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Germ line *BRCA1* and *BRCA2* gene mutations in Turkish breast cancer patients

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Abstract

Germ line *BRCA1* and/or *BRCA2* mutations were screened in 50 Turkish breast and/or ovarian cancer patients composed of hereditary, familial, early onset and male cancer groups. Genomic DNA samples were tested by heteroduplex analysis and DNA sequencing. Two truncating *BRCA2* mutations, one novel (6880 insG) and one previously reported (3034 delAAAC), were found in two out of six (33%) hereditary breast and/or ovarian cancer patients. A novel truncating (1200 insA) and a missense (2080A→G) *BRCA1* mutation was found in two of 27 (7%) individuals in the early onset group. A total of four (8%) disease-causing mutations in 50 breast cancer patients were identified in *BRCA1* and *BRCA2* genes. In addition, five *BRCA1* sequence variants have been identified in 23 patients. These results indicate that *BRCA1* and *BRCA2* genes are involved in some, but not all, forms of hereditary predisposition to breast cancer in the Turkish population. © 2000 Published by Elsevier Science Ltd.

Keywords: Hereditary breast/ovarian cancer; *BRCA1*; *BRCA2*; Germ line mutation

1. Introduction

Breast cancer is one of the most common malignancies affecting women [1] (http://www.nci.nih.gov/public/factbook98/incidence.htm). Inherited gene mutations may be responsible for 5–10% of breast cancer cases [1–3]. Ovarian cancer is also known to have a familial component [4].

Two genes associated with inherited predisposition to breast and/or ovarian cancer have been identified. These are *BRCA1* on chromosome 17q12-21 [1], and *BRCA2* on chromosome 13q12-13 [5]. Population genetics studies aimed at determining the relative contributions of these genes in hereditary breast and/or ovarian cancer have shown a wide variation among different populations [6]. For example, in families with three or more cases of female breast and/or ovarian cancer, *BRCA1* mutations are as low as 9% in Iceland and as high as 79% in Russia. A similar variation has been documented for *BRCA2* mutations as well, which can be exemplified by 8% in Finland and 64% in Iceland. In affected women before the age of 45 years with a first-degree relative, *BRCA1* mutations account for 7% of the families [7]. In families with both male and female breast cancer, *BRCA2* mutations were documented in 19% of North American, 33% of Hungarian and 90% of Icelandic populations [6]. In isolated male breast cancer cases, a very low frequency of *BRCA2* mutations has been reported [8]. In early onset breast and/or ovarian cancer patients not selected for family history, 4–9% have *BRCA1*, and 2–8% have *BRCA2* mutations [6,7].

Breast cancer is among the most common malignancies in Turkish women [9]. The frequency and the types of germ line mutations involved in Turkish breast/ovarian cancers are not well known. In order to determine the contributions of *BRCA1* and *BRCA2* mutations to the development of breast and/or ovarian

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cancer in the Turkish population, we screened preselected regions of these genes in four groups of patients composed of hereditary and familial cancer, as well as early onset and male breast cancer.

2. Patients and methods

2.1. Families

We analysed a total of 50 genomic DNA samples isolated from the white blood cells of breast cancer patients. The samples were collected from the Medical Schools of Hacettepe, Istanbul, Ankara Universities, and Ankara Oncology Hospital. Informed consent was obtained from all participants. Each proband was interviewed for pedigree construction including information concerning the family history of breast, ovarian and other cancers. Based on the pedigree analysis, patients were divided into four groups. Available medical and pathological records were reviewed to verify the diagnoses. The phenotypical characteristics of the families included in the study are summarised in Table 1 and the pedigrees for the hereditary and familial groups in Fig. 1.

2.2. Polymerase chain reaction (PCR) and heteroduplex analysis

DNA isolation was performed from 1 ml peripheral blood by phenol/chloroform extraction [10]. Exons 2, 5, 11 (10 overlapping fragments), 13, 20, 24 of *BRCA1*, and exon 11 (7 overlapping fragments) of *BRCA2* were screened for mutations by heteroduplex analysis using previously reported primers [11,12]. PCR was performed in a total volume of 10 μl, with 10× buffer, 1.5 U Taq polymerase, 1.5 mM $MgCl_2$, 200 μM dNTP, 10 pmol of each primer and 1μCi of [^{32}P] dCTP (specific activity 3000 Ci/mmol). PCR conditions were as follows: 94°C for 3 min, 30 cycles of 30 s at 94°C, 30 s at 55°C and 30 s at 72°C. Ten minutes of 72°C was added as the extension step. PCR products were denatured at 95°C for 10 min, and left at 37°C for 2 h. five microlitres of PCR products were loaded on to 6% native polyacrylamide gels and run at 400 V for 16 h. The gels were then dried and exposed to X-ray films overnight at −80°C.

