Age and Geographical Distribution in Families with *BRCA1/BRCA2* Mutations in the Slovak Republic

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Abstract

Molecular diagnostics of hereditary breast and/or ovarian cancer is mainly based on detection of *BRCA1* and *BRCA2* germline mutations in suspected families. The aim of the study was to determine the frequency, age and geographical distribution in 130 Slovak hereditary breast and ovarian cancer (HBOC) families diagnosed within the years 2000-2004. Mutation screening was performed by single-strand conformation polymorphism (SSCP), heteroduplex analysis (HDA) and sequencing of PCR products showing an abnormal migration pattern. Twenty of 130 (15.6%) HBOC suspected families were found to carry mutations in *BRCA1* or *BRCA2* genes. The glossary data from the National Cancer Registry of Slovakia (NCRS) were compared with the results from HBOC suspected kindreds. Age distribution of breast cancer onset in our study group showed the highest proportion of onset in HBC families within the 5th decade of life, while NCRS reports at least a ten year later onset. These findings confirmed that cases of breast cancer under 50 years of age can be used as one of the principal criteria to assign a family as a hereditary breast and/or ovarian cancer kindred. In contrast with unselected ovarian cancer cases, about 75% of all HOC index cases were diagnosed between 40 and 49 years of age. To study the geographical distribution of hereditary breast and/or ovarian cancer, Slovakia was divided into three parts. The distribution of HBOC suspected families approximately follows this division, with an increasing number in the western area of the country.

Introduction

Breast cancer is the most common malignancy affecting women in the Slovak Republic, accounting for 1,858 and 1,827 newly diagnosed breast cancer cases in years 2000 and 2001, respectively (World Standardized Rates 47.0/100,000 and 46.1/100,000). The complete data from NCRS show that it is also the leading cause of cancer-related mortality among Slovak women, the proportion being 15.1% of all cancer cases.

NCRS was created as one of the first in Central and Eastern Europe and contains 30-year-old data about newly diagnosed cancer patients of all types. According to NCRS evidence, 414 and 449 newly diagnosed cases of ovarian cancer were reported in years 2000 and 2001 (World Standardized Rates: 10.9/100,000 and 11.4/100,000). They represent 4.3% of all cancer deaths, and ovarian cancer is therefore the most frequent cause of death of all gynaecological malignancies.

Sona Ciernikova, Miroslav Tomka, Michal Kovac, Viola Stevurkova, Vladimir Zajac

A family history of breast and/or ovarian cancer is the most significant risk factor for developing the disease. An estimated 5-10% of all breast and ovarian cancer cases are hereditary in nature [1]. The search for genes associated with hereditary susceptibility to breast or ovarian cancer has been facilitated by the study of large kindreds with multiple affected individuals, and has led to the identification of several susceptibility genes, of which BRCA1 (MIM 113705) and BRCA2 (MIM 600185) are the most penetrant. Carriers of germline mutations in these genes have a 50-85% lifetime risk of breast cancer and a 15-45% risk of developing ovarian cancer during their lifetime [2, 3]. It was estimated that the combination of BRCA1/BRCA2 mutations was responsible for approximately 80% of all HBOC families [4, 5]. More recent estimates put this risk at about 30% and other low penetrance candidate genes involved in breast cancer susceptibility, CHEK2, ATM, TP53 and PTEN, have been identified [6, 7].

Here we aim to determine the frequency, age and geographical distribution of families carrying BRCA1/BRCA2 mutations within our collection of Slovak HBOC suspected kindreds. For this purpose, we have performed mutation screening of the entire coding region of BRCA1 and the first 16 exons of BRCA2 in 130 Slovak HBOC suspected families. Knowledge about HBOC families has been compared with the overall distribution of breast and ovarian cancer cases in 2001 and 2002, reported by NCRS.

Patients and methods

The collection of Slovak HBOC suspected families was obtained from the National Cancer Institute, the Centre of Clinical Genetics of the Faculty Hospital (Bratislava) and the departments of medical genetics of hospitals from all over the Slovak Republic. Informed consent was required prior to testing. Families having affected women were divided into potentially average, moderate or high risk of developing breast and/or ovarian cancer due to their family history. Potentially average-risk HBOC suspected families were defined by the presence of one breast cancer diagnosed under the age of 40, breast and ovarian cancer in the same individual at any age and bilateral breast cancer. Moderate risk was defined as two breast or ovarian cancer cases among first- and second-degree relatives (index case included) from the same ancestral lineage or a combination of breast and ovarian cancer among first-degree relatives. Families with high risk were characterized by the presence of at least three first- and second-degree affected relatives (index case included) on the same lineage of the HBOC suspected proband.

