Ashkenazi founder *BRCA1/BRCA2* mutations in Slovak hereditary breast and/or ovarian cancer families*

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Germline mutations in *BRCA1* and *BRCA2* have been predominantly associated with the breast and ovarian cancers. Two mutations in *BRCA1* (185delAG and 5382insC) and one mutation in *BRCA2* (6174delT) are common in Ashkenazi Jewish population. To determine the proportion of these founder mutations, we analyzed DNA samples of 120 Slovak hereditary breast and/or ovarian cancer (HBOC) suspected families. Two particular exons of *BRCA1* (2, 20) and 11N segment of *BRCA2* were screened by single strand conformation polymorphism (SSCP) followed by DNA sequencing of fragments showing abnormal migration pattern. Mutational analysis revealed that 7 out of 20 (35%) families with detected *BRCA1/BRCA2* pathogenic alteration harbored one of three Jewish mutations: five families with 5382insC, one family with 185delAG and one family with 6174delT. Interestingly, we have noted a very rare phenotype, when 5382insC in *BRCA1* co-segregated also with endometrial carcinoma. Similarly to the studies from other countries of Central and Eastern Europe, the most frequent pathogenic alteration found was 5382insC that accounted for 1/4 of all gene defects detected. Following the high proportion of Ashkenazi Jewish founder mutations in Slovak HBOC families, a pre-screening for at least 5382insC mutation in individuals at even moderate risk would be appropriate.

Key words: breast and ovarian cancer, BRCA1, BRCA2, Ashkenazi Jewish founder mutations, Slovak HBOC families

Breast cancer is one of the most common malignancies affecting women in developed countries with a lifetime risk of 10% [1]. According to the National Cancer Registry of Slovakia (NCRS), 1827 new cases of breast cancer were diagnosed in the year 2001 and they represent the highest proportion of all cancer (17.6%) among Slovak women. Epidemiological studies also show that breast cancer is the leading cause of cancer-related mortality with 15.6% of all cancer deaths. Incidence of this malignity has doubled during the past 20 years and still showing an increasing trend (data from NCRS).

Mutations in breast and ovarian cancer susceptibility genes *BRCA1* (MIM 113705) [2] and *BRCA2* (MIM 600185) [3] are found in a high proportion of multiple-case families with breast or ovarian cancer. A pooled analysis of 22 studies involving over 8000 breast and ovarian cancer cases estimates the average cumulative risks in *BRCA1*-mutation carriers of 65% (95% confidence interval (CI), 44–78%) for breast and 39% (95% CI, 18–54%) for ovarian cancer by age 70. The corresponding estimates for *BRCA2* were 45% (95% CI, 31–56%) and 11% (95% CI, 2.4–19%), respectively [4]. It was initially suggested that these two genes would be responsible for most cases of familiar incidence of breast and ovarian malignities [2, 3]. But more recent population-based studies have shown that other candidate genes – *CHEK2*, *ATM*, *TP53, PTEN* – play a role in early onset of disease [5, 6].

Although the spectrum of *BRCA1/BRCA2* mutations is very broad in most countries, founder mutations are responsible for a larger proportion of breast and ovarian cancer cases within certain inbred communities. The most thoroughly studied manifestations of the founder effects are among Ashkenazi Jewish population, where three common mutations in *BRCA1/BRCA2* are reported. The *BRCA1* mutation 185delAG has been found in 0.96–1.14%, the *BRCA1* mutation 5382insC in 0.15–0.28% and *BRCA2* mutation 6174delT

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in 0.62–1.36% of Ashkenazi women [7, 8]. Ashkenazi Jewish population represent the largest proportion of world Jewry today (82%) and are descendants of German Jews who migrated to Central and Eastern Europe [9]. Taken together, these three founder alterations account for 25% of early-onset breast cancer, and up to 90% of families with multiple cases of both breast and ovarian cancer in Ashkenazi population [10, 11]. On the other hand, their frequencies in non-Ashkenazi controls and cancer patients are much lower. Actually, 6174delT in *BRCA2* has not been reported outside the Jewish population [8, 12, 13].

The aim of this study was to estimate the contribution of Ashkenazi Jewish founder *BRCA1/BRCA2* mutations to the development of breast and ovarian cancer in our collection of Slovak families. To this purpose, we performed a mutation screening of particular exons of *BRCA1* and *BRCA2* in 120 Slovak HBOC suspected kindreds.

