The spectrum and incidence of *BRCA1* pathogenic mutations in Slovak breast/ovarian cancer families

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Pathogenic germline mutations in *BRCA1* and *BRCA2* account for the majority of hereditary breast/ovarian cancer cases. The analysis of *BRCA1* gene was carried out in 156 breast/ovarian cancer families: 82 families with strong family history and 59 families with medium family history. Generally, 31 families and 71 cases with *BRCA1* pathologic mutations (14 different types) were identified in this study by combination of SSCP and direct sequencing techniques. Using approved systematic nomenclature numbering, c.5266dupC (8 families, 21 cases), c.181T>G (5 families, 11 cases), c.68_69delAG (3 families, 5 samples) and c.843_846del4 (3 families, 4 samples) were the most frequently found mutations in *BRCA1* gene. Altogether these 4 mutations accounted for 61.3% of all detected pathogenic mutations in *BRCA1*. One novel mutation c.1166delG was detected in one family (4 cases). Frame-shift mutations were found in 21 families (46 cases), nonsense mutations in 4 families (8 cases) and missense mutations in 6 families (17 cases). Even though the 4 most frequent mutations account for 61.3% of all detected pathogenic mutations, screening of the whole *BRCA1* coding region is necessary, due to the large scale of low frequency disease causing mutations in breast/ovarian cancer families in Slovakia.

Key words: BRCA1, breast cancer, mutation analysis, ovarian cancer, Slovak population

Germline mutations in *BRCA1* (OMIM, 113705; GeneBank, U14680.1) and *BRCA2* (OMIM, 600185; GeneBank, U43746.1) are responsible for most of the familial breast and ovarian cancers. Altogether, mutations in these two genes are responsible for approximately 6–7% of breast and 10% of ovarian cancers in general, and an autosomal dominant pattern of inheritance was demonstrated [1]. In the majority of families with breast/ovarian cancer, the diseases are linked to the *BRCA1* gene [2]. The penetrance of the *BRCA* mutations is recently a much discussed problem. Breast cancer risk by the age of 70 has recently been estimated at 72% in *BRCA1* mutation carriers, and 75% in *BRCA2* mutation carriers. The corresponding ovarian cancer risks have been estimated at 38% and 49%, respectively [3].

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A large number of alterations (mutations, polymorphisms, and genetic variations of uncertain significance) in the *BRCA1* and *BRCA2* genes is described in the databases such as Breast Cancer Information Core (BIC) Database [4] or The Human Gene Mutation Database (HGMD) [5]. The proportion of families with breast/ovarian cancer cases attributable to *BRCA1*/2 mutations varies widely among populations. In some a high frequency of unique mutations throughout both genes is presented, for example in Italy, or Spain [6], while in other ethnic groups only one or a small number of founder mutations are responsible for a majority of inherited breast and ovarian cases [7], for example in Iceland (*BRCA2*, c.771_775del5), [8] or in Ashkenazi Jews (*BRCA1*, c.68_69delAG, c.5266dupC; *BRCA2*, c.5946delT), [9].

In our pilot study we analysed a set of 156 families with family history of breast/ovarian cancer, and evaluated the incidence, spectrum and association with cancer disease phenotypes of *BRCA1* pathogenic mutations.

Patients and methods

Patients and families. Women with breast/ovarian cancer or their relatives (subclinical cases) were referred for genetic counselling mainly to the Department of Oncological Genetics, National Oncological Institute, Bratislava, or to the Centre of Clinical Genetics, Faculty Hospital, Bratislava, or to other regional medical geneticists from several parts of Slovakia by regional gynecologists and general practitioners. The samples were collected in the time frame of 4 years (2001–2005).

All analysed individuals signed an informed consent and fulfill the criteria for genetic testing. Tested families were divided as follows:

Group A – Strong family history:

A1 – At least 2 cases of breast/ovarian cancer, 1 under the age of 45, the rest under the age of 65 years.

A2 – At least 1 case with breast/ovarian cancer under the age of 65 years.

A3 – At least 1 case with bilateral form of breast/ovarian cancer under the age of 65 years (bilateral form over the age of 65 years was counted as 2 independent cases).