Mutation screening

Genomic DNA was extracted from peripheral blood lymphocytes using QIAamp DNA blood kit (Qiagen, Hilden, Germany). All exons including intron-exon boundaries of BRCA1 and the first 16 exons of BRCA2 were amplified by PCR using the set of primers according to Wagner et al. [8]. Mutation screening methods included SSCP and HDA as previously described [9]. Fragments showing aberrant band profiling were sequenced on the ABI PRISM® 3100 Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Results

The study revealed that 20 of 130 (15.6%) HBOC suspected families carry mutations in BRCA1/BRCA2 genes using standard molecular techniques that included SSCP, HDA and subsequent automatic sequencing of PCR products with aberrant mobility. The efficiency of BRCA1/BRCA2 mutation detection in relation to the selection criteria is summarized in Table 1. Most analysed kindreds (80) were classified in the high-risk group, 40 families belong to the group with moderate risk and a relatively small number (10) was at average risk for genetic predisposition. The highest detection rate (17.5%) of disease-causing BRCA1/2 alleles was identified in the high-risk group of families with at least 3 breast or ovarian cancer cases. The majority (85%) of all detected mutations in our collection were attributable to BRCA1.

Table 1. The efficiency of mutation detection in relation to selection criteria

Selection Criteria	Number of Families	BRCA1 mutation (%)	BRCA2 mutation (%)	Overall mutation (%)
average-risk HBOC	10	1 (10%)	-	1 (10%)
moderate-risk HBOC	40	4 (10%)	1 (2.5%)	5 (12.5%)
high-risk HBOC	80	12 (15%)	2 (2.5%)	14 (17.5%)
total	130	17	3	20

Table 2. Age distribution in Slovak HBC and HOC suspected families

Decade	HBC families		HOC families	
	overall	with mutation	overall	with mutation
15-29	9 (8%)	1 (9%)	0 (0%)	-
30-39	30 (26.5%)	2 (18.2%)	1 (5.9%)	-
40-49	52 (46%)	4 (36.4%)	13 (76.5%)	5 (55.5%)
50-59	20 (17.7%)	4 (36.4%)	3 (17.6%)	4 (44.5%)
60-69	2 (1.8%)	-	-	-
total (100%)	113	11	17	9

Table 3. Age distribution in unselected breast and ovarian cancer cases according to NCRS*

	Breast Cancer Cases		Ovarian Cancer Cases	
Decade	NCRS*		NCRS*	
	2000 (%)	2001 (%)	2000 (%)	2001 (%)
15-29	4 (0.2%)	7 (0.4%)	17 (4.1%)	22 (4.9%)
30-39	100 (5.4%)	98 (5.4%)	33 (8%)	17 (3.8%)
40-49	360 (19.4%)	365 (20%)	84 (20.3%)	80 (17.8%)
50-59	444 (23.9%)	449 (24.6%)	84 (20.3%)	102 (22.7%)
60-69	429 (23%)	396 (21.6%)	95 (23%)	96 (21.4%)
70-79	376 (20.2%)	384 (21%)	76 (18.3%)	105 (23.4%)
80+	145 (7.8%)	128 (7%)	25 (6%)	26 (5.8%)
total (100%)	1858	1827	414	449

* NCRS – National Cancer Register of Slovakia

Table 4. Comparison of geographical distribution between Slovak HBOC suspected families and NCRS registered breast and ovarian cancer

	HBOC suspected families (%)	BRCA1/2 positive families/ carriers	Female Breast Cancer Cases (NCRS*)		Ovarian Cancer Cases (NCRS*)	
			2000 (%)	2001 (%)	2000 (%)	2001 (%)
Bratislava	46 (35,4%)	5/8	325 (17.5%)	314 (17.2%)	70 (16.9%)	75 (16.7%)
Western Slovakia	50 (38.5%)	7/29	682 (36.7%)	677 (37%)	142 (34.3%)	171 (38%)
Central and Eastern Slovakia	34 (26.1%)	8/14	851 (45.8%)	836 (45.8%)	202 (48.8%)	203 (45.2%)
Total (100%)	130	20/51	1858	1827	414	449

