MUTATION IN BRIEF

BRCA1 Mutation Analysis in Breast/Ovarian Cancer Families from Greece

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Germline mutations in BRCA1 gene account for varying proportions of breast/ovarian cancer families, and demonstrate considerable variation in mutational spectra coincident with ethnic and geographical diversity. We have screened for mutations the entire coding sequence of BRCA1 in 30 breast/ovarian cancer women with family history of two or more cases of breast cancer under age 50 and/or ovarian cancer at any age. Genomic DNA from patient was initially analyzed for truncating mutations in exon 11 with PTT followed by DNA sequencing. In the cases where no frameshift mutation was observed in exon 11, all other exons were screened with direct sequencing. Two novel (3099delT, 3277insG) and three already described (3741insA, 1623del5-TTAAA, 5382insC-twice) truncating mutations were identified. In addition, 6 point mutations (L771L, P871L, E1038G, K1183R, S1436S, S1613G) which are already classified as polymorphisms were identified. Three unclassified intronic variants (IVS16-68 G>A, IVS16-92 G>A, IVS18+65G>A) were also detected. These results show that BRCA1 deleterious mutations are present in a fraction (20%) of Greek breast/ovarian cancer families similar to other European countries. Mutations were detected in high- (≥3 members) as well as in moderate-risk (2 members) families. This is the first report of BRCA1 mutation analysis in Greece. **© 2000 Wiley-Liss, Inc.**

KEY WORDS: BRCA1, breast ovarian cancer, PTT, Greece

INTRODUCTION

BRCA1 (MIM# 113705) and BRCA2 (MIM# 600185) genes have been identified as being causative in familial breast/ovarian cancer (Miki et al., 1994; Futreal et al., 1994; Wooster et al., 1995). Carriers of germ-line mutations in these genes are at increased risk of developing breast and ovarian cancer. It is estimated that in the general

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population, approximately 6 to 7% of breast cancer cases and 10% of ovarian cancer cases averaged across all ages of onset result from mutations in breast cancer susceptibility genes (Claus et al., 1996). The frequency of BRCA1 and BRCA2 mutation carriers in women with breast or ovarian cancer (or both) depends on the study population and demonstrates considerable variation in coincidence with ethnic and geographical diversity (Neuhausen 1999). Preliminary studies carried out in Central-Eastern-European populations suggest a relatively high mutation frequency among moderate-risk breast/ovarian cancer families and the presence of strong founder effects in this region (Ramus et al., 1997a; Ramus et al., 1997b; Sobczak et al., 1997; Csokay et al., 1999; Olah, 1999).

The observed high variability concerning the geographic and ethnic distribution of some ancient mutations and the proportion of breast/ovarian cancer families attributable to BRCA1/BRCA2 as well as the ratios of the recurrent/unique mutations in Europe emphasize the importance of studies of yet uninvestigated European populations.

Several reports exist from Mediterranean countries including Italy, France and Spain (De Benedetti et al., 1998; Laplace-Marieze et al., 1999; Diez et al., 1999; Blesa et al., 2000). Few reports exist so far from the Balkan area including only Yugoslavia (Papp et al., 1999) and Turkey (Balci et al., 1999).

In this study, five frameshift mutations of BRCA1 were identified in 6 of the 30 Greek breast and breast/ovarian cancer women with family history studied, using the protein truncation test and direct sequencing. The founder mutation 5382insC was detected twice indicating an increased frequency of this mutation. Four other mutations were identified in exon 11. Mutation 3741insA has been reported only once in the BIC database in a Turkish family from Germany, 1623del5-TTAAA 6 times and 3099delT and 3277insG are novel mutations. In addition, already described polymorphisms (Dunning et al., 1997) and unclassified variants were identified, including L771L, P871L, E1038G, K1183R (exon11), S1436S (exon13), S1613G (exon16), IVS18+65G>A, IVS16-68 and IVS16-92; the last two were always observed simultaneously.

PATIENTS AND METHODS

Patients and Their Families

We have been constructing a genomic DNA bank from breast and/or ovarian cancer patients with family history in collaboration with Greek hospitals. The inclusion criteria for this study are having at least one first- or second-degree relative with breast cancer under age 50 or ovarian cancer at any age. Families for this study were selected under informed consent from patients attending the participating hospitals, in collaboration with the Hellenic Cooperative Oncology Group (HECOG).

Characteristics of the families where deleterious mutations were identified as well as mutations found are given in Table 1.

Mutation Screening

All nucleotide numbers refer to the wild-type cDNA sequence of BRCA1 as reported in GenBank (accession number U14680). Primer pairs were used to amplify all exons and intron-exon boundaries from genomic DNA extracted from patient samples with routine techniques. Primer selection was made from BIC database at http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/ Bic/Member/brca1_ mutation_ database. html. Genomic DNA was amplified by the Polymerase Chain Reaction (PCR) in a Perkin-Elmer 2400 Thermocycler (Perkin Elmer, CA, USA).