2.3. Sequencing

Fragments that showed an alteration in the heteroduplex analysis were re-amplified for sequencing. The primer pairs for *BRCA1* did not change. However, *BRCA2* exon 11 was subdivided into 26 overlapping fragments for DNA sequencing reactions [12]. PCR was performed in a total volume of 50 μl, with 10× buffer, 1.5 U Taq polymerase, 1.5 mM $MgCl_2$, 200 μM dNTP and 10 pmol of each primer. PCR conditions were as follows: 94°C for 3 min, 30 cycles of 30 s at 94°C, 30 s at 55°C and 30 s at 72°C. 10 min of 72°C was added as the extension step. PCR products were sequenced using Amersham Thermosequenase Dye Terminator cycle sequencing kit, according to the manufacturer's instructions, and analysed on an ABI 377 automated DNA sequencer or ABI 310 Genetic Analyzer.

Table 1
Phenotypes and mutations identified in Turkish hereditary, and familial breast and breast–ovarian cancer groups

Group	Family	Mutation	Predicted effect	Age of proband (years)	Relatives with breast and/or ovarian cancer			Other cancers in the relatives
					1st degree	2nd degree	3rd degree	
Hereditary cancer	1	–	–	45	2 (52, 53)	1 (53)	2	Liver (54)
	2	*BRCA2* 6880 insG	Stop2224	25[a]	1 (25)	–	–	–
	10	–	–	32[a]	1 (62)	–	1 (35)	Colon
	18	–	–	37	1 (63)	1 (55)	1 (45)	–
	20	–	–	53[b]	2 (57, 60)	–	–	Endometrium (58)
	22	*BRCA2* 3034 delAAAC	Stop958	76	2 (42, 77)	–	–	Stomach (68)
Familial cancer	3	–	–	50	1 (39)	–	–	Lung, gall bladder, liver, leukaemia
	4	–	–	50	1 (29)	–	–	–
	6	–	–	51	1 (35)	–	–	Liver
	7	–	–	37	1 (41)	–	–	Colon (63)
	19	–	–	38	1 (55)	–	1 (40)	–
	48	–	–	31	1 (63)	–	1 (45)	–
	61	–	–	36	1 (50)	–	–	Colon (35, 48)

[a] Bilateral breast cancer.
[b] Breast and ovarian cancer. The number in parentheses indicates the age at diagnosis.
Hereditary group consisted of probands with at least two affected first degree relatives, and those with bilateral breast cancer and one affected first-degree relative. Patients who have one affected first-degree relative diagnosed with breast cancer were in the familial group.

Mutations identified by direct sequencing were subjected to confirmatory analysis. For this purpose, PCR products were cloned into Promega pGEM TEasy TA cloning kit, according to the manufacturer's directions. Several miniprep plasmid DNA samples for each patient were isolated by phenol chloroform extraction and sequenced using the Perkin Elmer Big Dye cycle sequencing kit.

3. Results

Germ line *BRCA1* and/or *BRCA2* mutations were screened in 50 breast and/or ovarian cancer patients from Turkey. We employed a *BRCA1* and *BRCA2* mutation screening strategy which is based on the localisation of previously reported mutations in these genes.