A4 – Presence of breast cancer in male patients.

Group B – Medium family history:

B1 – At least 3 cases of breast/ovarian cancer over the age of 45 and under 65 years.

B2 – One case of breast/ovarian cancer under the age of 45 years.

B3 – At least 2 cases of prostate cancer in male patient under the age of 65, without any breast/ovarian cancer cases.

B4 – Only 1 case of breast/ovarian cancer over the age of 45 and under 65 years with at least 2 cases of associated carcinomas at any age.

Samples and DNA isolation. Blood samples of the patients or subclinical probands (n=248) indicated for genetic testing, were collected in our laboratory at the Department of Clinical Genetics, St. Elizabeth Cancer Institute in Bratislava between years 2001 and 2005. DNA was extracted by FlexiGene DNA Kit (Qiagen, Hilden, Germany) from about 10 ml of whole blood, which was anticoagulated using EDTA.

Mutation Screening. All coding exons and flanking intron sequences of *BRCA1* were analysed using PCR amplification and a combination of single strand conformation polymorphisms (SSCP) and direct sequencing (DS).

We divided the whole coding region of *BRCA1* gene into 39 PCR products. The sizes of PCR fragments varied from 197 bp to 450 bp. Primer sequences for amplification were used as described [10, 11].

All PCR products were screened by SSCP method. Atypical fragments were directly sequenced on a newly amplified PCR product. Each discovered sequence alteration was confirmed in independently repeated PCR amplification.

Mutations are described at the cDNA level according to the Human *BRCA1* reference sequence: U14680, GenBank and marked in the approved systematic nomenclature (12, 13, 14).

Results

Pathogenic germline mutations in *BRCA1* were detected in 31 (22%) out of 141 families, including 24 families with strong family history and 7 families with medium family history. Within 248 analysed DNA samples, 71 (28.6%) were positive for the presence of a germline mutation in *BRCA1*.

Table 1. The spectrum of *BRCA1* pathogenic germline mutations in breast/ovarian cancer families in Slovak population.

BIC traditional nomenclature	Approved nomenclature	Mutation type and predicted effect	Exon	Num. of families	Num. of samples	World appearance	Mutation published
185delAG	c.68_69delAG	FS; p.Glu23fsX39	2	3	5	Founder in Ashkenazi	BIC database (4)
234T-C	c.115T>C	MS; p.Cys39Arg	3	1	6	Rare mut. -Central/ Eastern EU	BIC database (4)
300T-G	c.181T>G	MS; p.Cys61Gly	5	5	11	Frequent in EU and USA	BIC database (4)
962del4 (CTCA)	c.843_846del4	FS; p.Ser281fsX296	11	3	4	Austria	Wagner et al (15)
1285delG*	**c.1166delG**	**FS; p.Ser389fsX393**	**11**	**1**	**4**	**Novel mut.**	**unpublished**
2057del10 (CAGTGAAGAG)	c.1938_1947del10	FS; p.Ser646fsX646	11	1	1	Only in Slovakia	Ciernikova et al. (16)
2072del4 (GAAA)	c.1953_1956del4	FS; p.Lys651fsX699	11	1	1	Western EU, USA	BIC database (4)
3135del4 (CATT)	c.3016_3019del4	FS; p.His1006fsX1022	11	1	1	Austria	Wagner et al. (15)
3819del5 (GATAA)	c.3700_3704del5	FS; p.Val1234fsX1241	11	2	3	Central-Eastern EU, Poland	BIC database (4)
3889delAG	c.3770_3771del2	FS; p.Glu1257fsX1265	11	1	6	Central-Eastern EU	BIC database (4)
4446C-T	c.4327C>T	NS; p.Arg1443X	13	1	1	France, French-Canada; one case in Finland	Castilla et al. (17); Syrjäkoski et al. (18)
4458C-T	c.4339C>T	NS; p.Gln1447X	13	1	2	Reported only once, very rare	BIC database (4)
5370C-T	c.5251C>T	NS; p.Arg1751X	20	2	5	Finland, Greece	Vehmanen et al. (19); Ladopoulou et al. (20)
5385dupC	c.5266dupC	FS; p.Gln1756fsX1829	20	8	21	Most frequent mutation, founder in EU, Ashkenazi	BIC database (4)

* Novel mutation, not yet published

Altogether, 14 different *BRCA1* mutations were identified, twelve caused a premature protein termination (86%), including nine frame-shifting (FS) deletions and insertions (64%) and three nonsense (NS) mutations (22%). The two remaining types of pathogenic mutations had missense (MS) character (14%) (Table 1).

