancer and CaOv – probands with ovarian cancer. The probands with breast cancer were younger by a mean of five years than the probands with ovarian cancer (p = 0.048).

Methods: The PCR-SSCP procedure was used to find mutations in the *BRCA1* gene. Fragments suspected of mutation were subjected to nucleotide sequencing.

Results: In the CaM group, which consisted of 17 women with breast cancer, the following mutations in the *BRCA1* gene were detected: 5382 insC, T300G, 3819del5 and IVS20+60ins12. The probands of the CaM group and their relatives developed a total of 49 breast and ovarian cancers. Among all these tumours the breast cancers of the probands made up 34.7%, the breast cancers of proband relatives made up 57.1% and the ovarian cancers of probands and their relatives made up only 8.2%.

The CaOv group consisted of 12 probands with ovarian cancers in whom we detected only two kinds of mutations: 5382insC and 185delAG. The probands of the CaOv group and their relatives developed a total of 38 ovarian and breast cancers. Among all these tumours the ovarian cancers of the probands made up 31.6%, the ovarian cancers of their relatives made up 34.2% and the breast cancers of the relatives 34.2% of tumours.

In the probands with breast or ovarian cancer the predominant mutation was 5382insC - in the *BRCA1* gene detected in 76.5%, and 91.7%, respectively. Despite the predominant presence of the same mutation in probands from both groups the ratio of the number of breast cancers to the number of ovarian cancers in their relatives differed significantly (p = 0.0003).

Conclusion: This data shows that the presence of the 5382insC mutation in the *BRCA1* gene is not always associated with the development of ovarian cancer. It is very likely that the development of ovarian cancer requires some additional factor, which was common among the familial ovarian cancer patients, and was almost inexistent among the familial breast cancer patients. On the other hand, the development of ovarian cancer at a later age than breast cancer in probands suggests that some factors exist which slow down the development of ovarian cancer or which accelerate the development of breast cancer.

Key words: BRCA1 gene mutation; Familial breast cancer; Familial ovarian cancer.

Introduction

Familial breast cancer and familial ovarian cancer appear in a number of different syndromes. The most common of these is a syndrome characterized by multiple appearances of both breast and ovarian cancer among family members [1, 2]. There are also less common syndromes, in the course of which breast cancer or ovarian cancer appear as solitary familial malignancies [3, 4]. Some authors maintain that the syndrome, in which only familial ovarian cancer is observed, is a specific form of the breast cancer-ovarian cancer syndrome, in which the breast cancer develops later and therefore may not be observed [5]. Certain predispositions towards developing ovarian cancer have been observed in families with the Lynch II syndrome and in families with hereditary non-polyposis colorectal cancer [2, 5].

The results of numerous studies devoted to the genetic background of familial breast cancer point to the fact that in over 90% of cases these malignancies appear in the course of *BRCA1* or *BRCA2* gene mutations [4, 6]. In patients with breast cancer *BRCA1* gene mutations are three times as common as *BRCA2* gene mutations [7, 8]. It is also being postulated that the development of breast and ovarian cancer is conditionally dependent on the localization of the *BRCA1* gene mutation. Some authors claim that mutations localized closer to the 5' region are more likely to be accompanied by ovarian cancer than mutations nearer to the 3' region [5, 8, 9], while others do not share this opinion [10].

In the course of studies aimed at screening for the presence of *BRCA1* and *BRCA2* gene mutations among women with familial breast or ovarian cancer we have found that the mutations known to be pathogenic were, in a majority, located in the *BRCA1* gene. *BRCA2* gene mutations in our population were very rare, and were

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found in only a few non-pathogenic polymorphisms and one mutation, the biological effect of which is not yet clear [11, 12]. Among the different pathogenic mutations found in the *BRCA1* gene of probands with familial breast cancer and/or familial ovarian cancer, the most common (82.8%) was the 5382insC mutation. This should suggest that among family members of these probands the frequency of breast cancer and ovarian cancer should be similar. However, this is not the case - there is a significant difference in the frequency of these two malignancies. The subject of this paper is a detailed discussion of this finding.

Material and Methods

In 48 women with familial breast cancer and 22 women with familial ovarian cancer the presence of pathogenic mutations in the *BRCA1* gene were investigated. The further subjects of this study were 17 patient-probands (group - CaM) with breast cancer and 12 patient-probands (group CaOv) with ovarian cancer in which pathogenic mutations in the *BRCA1* gene were detected.

Family history gathered from the probands provided information concerning the number of cases of breast and ovarian cancer which had developed among their blood relatives. The comparison of frequency of occurrence of breast and ovarian cancers in groups CaM and CaOv was based on the ratio of number of breast cancers to number of ovarian cancers in the respective groups.