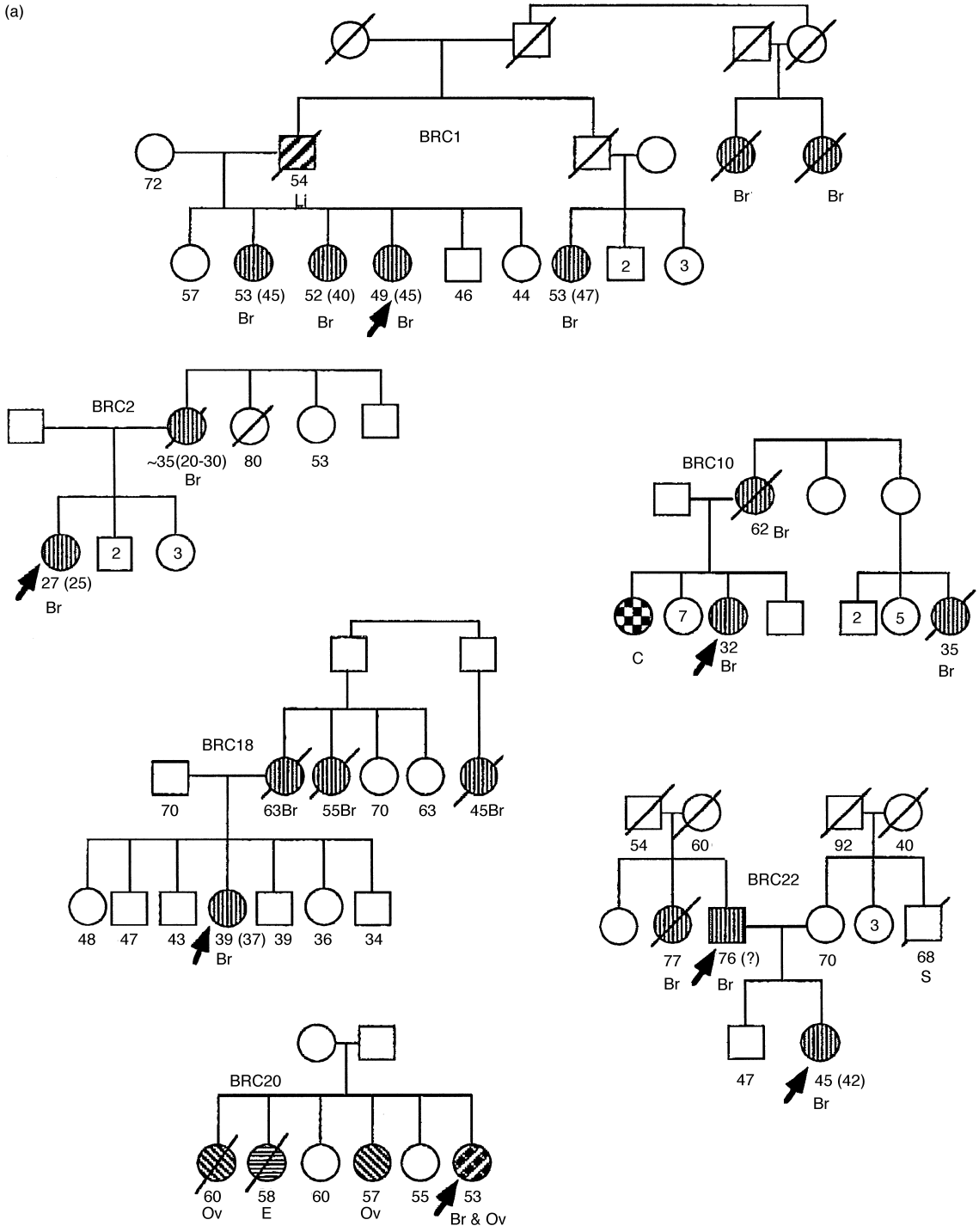

Fig. 1. Pedigrees of the hereditary (a) and familial (b) cancer groups. Ages of the individuals are indicated below the symbols, the age at diagnosis of the affected individuals are indicated in parentheses. Br, breast; Br and Ov, breast and ovarian; C, colon; E, endometrial; G, gall bladder; L, Leukaemia; Lg, lung; Li, liver, Ov, ovarian; S, stomach. *(continued overleaf)*.