The most frequently found mutations in *BRCA1* were: c.5266dupC – 8 families (25.8%) or 21 cases (30.4%); c.181T>G – 5 families (16.1%) or 11 cases (15.9%); c.68_69delAG – 3 families (9.7%) or 5 cases (7.2%) and c.843_846del4 – 3 families (9.7%) or 4 cases (5.8%). Altogether, these four *BRCA1* mutations were found in 61.3% of all detected positive families and 59.4% of all cases with identified mutations. A broad spectrum of other detected mutations demonstrates the diversity of the population in Slovakia.

The c.1166delG is a novel mutation in *BRCA1* formatting a stop codon in the position 393. This novel mutation was identified in 2 patients and 2 subclinical probands in family No. 200 with positive family history of breast and ovarian cancer diagnosed at the ages of 27, 52 and 41 years, respectively (not published by June 2006).

Table 2 shows the efficiency of mutation detection in relation to family history and age at onset. The prevalence of mutations in families with strong family history (A) was 29.3% (24 out of 82 analysed families). In the group of families with medium family history (B) the detection rate decreases to rare 12.5% (7 out of 59 families), considering the fact that there were no mutations detected in type B3 and B4 families.

Table 3 shows the results after dividing all analysed families into the groups by the presence of breast or ovarian cancer. A majority of families – 77 (49.4%) out of 156 were breast cancer only (BC), eight of them were positive for *BRCA1* mutations. Twentytwo (14.1%) of 156 families belong to the bilateral breast cancer group (BBC) and five were detected as positive for the presence of *BRCA1* mutations. In the group of 22 (14.1%) out of 156 families with breast and also ovarian cancer (BOC), there were twelve *BRCA1* positive families detected. There were 20 (12.8%) out of 156 families with ovarian cancer only (OC), in 4 we identified *BRCA1* mutations. In 2 out of 156 (1.3%) families with bilateral ovarian cancer (BOVC), only 1 presented a *BRCA1* mutation. In 2 out of 156 families (1.3%) there was a patient with ovarian and also breast cancer (OBC), and in 1 family we detected *BRCA1* mutations.

The estimation of cumulative risks for breast and ovarian cancer in *BRCA1* mutation carriers was accomplished in 71 samples within 31 *BRCA1* positive families (Table 4).

Table 2. The distribution of selected breast/ovarian families concerning the criteria of family history in Slovak population.

Criteria *	Number of families	*BRCA1* positive families (%)	Number of samples	*BRCA1* positive samples (%)
A1	61	19 (31.1%)	119	39 (32.7%)
A2	4	3 (75%)	11	10 (91%)
A3	18	2 (11.1%)	34	2 (5.9%)
A4	0	0	0	0
A. Strong family history	**82**	**24 (29.3%)**	**164**	**51 (31.1%)**
B1	34	6 (16.7%)	54	14 (26%)
B2	20	1 (5%)	25	6 (24%)
B3	1	0	1	0
B4	3	0	4	0
B. Medium family history	**59**	**7 (12.5%)**	**84**	**20 (23.8%)**

Table 3. The distribution of analysed families according to the presence of breast or ovarian cancer.

Presence of cancer type	Number of all families	*BRCA1* positive families (%)
Breast cancer only (BC)	76	8 (10.5%)
Bilateral breast cancer (BBC)	22	5 (22.7%)
Breast and ovarian cancer in one family (BOC)	22	12 (54.5%)
Only ovarian cancer (OC)	17	4 (23,5%)
Bilateral ovarian cancer (BOVC)	2	1 (50%)
Ovarian and breast cancer in one patient (OBC)	2	1 (50%)
Total	**141**	**31 (22%)**

Table 4. Estimated risk of breast and ovarian cancer in *BRCA1* mutation carriers in Slovakia.