BRCA1 mutation screening.

Genomic DNA was isolated from the peripheral blood of patients by the phenol-chloroform method using proteinase K according to Sambrook *et al.* [13]. The PCR-SSCP procedure, as described in detail elsewhere [14], was used to look for mutations in the *BRCA1* gene. The mutations found so far in the Polish population are exon 2-185delAG, exon 5-T300G and T309C, exon 22-G5465A and fragments of exon 11-110-3819del GTAAA and 11P-4153delA. Exons and fragments suspected of mutations were subjected to nucleotide sequencing. Moreover, the 5382insC mutation in exon 20, the most frequent mutation in the Polish population, was sought by direct sequencing without prior SSCP analysis. The primers used for the polymerase chain reaction (PCR) were those proposed by Friedman *et al.* [15].

The details of the PCR, SSCP analysis and nucleotide sequencing were performed according to the protocol described elsewhere by Paszko *et al.* [14].

Results

We examined 48 women with familial breast cancer and 22 women with familial ovarian cancer. In 17 of the breast cancer patients (35.4%) and in 12 of the ovarian cancer patients (54.6%) we found the presence of hereditary pathogenic mutations of the *BRCA1* gene. In the remaining 31 women with breast cancer (64.5%) and ten women with ovarian cancer (45.5%) no mutations of the *BRCA1* gene were found. Among the patients with *BRCA1* gene mutations we discerned 17 women with breast cancer - referred to as group CaM. Among these 17 women 14 had unilateral breast cancer and three had bilateral breast cancer (Table 1, positions 13, 14 and 17). Two of the patients with unilateral breast cancer also developed ovarian cancer (Table 1, positions 15 and 16), while one also developed cancer of the endometrium (Table 1, position 4). The mean age of the women from group CaM at the time of cancer diagnosis was 44.5 years (26-59 years).

Among the women in group CaM four different kinds of mutations of the *BRCA1* gene were found, of which three were exon mutations and one an intron mutation. The most common mutation was 5382insC in exon 20 (found in 13/17 women -76.5%). The second most common was T300G in exon 5 (2/17 - 11.7%). Both the 3819delGTAAA mutation in exon 11 and the intron IVS20+60ins12 mutation were found in one case each (5.9%, respectively).

In the CaM group the total number of cases of breast cancer was 45 (17 among the probands themselves and 28 among their relatives). There were also four cases of ovarian cancer - two among the probands and two among their relatives. The ratio of breast cancer cases to ovarian cancer cases among the probands' relatives was 14 (28/2), while in a calculation which also included the probands themselves the ratio was down to 11.3 (45/4). This data is presented in Tables 1 and 2.

Among the 22 women with familial ovarian cancer 12 were found to have *BRCA1* gene mutations. There were two different kinds of mutations - 5382insC in exon 20 found in 11 out of the 12 patients (91.7%) and 185delAG in exon 2 found only in one proband (8.3%).

The 12 women with *BRCA1* gene mutations formed the second study group - the CaOv. The mean age of the CaOv group of patients at the time of cancer diagnosis was 49.8 years (37-59 years). Careful collection of family history revealed 25 cases of ovarian cancer (12 among the probands and 13 among their relatives) and 13 cases of breast cancer, also among the probands' relatives. The ratio of breast cancer cases to ovarian cancer cases among the proband family members was one (13/13), while the same ratio after encompassing the probands into the calculation was down to 0.52 (13/25). This data is also shown in Tables 1 and 2.

The ratios of the number of breast cancer cases to the number of ovarian cancer cases differed significantly between the two groups, both when compared only among the probands' relatives, and among the probands and their relatives together ($\alpha = 0.0003$ or $\alpha = 0.0000$ Table 2).

Discussion

In all the investigated women with familial breast cancer *BRCA1* gene changes were found in 17/48 subjects (35.4%), while in the group of women with familial ovarian cancer they were found in 12/22 cases (54.6%). Therefore it is evident that *BRCA1* gene alterations in the entire examined population of women with familial

Table 1. — *Characteristics of probands patients with familial breast cancer or familial ovarian cancer.*