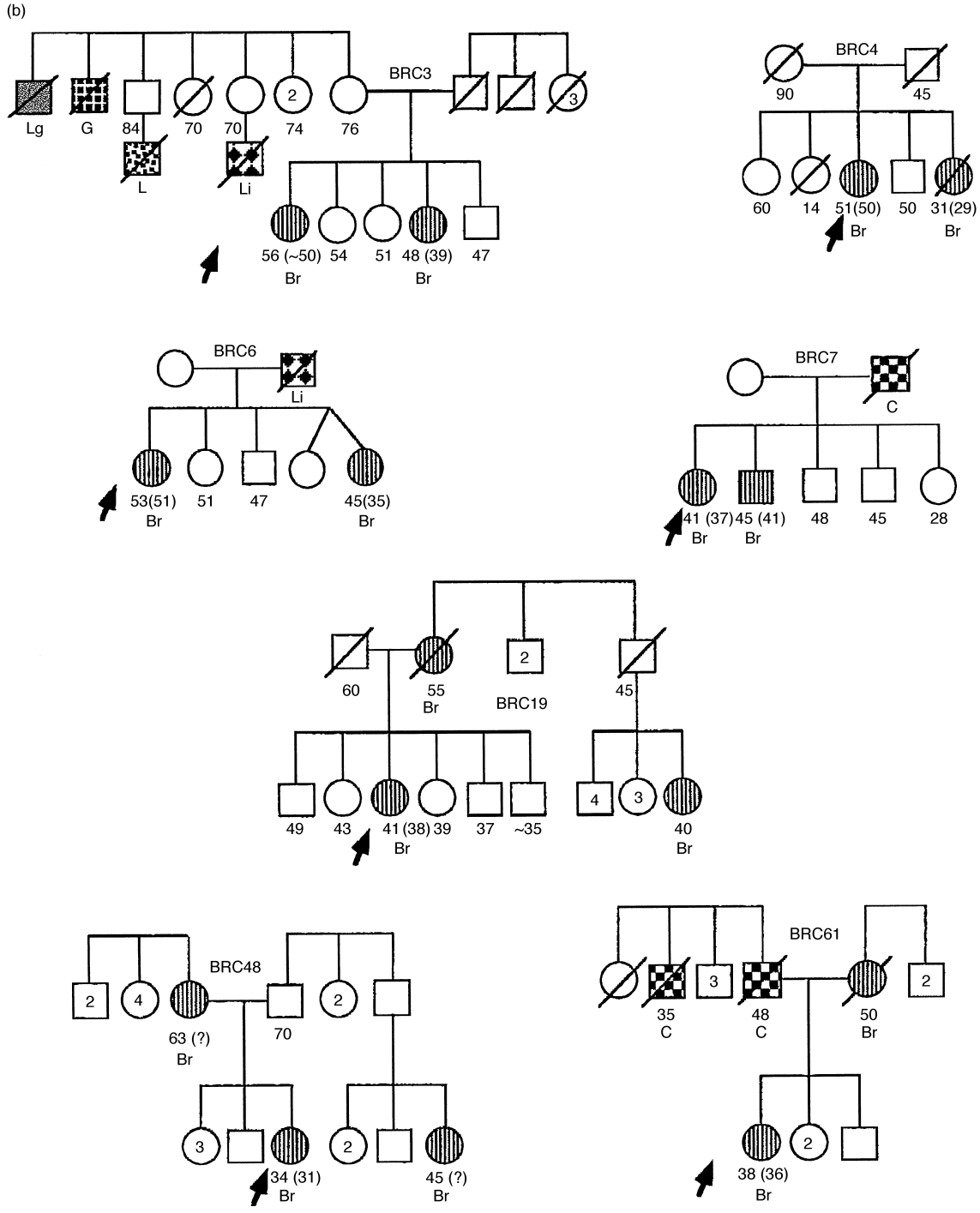

Fig. 1. *(continued)*.

Selected regions of *BRCA1* (exons 2, 5, 11, 13, 20 and 24), and *BRCA2* (exon 11) were subjected to heteroduplex analysis, and altered fragments were further analysed by DNA sequencing. According to the BIC database (http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/index.html), these *BRCA1* and *BRCA2* regions harbour 80% and 45% of the mutations, respectively.

The patients were divided into four groups of hereditary ($n=6$) breast/ovarian cancer, and familial ($n=7$), early onset ($n=27$) or male ($n=10$) breast cancer. The selected regions of both *BRCA1* and *BRCA2* genes were analysed in all four groups.

Nine different sequence alterations (seven for *BRCA1* and two for *BRCA2*) were identified in 25 patients. In *BRCA2*, one previously reported deletion (3034

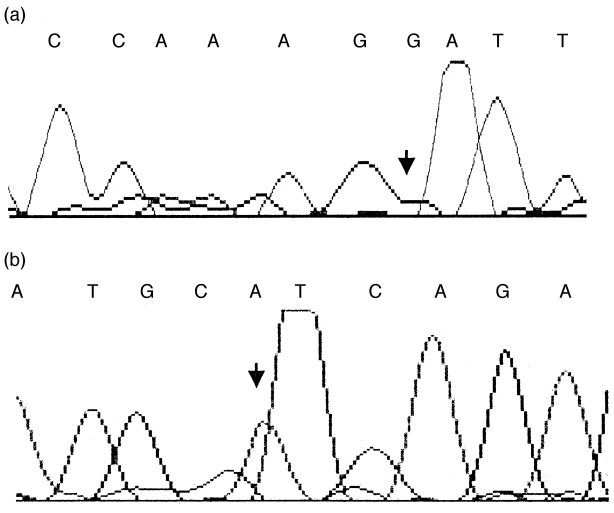

Fig. 2. (a) DNA sequence analysis of familial breast cancer case 96/2 with a novel *BRCA2* mutation (6880 insG). (b) DNA sequence of patient 97/641 a novel *BRCA1* mutation (1200 insA).