Age	*BRCA1* mutation carriers	Breast cancer		Ovarian cancer		Breast or Ovarian cancer	
17 – 49	48	9	18.8%	2	4.1%	**11**	**22.9%**
50 – 69	18	12	66.7%	3	16.7%	**15**	**83.4%**
70 –	5	2	40%	2	40%	**4**	**80%**
Summary	71	23	32.4%	7	9.9%	**30**	**42.3%**

Discussion

We used a combination of SSCP technique and direct sequencing to analyse all coding sequences of *BRCA1* gene, and detected 31 families (22%) with pathogenic germline *BRCA1* mutations within 141 breast/ovarian cancer families in the first study of this type of Slovak population. Compared to many other European studies of *BRCA1* positive families, the frequency is in agreement with the results found in the neighbouring populations: it was 23.6% in the Czech [21], 19.8% in the Austrian [15], and 23.3% in the Polish populations [22]. Frequency similarities show historical and geographic relationship among Central European populations.

The age-adjusted incidence of breast cancer in common Slovak population in the year 2002 was 48.6/100.000 (n=1945 cases), with average age 60.5 years at diagnosis (range of average ages from 22 to 87 years), most cases at the average age of 67 years (n=261). The age-adjusted incidence of ovarian cancer in the common Slovak population in the year 2002

was 11.5/100.000 (n=448 cases), average age was 59.3 years at diagnosis (range of average ages from 22 to 87 years), and most cases at the average age of 62 years (n=60) [23].

Our observed set of cases with the presence of *BRCA1* mutations comprised of 71 samples, 61 women and 10 men, 30 patients and 41 subclinical probands respectively. The age range at the time of cancer digagnosis in patients was from 27 to 63 years; in healthy subclinical probands at the time of DNA testing it was from 17 to 71 years. The average age of healthy subclinical probands at the time of DNA testing was 34 years. The average age of patients at the time of cancer diagnosis was 46.2 years. The average age at the time of breast cancer diagnosis was 41.4 years (range from 27 to 57 years), while in the patients with ovarian cancer it was 51.3 years (range from 41 to 63 years).

The breast/ovarian cancer risk by age 70 was estimated at 80%, by age 50 at 83.4%. These findings are relatively similar to the risks published in study Antoniou et al. (3). However, differences may be the result of many factors, e.g. selection of the families in the studies, or composition of observed set of mutation carriers.

In our study the *BRCA1* mutations were detected predominantly in families with both breast and ovarian cancer. The frequency of identified mutations among families with ovarian cancer (with or without breast cancer) was higher than in families with breast cancer only (Table 3). The observed effect in families with ovarian cancer is approximately the same in Czech and German studies [24, 25] but somewhat lower than in Polish or Swedish studies [22, 26].

Gayther et al. [27] reported that mutations in the 3' end of the *BRCA1* gene are associated with a lower proportion of ovarian cancer. The border for this phenotype correlation was located at exon 13, between codons 1435 and 1443. Further studies proved for this genotype-phenotype correlation [28], although other authors failed to confirm this observation [29]. In our series pathogenic mutations located in the region of exons 2–12 were detected in 61.3% of *BRCA1* positive families (19 out of 31), 8 families show the presence of breast cancer and 11 presence of ovarian cancer (8:11). Pathogenic mutations detected in the region of exons 13–20 were identified in 38.7% of *BRCA1* positive families (12 of 31), 5 families with presence of breast cancer and 7 with ovarian cancer (5:7).

The most frequently detected mutations in *BRCA1* were c.5266dupC, c.181T>G, c.68_69delAG, and c.843_846del4. Altogether these 4 mutations are responsible for 61.3% of all *BRCA1* positive families, so they can be considered as high-frequency *BRCA1* mutations. Similar results were presented in other studies of Central European populations [15, 21, 22, 24] except of mutation c.3700_3704del5 which is relatively common in some populations, but detected only in 2 (6.5%) breast/ovarian families in the Slovak population.