Patients and methods

Collection of HBOC suspected families. The families were obtained from the National Cancer Institute, The Centre of the Clinical Genetics of the Faculty Hospital and genetic centers of the hospitals all over the Slovak Republic. Informed consent was required prior the testing. The selection criteria used were at least two cases of breast or ovarian cancer among first and second-degree relatives from the same ancestral lineage or a combination of breast and ovarian cancer among first-degree relatives. Patients with very early-onset of breast cancer (under age 35), bilateral breast cancer, family history of male breast cancer or breast and ovarian cancer in the same individual at any age were also included. When available, index case's relatives were also tested for mutations identified to determine carrier status.

DNA isolation and PCR amplification. Genomic DNA was extracted from peripheral blood lymphocytes using QIAamp DNA blood kit (Qiagen, Hilden, Germany). Exons 2 (258 bp) and 20 (249 bp) of the *BRCA1* gene and exon 11N (536 bp) of *BRCA2* gene were amplified by PCR as described elsewhere [14, 15]. The PCR assay was conducted in 25-µl reaction volume including 100–200 ng of genomic DNA, 200 µM dNTPs, 1 x PCR buffer (Qiagen, Hilden, Germany), 1.5–2.5 mM $MgCl_2$, 1 U of Qiagen®Taq DNA Polymerase (Qiagen, Hilden, Germany) and 20 pmol of each primer. After an initial denaturation of 5 min at 94 °C, 30 cycles were performed with denaturation at 94 °C/1 min, annealing at 60 °C/1 min and elongation at 72 °C/1 min.

Single strand conformation polymorphism (SSCP) was used to screen all three particular exons of *BRCA1/BRCA2*. Amplified samples were denatured 5 min at 94 °C, then placed on ice for 5 min and loaded into a 6% polyacrylamide gel. Electrophoresis was performed under non-denaturing conditions at 4 °C for 20 hours at 68 V and the gels were consequently visualized by silver-staining.

DNA sequencing. DNA fragments showing abnormal banding pattern were purified by Exo-SAP-IT kit (USB, USA). The sequencing reaction was performed using an appropriate SSCP primer set and ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, USA). The extension products were afterwards purified by Dye Ex™ 2.0 Spin columns (Qiagen, Germany) and analyzed using an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). All alterations were confirmed by sequencing of both DNA strands in two independent PCR products.

Obtained sequences have been analyzed and compared with the cDNA of the Human *BRCA1* reference sequence: accession U14680; and the Human *BRCA2* reference sequence: accession U43746; from the NCBI GenBank sequence database.

Results

In this study we have analyzed DNA of 120 Slovak HBOC suspected kindreds to estimate the proportion of Ashkenazi Jewish founder *BRCA1/BRCA2* mutations. The study group was composed of 91 (76%) hereditary breast cancer (HBC) families, 5 (4%) hereditary ovarian cancer (HOC) families and 24 (20%) families with a family history of both breast and ovarian malignities. Mutation screening revealed that 7 out of 20 (35%) families with the *BRCA1* or *BRCA2* pathogenic alteration (data not presented) carry one of the Jewish founder mutations. The most frequent gene defect found was 5382insC in *BRCA1* gene, presented in five families; whereas 185delAG in *BRCA1* and 6174delT in *BRCA2* each of them were identified in one case. In total, 10 healthy carriers from 26 analyzed family members were identified. Example of SSCP analysis of the family with 5382insC is shown in Figure 1A. The presence of mutation was indicated by abnormal migration pattern of 4 family members and con-

Sequences of primers used for mutation screening [16].

Primer	Sequence 5'-3'	Position in gene*
BRCA1 exon 2 for:	5'GAA GTT GTC ATT TTA TAA ACC TTT	4557–4580
BRCA1 exon 2 rev:	5' TGT CTT TTC TTC CCT AGT ATG T	4793–4814**
BRCA1 exon 20 for:	5' ATA TGA CGT GTC TGC TCC ACC	71518–71538
BRCA1 exon 20 rev:	5' AAT GAA GCG GCC CAT CTC	71749–71766**
BRCA2 exon 11 Nfor:	5' AAC GAA AAT TAT GGC AGG TTG T	24534–24558
BRCA2 exon 11 Nrev:	5' GCT TTC CAC TTG CTG TAC TAA ATC C	25045–25069**