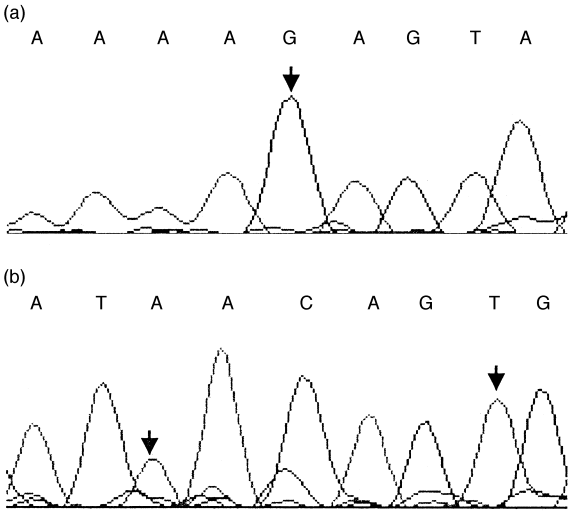

Fig. 3. (a) DNA sequence of patient 97/670 showing the *BRCA1* allele with missense mutation 2080 A→G. (b) DNA sequence of patient 97/670 showing the other *BRCA1* allele with two polymorphisms, 2196 G→A and 2201 C→T. Arrow indicates the mutations.

delAAAC), and one novel insertion (6880 insG) (Fig. 2a) type of frameshift mutations leading to protein truncation were observed. In *BRCA1*, one novel frameshift mutation (1200 insA) (Fig. 2b), one novel missense mutation (2080 A→G; K654E) (Fig. 3a), and one novel silent mutation (1013 T→C; N298N) were identified. In addition, four previously reported *BRCA1* polymorphisms (one silent and three missense mutations) were also found in these Turkish breast cancer patients (see Table 2).

The hereditary group consisted of patients with at least two affected first-degree relatives, and those with bilateral breast cancer plus one affected first-degree relative (Table 1). We identified two *BRCA2* frameshift mutations, 6880 insG (Fig. 2a) and 3034 delAAAC, in two of the six families (33%) in this group. Interestingly no *BRCA1* mutation was found except for a previously reported frequent polymorphism which is an A to G transition at nucleotide 3667 in three (50%) families.

Patients who have one affected first-degree relative also diagnosed with breast cancer constitute the familial group. No frameshift or nonsense *BRCA1* or *BRCA2* mutation was observed. However, previously reported *BRCA1* polymorphisms 3667 A→G in three (43%), and 2731 C→T in 2 (29%) patients were present. In addition, a novel silent *BRCA1* mutation (1013 T→C) was identified in 1 patient.

Women diagnosed as having breast cancer before the age of 35 years, none of whom were selected on the basis of family history status were in the early onset group (Table 3). A novel *BRCA1* frameshift mutation, 1200 insA (Fig. 2b), was identified in 1 patient. In addition, five different *BRCA1* sequence alterations were observed in 12 patients. These alterations include one novel (1013 T→C), and four previously reported polymorphisms (see Table 2). The 3667 A→G polymorphism was observed in 33% (9/27) of patients. Interestingly each allele of *BRCA1* displayed a missense

Table 2
Mutations and polymorphisms identified in turkish breast and breast–ovarian cancer patients

Number of times recorded	Gene	Exon	NT	Codon	Base change	AA change	Designation	Mutation type	Mutation effect
1	*BRCA2*	11	3034	938	delAAAC	Stop 958	3034 delAAAC	F	F
1	*BRCA2*	11	6880	2218	insG	Stop 2224	6880 insG	F	F
1	*BRCA1*	11	1200	361	insA	Stop 368	1201 insA	F	F
1	*BRCA1*	11	2080	654	A to G	Lys to Glu	K654E	M	M
1	*BRCA1*	11	2196	693	G to A	Asp to Asn	D693N	M	P
3	*BRCA1*	11	2731	871	C to T	Pro to Leu	P871L	M	P
16	*BRCA1*	11	3667	1183	A to G	Lys to Arg	K1183R	M	P
2	*BRCA1*	11	1013	298	T to C	Asn to Asn	N298N	P	P
1	*BRCA1*	11	2201	694	C to T	Ser to Ser	S694S	P	P

F, frameshift; M, missense; P, polymorphism; NT, nucleotide; Lys, lysine; Glu, glutamine; Asp, aspartic acid; Pro, proline; Leu, leucine; Arg, arginine; Asn, asparagine; Ser, serine; AA, amino acid.

