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Original Paper

Mutation Analysis of *BRCA1* and *BRCA2* in Turkish Cancer Families: a Novel Mutation *BRCA2* 3414del4 Found in Male Breast Cancer

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Since the identification of the *BRCA1* and *BRCA2* breast–ovarian cancer susceptibility genes, mutation analyses have been carried out in different populations. Here we screened 15 Turkish breast and breast–ovarian cancer families for mutations in both genes by conformation-sensitive gel electrophoresis (CSGE) and the protein truncation test (PTT), followed by DNA sequencing. Three families included a male breast cancer case, one without family history. Three germline mutations were identified, two in *BRCA1* and one in *BRCA2*. The two *BRCA1* mutations, 5382insC and 5622C→T, were found in breast–ovarian cancer families. The *BRCA2* 3414delTCAG is a novel mutation detected in a site-specific breast cancer family that included 1 case of male breast cancer. These first results of Turkish families show that the frequency of germline *BRCA1* or *BRCA2* mutations appears to be high in families with at least 3 breast and/or ovarian cancer cases. © **1999 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

BREAST AND ovarian cancer are the two most common malignancies in women in Turkey [1]. A positive family history is considered one of the most important cancer risk factors. The two cancer susceptibility genes *BRCA1* and *BRCA2* [2, 3] are responsible for approximately 6–10% of all breast and ovarian cancer cases unselected for family history [4]. Twenty-eight per cent of the high-risk breast and 80% of the breast–ovarian cancer families are attributed to *BRCA1*. *BRCA2* is responsible for approximately 37% of inherited site-specific breast and 15% of breast–ovarian cancer [5]. To date, more than 300 different mutations in *BRCA1* and approximately 200 in *BRCA2* have been reported (Breast Cancer Information Core (http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/BiC). A majority of them are frameshift and nonsense mutations causing premature protein truncation.

In males, breast cancer is approximately 100 times less common than in females [6]. Klinefelter's syndrome, androgen insufficiency and a family history of female or male breast cancer are known risk factors [7–9]. Seventy-seven per cent of male breast cancer seems to be associated with mutations in *BRCA2*. In addition, *BRCA1* has been found to account for approximately 19% of cases [5]. A small portion is also explained by germline mutations in the androgen receptor gene (*AR*) [10].

In most populations, there seems to be a wide spectrum of different germline *BRCA1* and *BRCA2* mutations (BIC). However, in some geographically isolated populations, clusterings of specific mutations have been observed. For instance, Ashkenazi Jews show a high prevalence (~2%) of three mutations (*BRCA1* 185delAG, 5382insC and *BRCA2* 1674delT) [11]. 5382insC is one of the most frequently

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reported *BRCA1* mutations and a majority of the families are from northern or eastern Europe [4, 12, 13].

In this study, we screened for *BRCA1* and *BRCA2* germline mutations in Turkish breast and breast–ovarian cancer families using conformation-sensitive gel electrophoresis (CSGE) and the protein truncation test (PTT), followed by DNA sequencing. Three families included a case of male breast cancer. We found mutations in three of the studied families: two in *BRCA1* and one in *BRCA2*. A novel mutation was found in one of the families with male breast cancer.

PATIENTS AND METHODS

Fifteen Turkish breast and breast–ovarian cancer families were analysed. The criteria for inclusion were: 2 or more cases of breast and/or ovarian cancer in first-degree relatives, male breast cancer, or early onset of disease. The phenotypical characteristics of the studied families are described in Table 1. The pedigree data and permission for genetic testing were requested during the counselling session. The diagnoses of index patients were confirmed by pathology reports. Blood samples were collected in Ankara during the years 1997–1998. DNA extraction from blood lymphocytes was performed using the standard phenol–chloroform methods. Exon 11 of *BRCA1* as well as exons 10 and 11 of *BRCA2* were screened for truncating mutations using PTT [14, 15]. The remaining coding regions and adjacent intronic regions of *BRCA1* and *BRCA2* genes were screened by CSGE [16, 17]. DNA sequencing was performed using the Cyclist Exo$^-$ *Pfu* DNA sequencing kit (Stratagene, La Jolla, California, U.S.A.). Additional primers were used for sequencing the samples positive after PTT [18, 19].