*primer position according to the reference sequences of *BRCA1* and *BRCA2* by NCBI GenBank, accession numbers L78833 and N000013, respectively; **the reverse complement of the given *BRCA1/2* sequence.

firmed by final sequencing (Fig. 1B). Figure 2A shows an aberrant SSCP profile of all 3 members of affected family No. 48, where the *BRCA1* mutation 185delAG was identified (Fig. 2B). The *BRCA2* mutation 6174delT in the index case of family No. 96 was revealed by combination of SSCP analysis (Fig. 3A) and sequencing (Fig. 3B).

Characteristics of the families with one of three Jewish founder mutations found, in terms of family history of breast and/or ovarian cancer, age of onset as well as mutation found and other types of cancer in family are given in Table 1 [17]. As described, most of them belong to the group of hereditary breast cancer families. Breast cancer alone was associated with the mutation in 3 of them (No. 54, No. 70, No. 90), while family No. 104 was characterized by the presence of 2 patients with ovarian cancer. Breast, colorectal and endometrial malignities were presented in the remaining 5382insC kindred (No. 120). Ovarian tumor in addition to breast cancer was observed in family with *BRCA1* mutation 185delAG (No. 48) and *BRCA2* mutation 6174delT in family No. 96 was associated only with breast cancer cases.

Discussion

Only a relatively small amount of data about genetic predisposition to breast and ovarian cancer in Slovak population has been published so far. To fill at least a part of this gap, we set out to determine the contribution of three Ashkenazi Jewish founder mutations (185delAG and 5382insC in *BRCA1*;

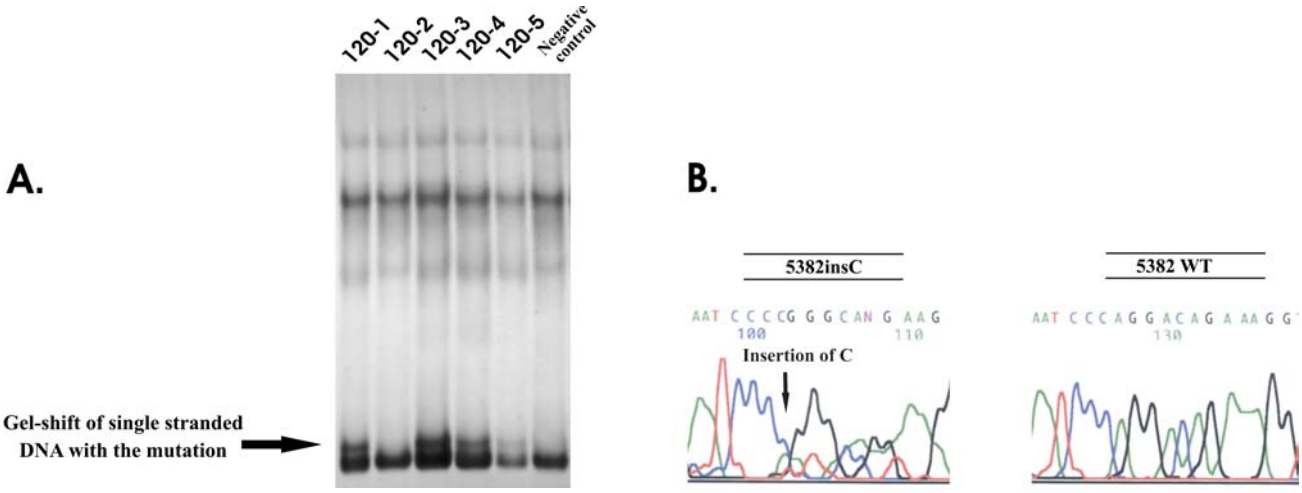

Figure 1. A. Members of family No. 120 screened by SSCP technique for *BRCA1* mutation 5382insC in exon 20 (249 bp). Abnormal migration pattern can be seen in the sample of index case (120-1) and other 3 healthy carriers of analyzed family (120-3, 120-4 and 120-5). B. Sequence analysis of samples showing altered migration confirmed the presence of 5382insC when compared to the sequence of member with standard SSCP profile (120-2).