PTT Analysis

Exon 11 was amplified in three fragments in the presence of forward primers containing a T7 promoter and transcription/translation initiation sequence as described (Hogervorst et al., 1995). The PCR products were then subjected to an in vitro transcription/translation initiation reaction in a reticulocyte lysate system (Promega), electrophoresed at a 12% sodium dodecyl sulfate polyacrylamide gel, fixed, dried and autoradiographed.

DNA Sequencing

PCR products were sequenced directly with the same forward and, when needed, reverse primers used for PCR amplification. Sequencing was done using an ABI PRISM DyeDeoxy Terminator Cycle Sequencing Kit and an ABI 310 Genetic Analyzer (Perkin Elmer, Applied Biosystems, CA, USA), according to manufacturer's instructions. Any mutation found was confirmed on a second DNA sample isolated from a duplicate tube of blood

followed by sequencing in both forward and reverse directions. We were not able to find blood samples from affected relatives of the 6 patients carrying the frameshift mutations mainly because most of them were not alive.

RESULTS AND DISCUSSION

Mutation analysis of BRCA1 was performed in 30 Greek breast and ovarian cancer women with family history of two or more cases of breast cancer under age 50 and/or ovarian cancer at any age. Preliminary search for mutations on the BRCA1 gene was performed by applying the PTT to exon 11, which spans more than half of the entire gene coding sequence. In the cases where no mutation leading to a premature protein termination was observed in exon 11, all other exons were screened with direct sequencing. In order to identify polymorphisms not detected by PTT, the full sequence of exon 11 was screened by direct sequencing in 10 patients.

PTT analysis led to the identification of truncated protein products, corresponding to different regions of exon 11, in patients 64, 98, 99 and 145 (see Fig. 1). Sequence analysis of the regions likely to contain the protein-terminating alterations revealed four different frameshift mutations (Table 1).

Table 1 Families with BRCA1 mutations

Family No.	Total No. of BrCa cases	No. of BrCa cases <50 years	No. of OvCa cases	Mutation	Effect	Comments/other cancers
64	2	2 (31 yrs)*		3741insA	ter 1218	One bilateral brca
97	2	1 (33 yrs)*		5382insC	ter 1829	
98		1 (31)*	1	1623del5-TTAAA	ter 505	One more case of endometrial cancer
99	1		4	3099delT	ter 999	One case is both brca & ovca
113	3	2 (39 yrs)*		5382insC	ter 1829	One more case of intestinal cancer
145	1		3	3277insG	ter 1059	One case is both brca & ovca

* age at diagnosis of the proband

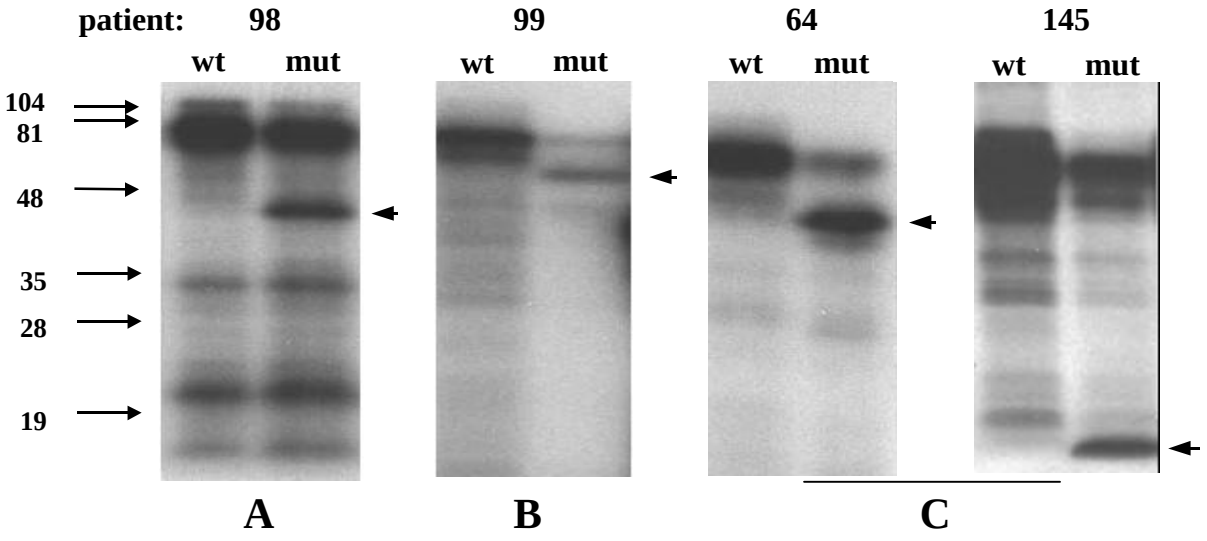

Figure 1. Identification of mutations by PTT applied to exon 11 of BRCA1 gene. In vitro synthesized polypeptides correspond to different fragments of exon 11 (Fragment A, nucleotide 793-2305; fragment B, nucleotide 1981-3381; fragment C, nucleotide 3061-4184). Arrows on the right side of the panels indicate abnormal peptides caused by frameshift mutations occurring in patients 64 (fragment A), 98 (fragment B), 99 and 145 (fragment C). Arrows on the left indicate sizes in kDa.