* NCRS – National Cancer Register of Slovakia

The age distribution of breast and ovarian cancer onset in the study group is shown in Table 2. Onset of breast cancer in the HBC group was 9 (8%), 30 (26.5%), 52 (46%), 20 (17.7%) and 2 (1.8%) for age intervals <29, 30-39, 40-49, 50-59, 60-69, respectively. The highest proportion of onset was observed within the 5th decade of life (interval 40-49), while NCRS reports a ten year later age of disease

Sona Ciernikova, Miroslav Tomka, Michal Kovac, Viola Stevurkova, Vladimir Zajac

onset for sporadic cases (see Table 3). The age distribution of index cases in 17 ovarian cancer families were 1 (5.9%), 13 (76.5%) and 3 (17.6%) for intervals 30-39, 40-49, 50-59, respectively. In contrast with the group of HOC families, where about 3/4 of all cases were diagnosed again in the 5th decade, more than half of the unselected ovarian cancer cases developed disease by the age of 60 years.

To study the geographical distribution of hereditary breast and/or ovarian cancer we have divided Slovakia into three parts: 1. Bratislava including the suburban area, 2. western Slovakia, 3. central and eastern Slovakia (Table 4). The incidence of HBOC suspected families approximately follows this geographical distribution, with a small increase in the western part of Slovakia. Families with detected BRCA1/2 mutations, however, are almost equally distributed in all three regions.

Discussion

In this report we focused on HBOC, age and geographical distribution of 130 suspected families from probands diagnosed with disease between and including the years 2000 to 2004. To date, no study addressing the presented features of Slovak HBOC suspected families with BRCA1/BRCA2 pathogenic mutations has been reported. Our Department of Cancer Genetics at the Cancer Research Institute in Bratislava was the first laboratory to provide molecular analysis for hereditary breast and/or ovarian cancer for families, and has state-wide relevance.

We used the incidence, age and geographical distribution of unselected breast and/or ovarian cancer patients from NCRS to compare with the data from the index cases of HBOC suspected families. NCRS was established in 1976 and provides a quality database of incidence and mortality of all cancer types in our country. To date, 250 members of 130 HBOC families have been screened for mutations in BRCA1/BRCA2. Of these 130 kindreds, 20 families with pathogenic alterations have been identified. Identification of 15.6% BRCA1/BRCA2 positive families correlates with mutation rates found in other European high-risk populations [10]. In total, 51 mutation carriers from 91 available family members were detected. Similar to the observations from mutation screening of women with familial or early onset breast and/or ovarian cancer in the Czech Republic [11], the majority (17.5%) of disease-predisposing BRCA1/BRCA2 alleles were identified in a group of high-risk HBOC families (Table 1).

The relative contribution of BRCA1 and BRCA2 appeared to be unequal and most of the pathogenic mutations were found in the BRCA1 gene (see Table 1). It has to be noted that our screening strategy including the first 16 exons of BRCA2 and the remaining exons of the gene needs to be analysed. Although the experimental approach used (a combination of SSCP, HDA and DNA sequencing) has a very high detection rate, some loss-of-function mutations and all large genomic rearrangements were not capable of being detected using this strategy. Such rearrangements are believed to be responsible for 10% to 15% of BRCA1 inactivating mutations [12].

Our findings confirmed that early age of breast and ovarian cancer onset (before the age of 50) is one of the best criteria for assigning the family as hereditary breast and/or ovarian cancer kindreds (Table 2). According to improvements in the overall education and prompt application of results from BRCA1/BRCA2 mutation screening, one can predict that the identification of women at risk of disease will shift to an earlier age and also to a lower stage of disease by virtue of active prophylactic screening measures.

When we look at the distribution of HBOC suspected families within the three regions of Slovakia, differences in the frequencies of families in particular areas can be seen. This is most likely caused by historical, industrial and information-level distinctions between the different geographical regions that are manifested as different frequencies of kindreds with proven mutations in BRCA1/BRCA2. The largest difference is primarily between western (which includes the capital, Bratislava) and eastern parts of the country. The change in HBOC frequency can be explained by the decreasing number of hospitals with departments of clinical genetics from the western to the eastern territories of the Slovak Republic. That therefore best explains the number of HBOC suspected families from these regions that did not appear to correlate with the overall distribution of unselected breast and ovarian cancer cases reported by NCRS.

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