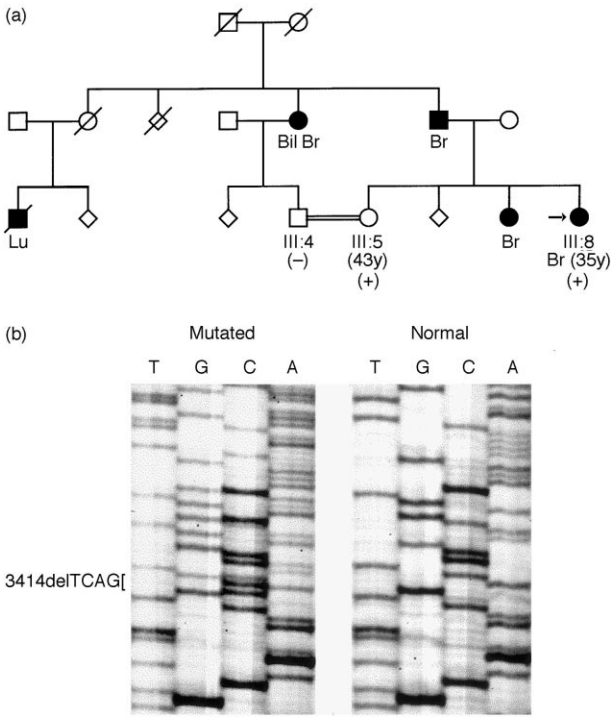

Figure 1. (a) Pedigree of the family carrying the novel *BRCA2* 3414delTCAG mutation. The number in parentheses indicates the age at diagnosis for an affected individual and the age at present for a healthy carrier. Br, breast cancer; Bil Br, bilateral breast cancer; Lu, lung cancer; + , mutation carrier; -, not a carrier; →, the proband. (b) Reverse primer DNA sequence analysis of part of *BRCA2* exon 11, revealing the 3414delTCAG mutation in the affected (III:8), but not in the control individual. Square bracket indicates the mutation site.

RESULTS

Mutation analysis of *BRCA1* and *BRCA2* was performed in 15 Turkish breast and ovarian cancer families. Three distinct disease-related mutations were found (Table 1 and Figure 1). The mutations occurred in the group of families showing the strongest history of cancer. Two of the mutations identified were found in *BRCA1*: 5382insC in exon 20 and 5622C→T in exon 24, both of which were detected by CSGE. 5382insC is a frameshift mutation causing premature protein termination at codon 1829. 5622C→T is a nonsense mutation leading to substitution of arginine to a stop codon at position 1835. One previously unidentified mutation, 3414delTCAG,

Table 1. *Phenotypical characteristics and mutations found in* 15 *Turkish families with breast or breast–ovarian cancer**

Family no.	No. of breast cancer cases (age of onset years)		No. of ovarian cancer cases (age of onset years)	Other cancers (no. of cases)	Mutation
	Female	Male			
1	3† (35, u, u)	1 (u)	–	Lu	*BRCA2* 3414delTCAG
2	3 (27, 42, u)	–	1 (38)	Bt, Lu (6), Os, Sto	*BRCA1* 5622C→T
3	3 (34, 40, 48)	–	–	Pan	–
4	2 (34, 36)	–	1 (40)	Sto (2)	*BRCA1* 5382insC
5	2† (27, 28/35)	–	–	–	–
6	2 (51, 58)	–	–	NHL	–
7	2 (46, 65)	–	–	–	–
8	2 (32, 55)	–	–	Leu	–
9	2 (31, 49)	–	–	Col, Leu	–
10	2 (42, u)	–	–	Csu	–
11	2 (28, 41)	–	–	Csu (2), Lu, Sto	–
12	1 (38)	–	–	Pro	–
13	1 (35)	1 (40)	–	–	–
14	–	1 (78)	–	–	–
15	1 (31)	–	–	–	–