Mutation 3741insA causes premature protein termination at codon 1218. This mutation was found in a case of early-onset bilateral breast cancer (age 31) in family 64, with only one second degree relative affected with breast cancer at age 35. It has been reported only once in the BIC database but not published elsewhere, found in a Turkish family living in Germany.

Mutation 1623del5-TTAAA results also in a premature protein termination at codon 505. This mutation was detected in a patient (family 98) who developed ovarian cancer. In the same family there was a case of early-onset breast cancer (at age 31) and a case of endometrial cancer. This mutation has been reported by Schofield and Haites 1997 in a British family and later by de Benedetti et al., 1998 in Italy and Blesa et al., 2000 in Spain. As of April 2000 there are 6 entries for this mutation in BIC.

Mutation 3099delT is a new frameshift mutation causing premature protein termination at codon 999. It was identified in a patient who developed ovarian cancer at age 33 belonging to a high-risk family (no. 99) with two more cases of ovarian cancer and one case of breast and ovarian cancer, all first degree relatives.

Mutation 3277insG is also a new frameshift mutation causing premature protein termination at codon 1059. This mutation was found in a family (145) with three cases of ovarian cancer with the proband being affected with breast cancer at age 62 and ovarian cancer at age 72. Her daughter developed ovarian cancer at age 40.

The known founder mutation 5382insC was found in two patients who developed early onset breast cancer (no. 97 and 113), the one (39 yrs) having a family history of three cases of breast cancer and one case of intestinal cancer and the other (34 yrs) with only one more case of breast cancer. Both families had only breast cancer cases.

We screened for each of these 5 mutations 20 more patients (total 50 patients) in order to verify if any of them is present in an elevated frequency, but none was observed. In all, the above four frameshift mutations of exon 11 were present only once and 5382insC of exon 20 twice in our study group of 50 patients.

Mutation 5382insC is the most common among Europeans and most frequently detected in Hungarian breast cancer families and Russian ovarian cancer families (Szabo and King, 1997) found also in a quite high frequency in Central, Eastern and Southern European countries including Poland, Yugoslavia, Latvia, Italy and Spain (Ramus et al., 1997b; Gayther et al., 1997; Sobczak et al., 1997; Papp et al., 1999; Csokay et al., 1999; Olah et al., 1999, 1997; De Benedetti et al., 1998; Diez et al., 1999). Therefore, it is not surprising that this mutation is found in a relatively elevated frequency also in Greece.

Furthermore, our results are consistent with the observation of Gayther et al. 1995 and Holt et al. 1996, suggesting that when a frameshift mutation of BRCA1 occurs in the first two-thirds of the gene, the risk of ovarian cancer relative to breast cancer in the family is significantly higher than when truncating mutations occur in the last one-third of the gene.

Six well-known polymorphisms were also identified, including L771L, P871L, E1038G, K1183R (exon11), S1436S (exon13) and S1613G (exon16) (Dunning et al., 1997). The three variants P871L, E1038G and S1613G are apparently the most frequent polymorphisms of the gene as they are found almost in all populations studied until today.

Three unclassified intronic variants, already described in BIC but not published elsewhere, IVS18+65G>A, IVS16-68, IVS16-92 were observed in a high frequency and most probably represent polymorphisms; the two intron 16 variants are always found together in a double heterozygote state but they were not studied further at the family segregation level.

In the present study of Greek breast / ovarian cancer families we found six patients carrying frameshift mutations of the BRCA1 gene from a total of 30 patients. The results indicate that the frequency (20%) of BRCA1 mutations in Greece seems to be relatively high even if families with moderate history of cancer are included in the study. Data from other southern European countries report very similar frequencies, of 14% in Spain (Blesa et al., 2000), 18% in Italy (de Benedetti et al., 1998) and 24% in France (Laplace-Marieze et al., 1999). Furthermore, the presence of novel and probably unique mutations observed in our study, indicates an elevated heterogeneity of the mutational spectrum of BRCA1 in Greece, consistent with the observations in the previously cited studies of southern European countries. It has to be underlined at this point that varying proportions found in different countries may be due in a great part to a) the sensitivity of detection methods b) the complexity of mutational spectrum and c) the severity of inclusion criteria in the study. Especially with respect to the inclusion criteria, it is now well established that as the number of affected relatives increases, the frequency of deleterious mutations identified increases proportionally (Frank et al., 1998). The presence also of multiple cases of ovarian cancer in the family (as in families 99 & 145) is highly indicative for the presence of BRCA1 truncating mutations. Overall, our data justify the importance of genetic testing in high- and moderate-risk families in Greece.

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