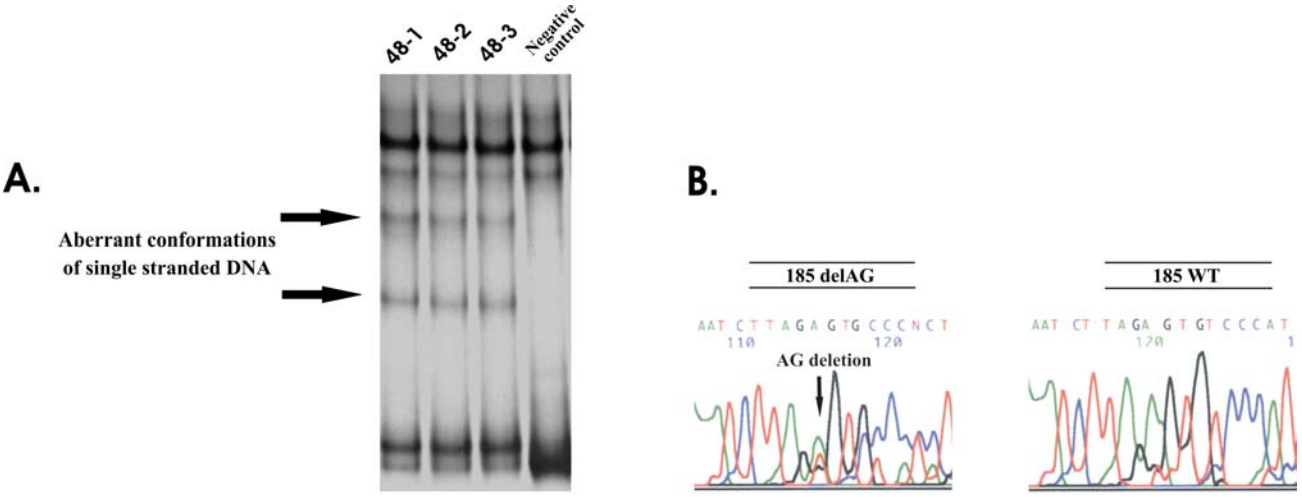

Figure 2. A. Members of family No. 48 screened by SSCP technique for *BRCA1* mutation 185delAG in exon 2 (258 bp). The arrows indicate the aberrant conformations present in all three members of affected family and absent in negative control. B. Sequence analysis of all three members revealed the presence of 185delAG when compared to the sequence of wild-type homozygote.