*The mutation carrier status was not defined for all individuals. †One of the cases was bilateral breast cancer. Bt, brain tumour; Col, colon cancer; Csu, cancer site unknown; Leu, leukaemia; Lu, lung cancer; NHL, non-Hodgkin's lymphoma; Os, osteosarcoma; Pan, pancreas cancer; Pro, prostate cancer; Sto, stomach cancer; u, age unknown.

occurred in *BRCA2* exon 11. This mutation was detected by PTT. 3414delTCAG alters the reading frame and causes premature protein termination at codon 1076. In addition, a number of different DNA polymorphisms were found in the coding regions of exons 9 (710C→T), 13 (4427T→C) and 16 (4956A→G, 5075G→A) of *BRCA1* and in adjacent intronic regions (data not shown).

DISCUSSION

In this pilot study of Turkish cancer families, we found previously known *BRCA1* mutations in two breast–ovarian cancer families and one novel *BRCA2* mutation in a site-specific breast cancer family showing a case of male breast cancer. The *BRCA1* 5382insC mutation occurred in a breast–ovarian cancer family (no. 4), in a sister-pair diagnosed with early-onset breast cancer (Table 1). Also, 1 early-onset ovarian and 2 stomach cancer cases were seen in this family. 5382insC is the third most common mutation in Ashkenazi Jews [20] and the most common mutation in Russia [12]. 5382insC is thought to be of Baltic origin and the estimated age of this mutation is 38 generations [4, 21]. Because of the geographical location of Turkey between Russia and Israel, it would be interesting to compare the disease-linked haplotypes.

The *BRCA1* 5622C→T mutation was also found in an affected sister-pair (of family no. 2), one of which had breast and the other ovarian cancer at a young age. Additional malignancies were found in both sides of the family, including one brain tumour, one osteosarcoma, as well as two breast, six lung and one stomach cancer (Table 1). This mutation was initially identified in a breast–ovarian cancer family [22] and has since been reported 11 times in BIC, but detailed information about families and the origin of this mutation is not presently available.

The novel *BRCA2* 3414delTCAG mutation was detected in a high-risk family (no. 1) with 1 male and 3 female breast cancer cases, 1 of which was bilateral (Table 1 and Figure 1). In addition, there was 1 case of lung cancer in this family. This mutation was also screened for in 2 healthy individuals (III:4 and III:5, Figure 1), with consanguineous marriage. Fortunately, only III:5 was found to be a carrier. Although the mutation is located in the ovarian cancer cluster region (OCCR, between nucleotides 3035 and 6629) [23], no ovarian cancer was observed in this family.

As expected, the male breast cancer family no. 1 was found to carry a germline *BRCA2* mutation (Table 1). The result was concordant with the previous study by the Breast Cancer Linkage Consortium, in which 77% of the high-risk families containing at least one male breast cancer was attributed to *BRCA2* [5]. However, one-fifth of male breast cancer cases without any family history has been suggested to associate with germline *BRCA2* mutations [24]. Therefore, a larger series of male breast cancer families would have been needed to test this hypothesis in the Turkish population. Since the other male breast cancer cases did not show any features of androgen insufficiency, mutation analysis of the *AR* gene would probably not reveal any additional mutations. In the remaining 10 families, no additional disease-related *BRCA1* or *BRCA2* mutations were observed. In most of the female breast cancer families, the number of affected individuals was low, and the only inclusion criterion was the early onset of the disease. Therefore, it seems that young age alone is not a very good predictor of finding a *BRCA1* or *BRCA2* mutation.

These preliminary results indicate that the frequency of *BRCA1* and *BRCA2* mutations in Turkey seems to be relatively high in families with a strong history of cancer. To evaluate further the role of *BRCA1* and *BRCA2* mutations in Turkey, a greater number of families should be studied.

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