The *BRCA1* mutation c.5266dupC was first observed in Baltic families, being very frequent in Russia and Hungary, and also among the Ashkenazi Jews (30). The common finding of haplotype c.5266dupC carriers and their concentration in Eastern Europe suggested a Baltic origin during the medieval period [7]. It is in disagreement with results of Latvian population (Baltic) study, where most of the c.5266dupC mutation carriers were of Russian origin (31). In Slovak population *BRCA1* mutation c.5266dupC in exon 20 was detected in 25.8% of breast/ovarian families (8 of 31). This mutation was frequently detected in *BRCA1* positive families in the Central-Eastern European region [21, 22, 24, 31, 32, 33] and surprisingly in Greece (19). It was also found at lower rates in Germany [34], Austria [15] or Sweden [26], but was absolutely rare in Italy [35], Turkey [36] or Spain [6]. These findings may indicate the possible Slavic origin of c.5266dupC mutation concerning the high occurrence in Slavic or Slav-neighbouring populations, decreasing in Germanic populations and very rare in Romanic populations.

Mutations c.843_846del4 (3 families) and c.3016_3019del4 (1 family) were detected in Austrian population and were not previously reported in families of non-Austrian origin [15]. This is the first time these mutations are detected in Slovak population and in non-Austrian population.

Nonsense mutation c.5370C>T found in 2 breast/ovarian families (6.5%) is also relatively rare (19, 20); this is the first time it is detected in Central European population.

Mutation c.115T>C is supposed to be a very rare substitution and has been detected only in three Czech families [21], and now in one family in Slovak population.

Mutation c.1938_1947del10 (deletion of CAGTGAAGAG in *BRCA1* exon 11) was detected and published only once in Slovak population. The new stop codon TAA is formed directly at the place of deletion, and produces a significantly truncated protein [16].

Two mutations were found in exon 13 of *BRCA1* gene, and are localised in a close region. Mutation c.4327C>T was detected in French and French-Canadian breast/ovarian families as a founder mutation, but it is suggesting to have multiple origins [37]. The presented mutation was identified in one Slovak family. Mutation c.4339C>T, is according to BIC database considered as a very rare. It is for the first time reported in Slovak population, thus it might be regarded as an origin-independent rare mutation arising in the hotspot region of exon 13.

As shown above, mutations in *BRCA1* gene in Slovakia may be separated into two population specific groups, high frequency mutations (c.5266dupC, c.181T>G, c.68_69delAG and c.843_846del4) and low frequency mutations (c.115T>C; c.1938_1947del10; c.1953_1956del4; c.3016_3019del4; c.3770_3771del2; 4446C>T and c.4339C>T). A set of several low frequency mutations may be supposed as special and unique for each of closely related populations, e.g. in Central European region.

For *BRCA1* mutation screening we used SSCP method with detection sensibility rate range around 70–80%. On the other hand, the set of 14 different mutations, and the frequency of *BRCA1* positive families approach or exeed that of some other studies. Even though we may expect that about 10% of fami-

lies probably escape the detection. To increase the detection rate, DS of large exons and fragments (such as exon 11 of *BRCA1*), should be used in the future. There can be even some cases, which escapes detection because of large *BRCA1* rearrangements and are able to be identified by screening with MLPA analysis only.

In summary, in the pilot study of Slovak population we have presented the *BRCA1* mutational analysis of 141 families with familial or early-onset breast/ovarian cancer and demonstrated the dominant role of c.5266dupC, c.300T>G, c.68_69delAG and c.843_846del4 mutations (61.3% of all detected mutations). One novel frame-shift mutation c.1166delG is reported in exon 11 of *BRCA1* in one family. We found pathogenic *BRCA1* germline mutations in 22% of selected breast/ovarian families, or 28.6% of cases. Mutation detection rate was higher in the families with both breast and ovarian cancer presence. This observation leads to the conclusion, that occurrence of both diseases in one family had considerable influence on the presence of the *BRCA1* mutation.

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