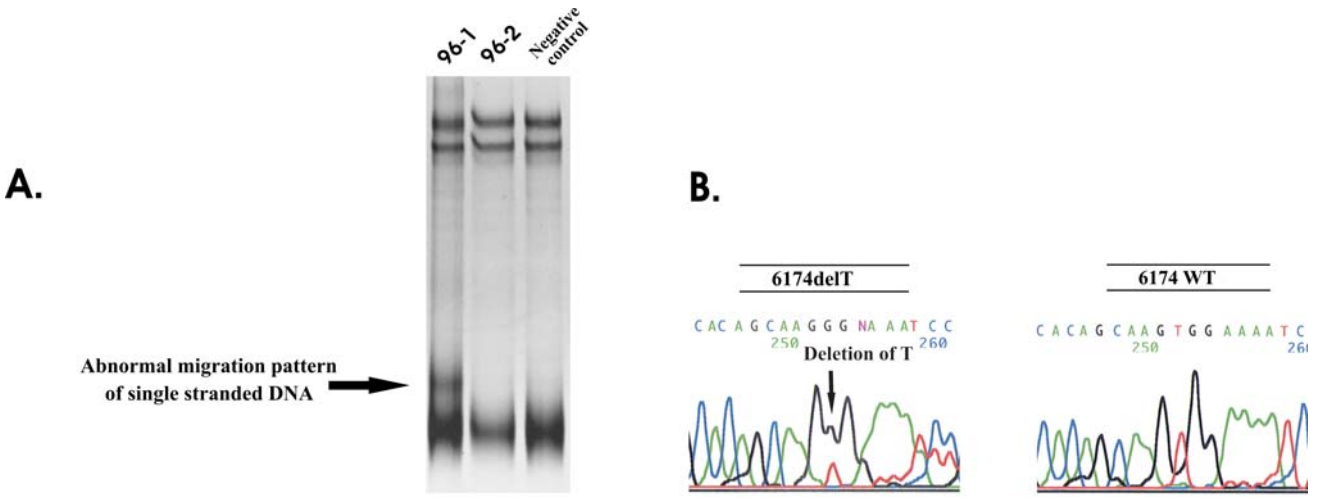

Figure 3. A. Members of family No. 96 screened by SSCP technique for *BRCA2* mutation 6174delT in exon 11, fragment N (536 bp). The abnormal mobility of single stranded DNA can be seen in the sample of index case (96-1). Bands' profile of her relative (96-2) and negative control shows no additional bands. B. Sequence analysis of index case confirmed the presence of 6174delT when compared to the sequence of family member showing standard migration pattern.

6174delT in *BRCA2*) to the development of breast and ovarian cancer in 120 Slovak HBOC suspected families. By using of standard screening techniques we have identified seven families carrying one of three particular Jewish mutations. Members of 5 affected kindreds come from the central part of Slovakia; next 2 families were originated in western region of the country.

To date, 10 healthy carriers of Jewish mutations were identified within the families revealed. ANTONIOU et al [18] estimated the average risk of breast cancer by age 70 for carriers of the 185delAG, 5382insC and 6174delT mutation as 64%, 67% and 43%, respectively. The corresponding ovarian cancer risks were 14%, 33% and 20% for carriers of these mutations. According to such high penetrances, it is very important to test as many family members as possible and assign the asymptomatic carriers to the program of preventive health care. They should be follow-up to discover any incipient neoplasia at sites associated with mutations above.

Table 1. Characteristics of affected families harboring one of three particular Jewish founder mutations. Numbers in parenthesis represent the age at diagnosis of respective tumors

Proband ID	Tumor type	Age of onset	Disease-associated mutations					Family history
			Exon	BRCA1 mutation‡	BRCA2 mutation‡	Change	Mutation effect	
48-1	Ovarian cancer	53	2	185delAG		39stop	Frameshift	Mother: Bilateral breast cancer (50) Sister: Breast and ovarian cancer (40) Niece: Breast cancer (26)
54-1	Breast cancer	45	20	5382insC		1829stop	Frameshift	Mother: Breast cancer (49) Sister: Breast cancer (45)
70-1	Breast cancer	41	20	5382insC		1829stop	Frameshift	Mother: Breast cancer (40) Sister: Breast cancer (33)
90-1	Breast cancer	46	20	5382insC		1829stop	Frameshift	Mother: Breast cancer (56) Sister: Breast cancer (42)
96-1	Breast cancer	56	11		6174delT	2003stop	Frameshift	2 sisters: Breast cancer (33–68) 2 aunts: Breast cancer
104-1	Ovarian cancer	47	20	5382insC		1829stop	Frameshift	Sister: Ovarian cancer (43)
120-1	Breast cancer	47	20	5382insC		1829stop	Frameshift	Mother: Bilateral breast cancer (43) Endometrial carcinoma (49) Aunt: Endometrial carcinoma (50) Father: Colorectal cancer (53)

‡ nomenclature is according BEAUDT and TSUI [17].

The studies on genotype-phenotype correlations showed that pathogenic variants located in first 2/3 of *BRCA1* gene were common in families with occurrence of both breast and ovarian cancer, while mutations close to 3' end were associated mainly with breast cancer in family history [19, 20]. Our results confirmed this trend in most cases (Tab. 1). The exemption represents the family No. 104 with 5382insC, characterized just with two ovarian cancer cases. When we look at the spectrum of malignities in families detected, the most diverse cancer phenotype can be seen in family No. 120 (Tab. 1). Here, 5382insC in *BRCA1* co-segregates also with endometrial carcinoma. Rarely, endometrial tumors have been distinguished in *BRCA* mutation carriers through isolated cases or in small case series only. The first evidence of an association between this *BRCA1* insertion and endometrial carcinoma was described by HORNREICH et al [21]. They observed two sisters harboring 5382insC; one sister was diagnosed with a papillary serous carcinoma of endometrium and the other with a papillary serous ovarian carcinoma. The patients were of Ashkenazi Jewish descent and their family history included breast, ovarian and colon cancer. LEVINE et al [22] genotyped 199 consecutive Ashkenazi Jewish patients with endometrial carcinoma for the presence of three *BRCA* founder mutations. Screening of this cohort revealed the mutation in 3 of 199 (1.5%) patients, compared to a frequency of 2.0% in this population generally. None of the tumors were associated with 5382insC. These data and also larger study reported by GOSHEN et al [23] indicate that occurrence of endometrial carcinoma does not appear to be elevated in the *BRCA* mutation carriers.

