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BRCA1 and BRCA2 Mutations are they Related to Breast Cancer in a Sample of Tunisian Population?

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Abstract

Mutations in the BRCA1/BRCA2 genes account for varying proportions of breast cancer families studied, and demonstrate considerable variation in mutational spectra coincident with ethnic and geographical diversity. This work aimed to identify mutations in BRCA1 and BRCA2 genes to explore the existence of population-specific recurrent or founder mutations, in Tunisian breast cancer families. We have screened for germline mutations in seventeen Tunisian high-risk breast cancer patients using direct sequencing. Index patients, diagnosed before age 45, possessing a positive family history or bilateral breast cancer were asked for detailed information on family history of breast or any other cancer type in their families. One family out of 17 (6%) carried BRCA 1 mutations and no BRCA2 mutations was found. One recurrent mutation in BRCA 1 was identified, c.798-799delTT, which appear to represent founder mutation in this population. Thirty-one variants were considered of unknown clinical significance according to BIC and UMD-BRCA1/BRCA2 databases.

Keywords: BRCA 1; BRCA2; Breast cancer; Tunisia

Abbreviations: BRCA1: Breast Cancer Susceptibility Gene 1 ; BRCA2: Breast Cancer Susceptibility gene 2; PCR: Polymerase Chain Reaction

Introduction

Breast cancer is one of the most prevalent diseases affecting women [1], it represents a heterogeneous and phenotypically complex disease, with several biological subtypses that present distinct behaviours and responses to therapy [2]. According to the 1999-2003 North Tunisia Cancer Registry, breast cancer is the first female malignancy with a standardised incidence of 29.2/100000 habitants and a mean age of occurrence of 50.7 years with 10.9% of them younger than 35 years [3]. Although the mecanism of breast carcinogenesis is still not fully understood, a variety of risk factors have already been identified, including those directly inducing endogenous and exogenousn DNA damage [4]. Two genes, breast cancer susceptibility gene 1 (BRCA1) and breast cancer susceptibility gene 2 (BRCA2) have been identified as being causative in familial breast cancer [5].

BRCA1 and BRCA2 proteins appear to share a number of functional similarities that may suggest why mutations in these genes lead to specific hereditary predisposition to breast cancer [6]. BRCA genes contribute to DNA repair and transcriptional regulation in response to DNA damage and cell cycle control. Recent studies suggest that BRCA proteins are required for protecting the genome from damage [7].

Deleterious mutations in either of these genes, leading to protein dysfunction, have been established to increase women ' risk for breast cancer across their lifespan (70 years): the meta-analytic mean cumulative breast cancer risk for mutation carriers: of 57% (95% CI, 47% to 66%) for BRCA1 and 49% (95%CI, 40% to 57%) for BRCA2 mutation carriers [8].

Troudi et al. reported that among 36 patients who had at least one first degree relative with breast and/or ovarian cancer, the frequency of germline BRCA mutations in Tunisian familial breast cancer patients is 14% in BRCA1 and 5.5% in BRCA2 [9]. One more recent study by Mahfoudh et al carried on 16 Tunisian high-risk breast cancer families screened for germline mutations in BRCA1 reported that the prevalence of BRCA1 mutation is 37.5 % and suggest that the c.798_799delTT mutation is a Tunisian founder mutation [10].

The frequency and spectrum of mutations within BRCA1/2 genes vary widely among populations. In some ethnic or geographically isolated groups, founder mutations can explain the majority of inherited breast and ovarian cancer cases [11-14]. The analysis of the BRCA1/2 genes conferring increased risk of hereditary breast cancer was poorly studied in Tunisian and North Africa populations. Few reports, reported BRCA1/2

mutations associated with hereditary breast cancers in a small number of families from Middle Eastern and North African countries [9,10,15-19].

The aim of the present work is to identify mutations in BRCA1 and BRCA2 genes to explore the existence of population-specific recurrent or founder mutations, in Tunisian breast cancer families.

Materials and Methods

Patients

Seventeen breast cancer patients, derived from 17 families were selected from patients attending the Department of Medical Oncology at the University Hospital Farhat Hached of Sousse. The study, in accordance with Helsinki Declaration, was reviewed and approved by the local ethics committee and included only individuals that agreed to participate after reading and signing a free and informed consent form. Selected index patients were preferred to be diagnosed with breast cancer before age 45, possessing a positive family history or bilateral breast cancer. Index patients were asked for detailed information on family history of breast or any other cancer type in their families. Blood samples (5 ml each) were collected from the patients (17 women) in EDTA tubes.