Group - CaM - probands with breast cancer						Group - CaOv - probands with ovarian cancer					
No. of patients	Kind of mutations in *BRCA1* gene	Age of patients	Number of probands with breast cancer	Number of tumours in proband's relatives breast	ovary	No. of patients	Kind of mutations in *BRCA1* gene	Age of patients	Number of probands with ovarian cancer	Number of tumours in proband's relatives ovary	breast
1	T300G	37	1	3							
2	T300G	48	1	4							
3	3819del5	51	1	3							
4§	IVS20+60ins12	59/63	1	1							
5	5382insC	26	1	1							
6	5382insC	50	1	1		18	5382insC	49	1	2	5
7	5382insC	47	1	1		19	5382insC	45	1	1	1
8	5382insC	50	1	1		20	5382insC	50	1		1
9	5382insC	39	1	1		21	5382insC	52	1		1
10	5382insC	38	1	1		22	5382insC	55	1	1	1
11	5382insC	39	1	3		23	5382insC	53	1	3	
12	5382insC	48	1	2		24	5382insC	59	1	1	1
13*	5382insC	48	1			25	5382insC	53	1		
14*	5382insC	51	1	3	2	26	5382insC	46	1	2	2
15#	5382insC	45	1	3	1	27	5382insC	49	1	1	1
16#	5382insC	46/53	1		1	28	5382insC	37	1		
17*	5382insC	35	1			29	185delAG	50	1	2	
Sum of tumours in group			17	28	4	Sum of tumours in group			12	13	13

*: bilateral breast cancer in proband, #: additional ovarian cancer in proband at age 53, §: additional endometrial cancer at age 63.

Table 2. — *Statistics of selected characteristics of probands patients and their relatives.*

Parameters of patients	Group CaM probands with breast cancer	Group CaOv probands with ovarian cancer	Statistic
Age			
Mean	44.5	49.8	
Median	47	50	Wilcoxon test = 1.97
Range	26 - 59	37 - 59	Significance 0.04817
SE	1.9	1.6	
n	17	12	
Mutations in BRCA1			
5382insC	13/17 - 76.5%	11/12 - 91.7%	
IVS20+60ins12	1/17 - 5.9%		
Mutation in 20th exon and intron	14/17 - 82.4%		
T300G	2/17 - 11.7%		
3819del5	1/17 - 5.9%		
185delAG		1/12-8.3%	
Number and	% of tumours in	groups*	
Breast cancer in probands	17 - 34.7%		
Breast cancer in relatives	28 - 57.1%	13 - 34.2%	
Ovarian cancer in probands	2 - 4.1%	12 - 31.6%	
Ovarian cancer in relatives	2 - 4.1%	13 - 34.2%	
Ratio of the number of breast cancers/to the number of ovarian cancers in relatives	28/2 = 14	13/13 = 1	Test χ^2 = 13.34 α = 0.0003
Ratio of the number of breast cancers/to the number of ovarian cancers in the whole group	45/4 = 11.3	13/25 = 0.52	Test χ^2 = 31.98 α = 0.0000

*: % of total number of breast cancers and ovarian cancers in the whole group.

ovarian cancer are 19.2% higher than among the population of women with familial breast cancer.

There is numerous data in the literature that the development of breast and ovarian cancers within families is associated with *BRCA1* and *BRCA2* gene mutations in 43-55% of proband-patients [6, 14, 15]. Our results provide similar data concerning the *BRCA1* gene mutations only. In our population *BRCA2* gene mutations are very rare [16, 17].

Further considerations concern the relation between the presence of *BRCA1* gene mutations among probands and the frequency of development of breast cancer and ovarian cancer probands and their relatives. To perform such an analysis we divided the patients with *BRCA1*

gene mutations into two groups. The criteria of dividing our patients was based on the diagnosis of either breast cancer (CaM group) or ovarian cancer (CaOv group). The probands with breast cancer - CaM group - were on average five years younger than the probands with ovarian cancer - CaOV group ($p = 0.0482$) (Table 2). There have been literature reports of similar observations, i.e. that the risk of developing breast cancer in younger women (below 50 years of age) is higher than the risk of developing ovarian cancer [18].

Although all the probands of the CaM group ($n = 17$) had breast cancer, only 82.3% of the group could be considered as homogeneous. Two of the women additionally developed ovarian cancer, and one-cancer of the endometrium. In three other women bilateral breast cancer developed (Table 1). Breast cancer was more frequent than ovarian cancer - both among the probands and their relatives. The ratio of the number of breast cancers to the number of ovarian cancers was 14 among the probands' relatives, and 11.3 in the entire analyzed group (i.e. both probands and their relatives) (Table 2).

The second group which we created – the CaOv – was diagnostically much more homogeneous. All the probands ($n = 12$) developed only ovarian cancer. Among the probands' relatives the frequency of ovarian cancer was the same as the frequency of breast cancer, with a ratio of one (13/13). When the entire CaOv group was analyzed this ratio was lower (0.52) (Table 2).