The variation in the population dynamics of *BRCA1* and *BRCA2* can be seen in different countries, reflecting the historical influences of migration, cultural and geographical isolation**.** Most of the Slovak population is of Caucasian origin, but there exist a significant influx of other nations during the past centuries. Before the World War II, approximately 138 000 Jewish citizens were living in the Slovak part of the Czechoslovakia. But holocaust and subsequent emigration waves diminished the current Jewish population in Slovak Republic to 3000–5000 (www.holokaust.sk). In spite of mentioned facts, it become interesting that more then 1/3 of all *BRCA1/BRCA2* mutations detected in Slovak HBOC suspected families are of Ashkenazi Jewish origin. On the other hand, it needs to be mentioned that *BRCA1* mutation 5382insC occurs very frequently even in other Central and Eastern European countries (Czech Republic, Poland, Hungary, Russia, Austria), as well [24–28]. In contrast to these findings, analyses of patients from Scandinavian area, Belgium, Netherlands and Great Britain have noted the absence or rather very small frequencies of presented sequence variation [29].

Following the high proportion of Ashkenazi Jewish founder mutations in Slovak HBOC families, a pre-screening for these pathogenic alterations, or at least 5382insC mutation, in individuals at even moderate risk could enable rapid detection of cancer predisposition. In addition, more information about patient's ancestry may also reduce time and cost of mutational analysis and bring the profit for tested members of affected family.

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References

[1] CASEY G. The BRCA1 and BRCA2 breast cancer genes. Curr Opin Oncol 1997; 9: 88–93.

[2] MIKI Y, SWENSEN J, SHATTUCK-EIDENS D, FUTREAL PA, HARSHMAN K et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 1994; 266: 66–71.

[3] WOOSTER R, BIGNELL G, LANCASTER J, SWIFT S, SEAL S et al. Identification of the breast cancer susceptibility gene BRCA2. Nature 1995; 378: 789–792.

[4] ANTONIOU A, PHAROAH PDP, NAROD S, RISCH HA, EYFJORD JE et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003; 72: 1117–1130.

[5] DE JONG MM, NOLTE IM, TE MEERMAN GJ, VAN DER GRAAF WTA, OOSTERWIJK JC et al. Genes other than *BRCA1* and *BRCA2* involved in breast cancer susceptibility. J Med Genet 2002; 39: 225–242.

[6] MEIJERS-HEIJBOER H, VAN DEN OUWELAND A, KLIJN J, WASIELEWSKI M, DE SNOO A et al. Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations. Nat Genet 2002; 31: 55–59.

[7] STRUEWING JP, HARTGE P, WACHOLDER S, BAKER SM, BERLIN M et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. New Engl J Med 1997; 336: 1401–1408.

[8] ABELIOVICH D, KADURI L, LERER I, WEINBERG N, AMIR G et al. The founder mutations 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 appear in 60% of ovarian cancer and 30% of early-onset breast cancer patients among Ashkenazi women. Am J Hum Genet 1997; 60: 505–514.

[9] BAHAR AY, TAYLOR PJ, ANDREWS L, PROOS A, BURNETT L et al. The frequency of founder mutations in the BRCA1, BRCA2, and APC genes in Australian Ashkenazi Jews: implications for the generality of U.S. population data. Cancer 2001; 92: 440–445.

[10] ELLISEN LW, HABER DA. Hereditary breast cancer. Annu Rev Med 1998; 49: 425–436.

[11] BRODY LC, BIESECKER BB. Breast cancer susceptibility genes BRCA1 and BRCA2. Medicine 1998; 77: 208–226.

[12] SZABO CI, KING M-C. Population genetics of BRCA1 and BRCA2. Am J Hum Genet 1997; 60: 1013–1020.

[13] NEUHAUSEN SL, GODWIN AK, GERSHONI-BARUCH R, SCHUBERT E, GARBER J et al. Haplotype and phenotype analysis of nine recurrent BRCA2 mutations in 111 families: re-

sults of an international study. Am J Hum Genet 1998; 62: 1381–1388.