DNA Isolation

Genomic DNA was extracted from peripheral blood lymphocytes using the standard salting out procedure [20].

Genotyping

Universal primers (designed by Centre Jean Perrin; sequences available on demand) were used to amplify all regions of the BRCA1 and BRCA2 genes. Genomic DNA was amplified by polymerase chain reaction (PCR) cycling program with an initial denaturation at 95°C for 10 min, followed by 30 cycles of 94°C for 20 s, annealing at specific temperatures for each primer pair and extension at 72°C for 45 s. Twenty-four exons of the BRCA1 gene and 27 exons of the BRCA2 gene were analyzed. The BRCA1 exon 11 was separated into nine fragments, the BRCA2 exon 11 was separated into 13 fragments and exon 10 was separated into three fragments. Reaction products were purified on a Sephadex G50, and then electrophoresed on an agarose gels to 2%. The purified DNA was used in the sequencing reaction (PRISM Ready Kit Reaction, Applied Biosystems) whose product was deposited on a denaturing polyacrylamide gel to 6%. The migration was performed in an automatic sequencer. Sequence analysis was performed using SEQMAN (DNAstar, Madison, WI) and SEQSCAPE V2.5 (Applied Biosystems) software.

Results

In this study, seventeen patients from Tunisian high-risk breast cancer families varying in age of onset disease from 33 to 64 (median age 45.8 years) were analyzed for hereditary breast cancer. Thirteen index cases were diagnosed with site-specific breast cancer and four with bilateral breast cancer. Characteristics of population study and their family antecedents, related to the first, second or third degree, are presented in Table 1.

Table 1: Characteristics of breast cancer patients and relatives.

Patient	Age	First degree relatives n(age)	Second/Third degree relatives n(age)	Comments/other cancers
1	46	0	1(37)	One bilateral BrCa case
2	48	2(49+56)	1 (52)	Colon, uterus
3	33	1(43)	0	
4	38	0	1 (80)	
5	50	0	2 (50+40)	
6	42	1(31)	0	
7	37	0	1 (40)	
8	47	0	1 (37)	
9	54	0	3(43+42+48)	One bilateral BrCa case
10	39	2(33+60)	0	Prostate
11	41	0	1 (30)	
12	40	0	2 (50+70)	Colon
13	57	3(39+61+62)	4 (74+54+58+52)	One bilateral BrCa case, bone, bowel, prostate
14	37	0	2 (60+72)	
15	62	2(66+60)	2 (64+60)	One bilateral BrCa case
16	64	1(35)	0	
17	43	2(39+62)	1 (66)	

BrCa: Breast Cancer

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Mutation analysis of the BRCA1 and BRCA2 was performed in these 17 index cases. A total of 9 variants in the BRCA1 gene and 6 in the BRCA2 gene were identified. An overview of the mutational spectrum is listed in Table 2. Overall, only one deleterious BRCA1germline mutation, was detected in 1/17 (6%) familial cases, c.798-799delTT, a Tunisian founder mutation, this is a small deletion resulting in frameshifts causing premature protein termination at codon 285. The 31 remaining identified variants classified as polymorphisms or unknown variants (Table 2).

Table 2: BRCA1/2 mutations identified in 17 breast cancer families from Tunisia.

Mutation type	Exon	Sequence variant	Effect
BRCA1			
Deleterious			
	11	c.798_799delTT	p.Val266-Ser267ValLys
Sequence variant of unknown significance			
	8	c.536A>G	p.Tyr179Cys
	9	c.548-12dupG	-
	11	c.981A>C	p.Thr327
	11	c.1456T>C	p.Phe486Leu
	11	c.1648A>C	p.Asn550His
	11	c.2733A>G	p.Gly911
	11	c.3024G>A	p.Met1008Ile
	11	c.2082C>T	p.Ser694
	11	c.2077G>A	p.Asp693Asn
	11	c.2 341T>C	-
	11	c.2612C>T	p.Pro871Leu
	11	c.3119A>G	-
	11	c. 3113A>G	-
	11	c.3548A>G	p.Lys1183Arg
	11	c.2521C>T	p.Arg841Trp
	13	c.4308T>C	p.Ser1436
	16	c.4837A>G	p.Ser1613Gly
	23	c.5412C>T	p.Val1804
BRCA2			
Sequence variant of unknown significance			
	10	c.1788T>C	p.Asp596
	10	c.865A>C	p.Asn289His
	10	c.1114C>A	p.Asn372
	10	c.1365A>G	p.Ser455
	11	c.2971A>G	p.Asn991Asp
	11	c.3396A>G	p.Lys1132
	11	c.3807T>C	p.Val1269
	11	c.5744C>T	p.Thr1915Met
	11	c.4747A>G	p.Ile1583Val
	11	c.6100C>T	p.Arg2034Ser
	11	c. 6347A>G	p.His2116Arg
	20	c.8503T>C	p.Ser2835Pro
	27	c.10233A>G	p.Gln3411