Among the 17 probands from the CaM group 13 (76.5%) were found to have, presumably, an identical ethiopathogenetic background, i.e. the 5382insC mutation of the *BRCA1* gene. Assuming that the intron IVS20+60ins12 mutation found in the near vicinity of exon 20 had consequences similar to the 5382insC mutation, the percentage of probands with a similar ethiopathogenetic background of breast cancer increases to 82.3%. The remaining 17.7% of probands form a subgroup presenting much more rare *BRCA1* gene mutations – T300G in two cases and 3819del 5 in one case.

The second group of patients, the CaOv, was more homogeneous not only in the diagnostic aspect but also as far as the *BRCA1* gene mutations were analyzed. Among the 12 probands who had developed ovarian cancer, 11 (91.7%) presented with the 5382insC mutation and only one with a different change - 185delAG (8.3%).

All the *BRCA1* mutations, which we have found among our breast cancer and ovarian cancer probands, are quite common in the Polish population as reported by Sobczak *et al.* [19]; Paszko *et al.* [20, 21]; Górski *et al.* [22, 23]; Grzybowska *et al.* [24]; Jakubowska *et al.* [25]. Our study has only confirmed the previous data, that the 5382insC mutation of the *BRCA1* gene is the most common mutation present in Polish women with breast cancer and ovarian cancer [21, 23].

Another issue, which ought to be discussed, is the localization of *BRCA1* gene mutations. It is postulated that some localizations of mutations within the *BRCA1* gene account for an increased risk of breast cancer or ovarian cancer [5]. Some authors maintain that mutations located nearer the 5' region are more common in cases of ovarian cancer than mutations nearer to the 3' region [8, 9]. On the contrary, breast cancer is considered to be more frequently accompanied by mutations of the 3' region [8]. Therefore it is all the more interesting that in both our groups of probands (in 11/12 patients with familial ovarian cancer and in 14/17 patients with familial breast cancer) the *BRCA1* gene mutation was found near the 3' region (5382insC in exon 20). Only in two probands with familial breast cancer and in one proband with familial ovarian cancer - did we find a *BRCA1* gene mutation near the 5' region (T300G-exon 5 and 185delAG-exon 2, respectively). Similar data showing no correlation between the location of *BRCA1* gene mutations and an increased risk of developing ovarian cancer, has been reported by Shattuck-Eidens *et al.* [10].

The presented data shows that among all the women investigated in the CaM group (both the probands and their relatives) the ratio of the number of breast cancers to the number of ovarian cancers was 11.3 (Table 2). On the other hand, among all the women investigated in the CaOv group, the ratio of the number of breast cancers to the number of ovarian cancers equals 0.52. It is evident that in the CaM group the frequency of ovarian cancers was much lower than in the CaOv group.

These relationships between breast and ovarian cancer are accompanied by a common feature of both groups of patients – the predominant presence of the same *BRCA1* gene mutation, i.e. 5382insC, which was found in 76.5% of CaM patients and in 91.7% of CaOv patients. Thus we arrive at one of the more crucial issues – why, despite the predominance of the same *BRCA1* gene mutation – 5382insC among the investigated probands, do we observe such significant differences in the frequency of breast cancer and ovarian cancer development among their relatives? If we assume that the 5382insC mutation of the *BRCA1* gene is the cause behind the development of both breast and ovarian cancer in both groups of patients and their relatives then why is the ratio of the number of breast cancers to the number of ovarian cancers in both group different?

In view of these above considerations it may be assumed that in relatives of probands from the CaM group some factor exists that slows down the development of ovarian cancer. Such an explanation can be supported by the fact that the appearance of ovarian cancer is delayed in our patients on average by some five years as compared to breast cancer.

Conclusions

1. The occurrence of changes in *BRCA1* gene in women with familial ovarian cancer is more frequent than in women with familial breast cancer.

2. The ratio of the number of breast cancers to the number of ovarian cancers in familial breast cancer patients and their relatives is significantly higher than the ratio in familial ovarian cancer patients and their relatives.

3. In probands with familial breast cancer and in probands with familial ovarian cancer, the predominant pathogenic mutation of the *BRCA1* gene was the 5382insC (76.5% and 91.7%, respectively). The presence of the 5382insC mutation in the *BRCA1* gene does not always accompany the development of familial ovarian cancer. It is very likely that the development of ovarian cancer requires some additional factor, which is common among the familial ovarian cancer group of patients, and almost inexistent among the familial breast cancer group of patients.

4. Despite the predominance of the same mutation - 5382insC - in breast cancer and ovarian cancer patients the neoplastic expression in probands with breast cancer appears on average five years earlier than in probands with ovarian cancer ($p = 0.04817$). It may therefore be assumed that some factors exist which slow down the development of ovarian cancer or which accelerate the development of breast cancer.

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