[14] TOMKA M, SEDLAKOVA O, REINEROVA M, VESELOVSKA Z, STEVURKOVA V et al. Mutation screening of the BRCA1 gene in Slovak patients. Neoplasma 2001; 48: 541–455.

[15] ČIERNIKOVÁ S, TOMKA M, SEDLÁKOVÁ O, REINEROVÁ M, ŠTEVURKOVÁ V et al. The novel exon 11 mutation of BRCA1 gene in a high-risk family. Neoplasma 2003; 50: 403–407.

[16] WAGNER T, STOPPA-LYONNET D, FLEISCHMANN E, MUHR D, PAGÉS S et al. Denaturing high-performance liquid chromatography detects reliably BRCA1 and BRCA2 mutations. Genomics 1999; 62: 369–376.

[17] BEAUDET AL, TSUI LC. A suggested nomenclature for designating mutations. Hum Mutat 1993; 2: 245–248.

[18] ANTONIOU A, PHAROAH PDP, NAROD S, RISCH HA, EYFJORD JE et al. Breast and ovarian cancer risks to carriers of the BRCA1 5382insC and 185delAG and BRCA2 6174delT mutations: a combined analysis of 22 population based studies. J Med Genet 2005; 42: 602–603.

[19] GAYTHER SA, WARREN W, MAZOYER S, RUSSELL PA, HARRINGTON PA et al. Germline mutations of the BRCA1 gene in breast and ovarian cancer families provide evidence for a genotype-phenotype correlation. Nat Genet 1995; 11: 428–433.

[20] HÄKANSSON S, JOHANNSSON O, JOHANSSON U, SELLBERG G, LOMAN N et al. Moderate frequency of BRCA1 and BRCA2 germ-line mutations in Scandinavian familial breast cancer. Am J Hum Genet 1997; 60: 1068–1078.

[21] HORNREICH G, BELLER U, LAVIE O, RENBAUM P, COHEN Y, LEVY-LAHAD E. Is uterine serous papillary carcinoma a BRCA1-related disease? Case report and review of the literature. Gynecol Oncol 1999; 75: 300–304.

[22] LEVINE DA, LIN O, BARAKAT R, ROBSON ME, McDERMOTT D et al. Risk of endometrial carcinoma associated with *BRCA* mutation. Gynecol Oncol 2001; 80: 395–398.

[23] GOSHEN R, CHU W, ELIT L, PAL T, HAKIMI J et al. Is uterine papillary serous adenocarcinoma a manifestation of the hereditary breast-ovarian cancer syndrome? Gynecol Oncol 2000; 79: 477.

[24] POHLREICH P, ZIKAN M, STRIBRNA J, KLEIBEL Z, JANATOVA M et al. High proportion of recurrent germline mutations in the BRCA1 gene in breast and ovarian cancer patients from the Prague area. Breast Cancer Res 2005; 7: R728–R736.

[25] GÓRSKI B, BYRSKI T, HUZARSKI T, JAKUBOWSKA A, MENKISZAK J et al. Founder mutations in the BRCA1 gene in Polish families with breast-ovarian cancer. Am J Hum Genet 2000; 66: 1963–1968.

[26] VAN DER LOOIJ M, SZABO C, BESZNYAK I, LISZKA G, CSOKAY B et al. Prevalence of founder BRCA1 and BRCA2 mutations among breast and ovarian cancer patients in Hungary. Int J Cancer 2000; 86: 737–740.

[27] GAYTHER SA, HARRINGTON P, RUSSELL P, KHARKEVICH G, GARKAVTSEVA RF, PONDER BA. Frequently occurring germ-line mutations of the BRCA1 gene in ovarian cancer families from Russia. Am J Hum Genet 1997; 60: 1239–1242.

[28] WAGNER TM, MÖSLINGER RA, MUHR D, LANGBAUER G, HIRTENLEHNER K. et al. BRCA1-related breast cancer in Austrian breast and ovarian cancer families: specific BRCA1 mutations and pathological characteristics. Int J Cancer 1998; 77: 354–360.

[29] BACKE J, HOFFERBERT S, SKAWRAN B, DÖRK T, STUHRMANN M et al. Frequency of BRCA1 Mutation 5382insC in German Breast Cancer Patients. Gynecol Oncol 1999; 72: 402–406.