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Discussion

In this study, we have identified BRCA1- 2 germline mutations in patients with a positive family history of breast cancer from Tunisia. Prevalence of BRCA1-2 mutations may indeed vary among distinct populations due to concurrence of different environmental factors and genetic backgrounds; in other words, patient's origin may strongly account for different mutation rates in candidate genes.

In the present study, a germline pathogenic mutation in BRCA1 was identified in one (6%) of the familial breast cancer patients. This proportion, although lower than predicted by statistical analysis of a cohort of families collected for the linkage analysis, is in agreement with that observed in other studies. Interestingly, findings reported by Mahfoudh et al. [10] in which this mutation was shared by five patients belonging to three apparently unrelated families and accounted for 18% BRCA1 mutated families and suggests that c.798_799delTT mutation is a Tunisian and North African founder mutation. This mutation was also observed in two Algerian families [19]. It has been cited four times in the BIC database but only once referring to a Spanish origin (Galician). To our knowledge, it has been reported in two breast-ovarian cancer families from northeastern France [21] and also in two breast-ovarian cancer families from southern Italy (Sicily) [22]. Recently, it has been identified in three unrelated families from the middle and south Sardinia area with many Phoenician and Carthaginian archaeological sites [23]. This restricted geographical distribution of the c.798_799delTT mutation in these close Mediterranean countries may suggest a common founder ancestor.

Only one index case, diagnosed with bilateral breast cancer was a carrier of the c.798_799delTT mutation. The selection criterion used in this study, may somehow explain this low prevalence rate, though it is not possible to exclude that a specific genetic background may play a role on breast cancer susceptibility among Tunisian population. Noteworthy, a similar low prevalence of BRCA mutations was reported in the Finnish population which has genetic features comparable to the Tunisian one, where, it may have selected specific genetic variants as susceptibility genes for breast cancer [24].

The identification of this founder mutation in the population is an extremely important step towards the improvement of genetic counselling since molecular testing can be targeted to the founder mutation allowing for a more rapid and less expensive test.

For genetic counseling purposes, it is extremely important to differentiate between deleterious and polymorphic splice-site alterations. Analysis at the mRNA level will allow us to identify whether this variant leads to an aberrant splicing and can contribute to a correct and definitive clinical interpretation. Apart from the deleterious mutation, we have also identified 18 BRCA1 and 13 BRCA2 variants classified as polymorphisms or variants with unknown significance (Table 2). These variants are known to occur worldwide and in populations from various geographical areas.

The proportion of breast cancer families attributable to BRCA1 and BRCA2 mutations in this study is relatively lower when compared to many studies [25-38]. However, the finding of only one of seventeen breast families with BRCA1 mutations is less than has been reported elsewhere. However, due to the small sample used in this study we probably underestimated the actual role of BRCA1 in occurrence of breast cancer in Tunisian women; a study with a much larger effective would verify our results. Additionally, we failed to detect any instances of mutations in BRCA2. These data support the observation that although the overall contribution of BRCA1/BRCA2 to familial breast cancer may be the same among study groups, the distribution of mutations is quite variable with respect to ethnogeographical differences.

Conclusion

Our results demonstrated that prevalence of BRCA1 mutations is 6% and BRCA2 is 0% in our 17 cases of familial breast cancer. Our findings further confirm that mutation frequency for BRCA1 and BRCA2 as cancer susceptibility genes for breast carcinoma needs to be evaluated with a larger sample. The absence of any BRCA2 mutations among the sixteen remaining families with breast cancer supports the hypothesis that selection criterion used in this study is failed. Finally, patients tested for BRCA1 and BRCA2 mutations and found to be negative should, thus, be informed of the potential of mutations in other as yet unidentified breast cancer susceptibility genes contributing to continue increased cancer risk.

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