Table 3
Age of early-onset breast cancer cases

Case	Age (years)	Case	Age (years)
96/5	33	97/656	35
96/10	32	97/661	26
97/114	34	97/662	36
97/270	29	97/670	27
97/343	35	97/674	27
97/344	32	97/681	33
97/359	32	97/682	36
97/472	32	97/683	32
97/473	31	97/684	29
97/508	26	97/703	27
97/632	31	98/12	27
97/639	38	98/17	29
97/641	31	98/18	28
97/655	31		

mutation in 1 patient (Fig. 3a and b): a previously described rare polymorphism (2196 G→A; D693N) and a novel missense mutation (2080 A→G; K654E). This patient also carried a silent mutation (2201 C→T) co-segregating with the 2196 G→A mutation. The phase of these transitions was determined by cloning of the patient's PCR product and sequencing of the multiple clones. This finding establishes that sequence alterations 2080 A→G and 2196 G→A, which lead to amino acid substitutions K654E and D693N, respectively, were independently inherited. Sequence analysis showed that the 2080A→G mutation was absent in 100 independent alleles from a control population. The novel K654E substitution is not in a conserved residue but when the *BRCA1* sequence is subjected to secondary structure prediction programs SOPMA and GOR4 (http://pbil.ibcp.fr/cgi-bin/secpred) this region forms a short alpha helix, carrying four consecutive lysine residues. Even though the Lys to Glu substitution may increase the helix stability, it neutralises the positive charges on the helix, which may have an important role for the structure and function of the protein. Occurrence of two mutant alleles in the same patient with a sporadic early onset malignancy (age 27 years) suggests that these mutations may be involved in the development of breast cancer.

Table 4
Age of male breast cancer cases

Case	Age (years)
97/631	71
97/694	73
97/695	57
97/699	56
98/3	66
98/13	56
98/14	40
98/15	70
98/16	77
98/59	68

Table 5
BRCA1 and *BRCA2* mutation frequencies in different patient groups

Patient groups	*BRCA1*	*BRCA2*
	n (%)	*n* (%)
Hereditary breast and/or ovarian cancer ($n=6$)	0	2 (33)
Familial breast and/or ovarian cancer ($n=7$)	0	0
Early onset breast cancer ($n=27$)	1 (4)	0
Male breast cancer ($n=10$)	0	0

The fourth group we studied was composed of isolated male breast cancer cases. We were unable to detect mutations in the selected regions of *BRCA1* and *BRCA2* genes in this group. The age of the male breast cancer cases is shown in Table 4.

In conclusion, among 50 Turkish breast cancer cases, we detected two (4%) *BRCA2* and one (2%) *BRCA1* disease-causing frameshift mutations. In addition, one (2%) missense *BRCA1* mutation, and five *BRCA1* polymorphisms in 23 patients (46%) were identified.

4. Discussion

A wide variation in the *BRCA1* and *BRCA2* mutation spectrum and frequency has been reported for different populations [6]. The results of our Turkish data are summarised in Table 5. In the hereditary breast cancer group, *BRCA2* mutations accounted for 33% of cases. This frequency is rather high and similar to the Icelandic population. Interestingly, *BRCA1* mutations in our study group appear to be rare — if not exceptional, similar to patients from Iceland, Norway and Japan. However, in a recently published paper, Balci and colleagues reported *BRCA1* mutations in 2 out of 5 hereditary breast cancer cases compared with one *BRCA2* mutation [13]. We did not detect *BRCA1* or *BRCA2* mutations in the familial breast cancer cases. Our *BRCA1* data confirm the observations of Malone and colleagues who identified *BRCA1* mutations in only 7% of cases [7]. To the best of our knowledge, *BRCA2* mutations in this group have not been reported previously. Although we did not screen all exons of *BRCA2* our observations suggest that the *BRCA2* gene is also infrequently involved in familial breast cancer. The results obtained from this study indicate that *BRCA1* and *BRCA2* genes are involved in the development of some, but not all, hereditary breast cancers in the Turkish population as reported for other populations [14,15].

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