Research Paper

Mutation Screening of the BRCA1 Gene in Early Onset and Familial Breast/Ovarian Cancer in Moroccan Population

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Abstract

Worldwide variation in the distribution of *BRCA* mutations is well recognised, and for the Moroccan population no comprehensive studies about *BRCA* mutation spectra or frequencies have been published. We therefore performed mutation analysis of the *BRCA1* gene in 121 Moroccan women diagnosed with breast cancer. All cases completed epidemiology and family history questionnaires and provided a DNA sample for *BRCA* testing. Mutation analysis was performed by direct DNA sequencing of all coding exons and flanking intron sequences of the *BRCA1* gene. 31.6 % (6/19) of familial cases and 1 % (1/102) of early-onset sporadic (< 45 years) were found to be associated with *BRCA1* mutations. The pathogenic mutations included two frame-shift mutations (c.798_799delTT, c.1016dupA), one missense mutation (c.5095C>T), and one nonsense mutation (c.4942A>T). The c.798_799delTT mutation was also observed in Algerian and Tunisian BC families, suggesting the first non-Jewish founder mutation to be described in Northern Africa. In addition, ten different unclassified variants were detected in *BRCA1*, none of which were predicted to affect splicing. Most unclassified variants were placed in Align-GVGD classes suggesting neutrality. c.5117G>C involves a highly conserved amino acid suggestive of interfering with function (Align-GVGD class C55), but has been observed in conjunction with a deleterious mutation in a Tunisian family. These findings reflect the genetic heterogeneity of the Moroccan population and are relevant to genetic counselling and clinical management. The role of *BRCA2* in BC is also under study.

Key words: Breast cancer, *BRCA1* mutations, unclassified variants.

Introduction

Breast cancer (BC) is the most prevalent malignancy and primary cause of cancer death in women worldwide, accounting for 23 % of all cancers among women. The incidence of BC is higher in developed countries compared to the developing world, with incidence varying from 19.3 per 100,000 women in

Eastern Africa to 89.7 per 100,000 women in Western Europe. In all, BC accounts for 14.1 % of female cancer deaths and is the second most common cancer overall when both sexes are considered together. Most alarmingly, incidence rates have continued to increase worldwide, with an overall annual increase of approximately 0.5 % since 1990. However, changes in incidence rates are greater in developing countries, attaining annual increases of 3-4 % [1].

The incidence of BC in North Africa (including Morocco, Algeria, Tunisia, Libya and Mauritania) is rising and is rapidly becoming the leading form of cancer in women. Age-standardized incidence per 100,000 for BC was 23.5 and 16.7 in Algeria and Tunisia, respectively, versus 91.9 in France [2]. The size and grade of breast tumors in North Africa are higher, while the median age of onset (48) is more than ten years younger than the European/North American median of 61 [3]. In Morocco, BC has become the most common cancer, accounting 36 % of all cancers [4]. In the urban cancer registries of Casablanca and Rabat [5-7], BC has rapidly overtaken cervical cancer in frequency. The highest cancer incidence rate recorded among women at The Cancer Registry of the Grand Casablanca is BC (ASR per 100,000 for BC and for cervical cancer in 2004 were 35.0 and 13.5, respectively). The average age at diagnosis was 48.1 years (± 11.3). The age specific rates were observed to rise from 35 onwards and reached a peak in the 40 to 54 year age group. The rates decreased in the older age groups. Infiltrating ductal carcinoma was the most frequent, accounting for 70 % of cases [5]. The combination of lower incidence and lower age of onset of BC suggests that the contribution of genetic factors such as mutation of *BRCA1* may contribute to a larger proportion of BC overall.

The detection of *BRCA1* gene alterations makes it possible to recognize subjects who are carriers of germline mutation in this gene and at high risk of developing breast and/or ovarian cancer (BC and/or OC) [8]. Among carriers of *BRCA1* mutations up to 70 years old, 56-80 % and 10–30 % develop BC or OC, respectively. In addition, female carriers of a known *BRCA1* mutation affected by BC show a 40-60 % risk of developing a second breast tumor [9]. *BRCA1* mutations also appear to be associated with a higher risk of developing prostate, colorectal and pancreatic tumors [10].

The spectrum of mutations in the *BRCA* genes varies between populations, with some showing a high frequency of unique mutations [11]. Many such alterations may be recurrent, often being identified in isolated populations as a founder effect [12] and may contribute to differences in cancer risk between populations [13]. Ashkenazi Jewish, Norwegian, Dutch, and Icelandic people have a higher rate of certain genetic alterations in *BRCA1* [14]. However, the contribution of mutations in *BRCA* genes to BC patients in the Moroccan population remains unexplored and few molecular genetic study of *BRCA* status has been reported [15]. In order to investigate the contribution of germline mutations of *BRCA1* to BC in the Moroccan population, we screened 121 BC cases for alterations in all coding exons and splice junctions of *BRCA1* gene.

Materials and methods

Study subjects

Breast cancer cases were identified at the department of Obstetrics and Gynecology of Mohammed V Military Teaching Hospital in Rabat, Morocco. Cases were chosen according to the following criteria: age at diagnosis < 45 years for sporadic cases; two or more first degree relatives with BC and/or OC for familial cases. A total of 121 cases were enrolled between December 2009 and June 2010. Informed consent was obtained from all participants at the time of peripheral blood draw. All cases completed epidemiology and family history questionnaires and provided a DNA sample for *BRCA* testing. Clinical and pathological characteristics including age at diagnosis, mono- or bilateral tumour location, stage of disease, tumour-node-metastasis system status, tumour grading, and estrogen (ER)/progesterone receptor (PR) and HER2-*neu* status were determined from medical records and pathology reports. ER and PR were considered negative if staining of tumor cell nuclei was < 10 %. A HER2 negative result was defined as immunohistochemistry 0 or 1+. Triple negative BC was characterized by the lack of ER, PR, and HER2/*neu*.

Molecular analysis

Genomic DNA was extracted from peripheral blood lymphocytes using a standard phenol-chloroform extraction method. Blood was first digested with lyses buffer I (30 mM Tris, 5 mM EDTA and 50 mM NaCl) and lyses buffer II (20 % SDS, 100 µg/ml Proteinase K) followed by the extraction with Tris saturated phenol and Chloroform-isoamyl alcohol (24:1) and finally recovered by ethanol precipitation. The quantity and the quality of the DNA samples were determined by UV absorbance and agarose electrophoresis.

Mutation analysis was performed by direct DNA sequencing of all coding exons and of each flanking intron of *BRCA1* gene. PCR reactions were carried out

in a volume of 15 µl with 25 ng genomic DNA, 1x reaction buffer, 0.3 mM dNTPs, 1 nM of both forward and reverse primer, and 0.5 units Taq polymerase (primers from Sigma Aldrich, France, and all other reagents from Applied Biosystems, Lifetech, France). PCR was performed in an MWG Bioblock thermocycler with initial denaturation of 94 °C for 2 min, followed by 30 to 35 cycles of (94 °C 20s, 54 °C 20s, 72 °C 20 s), except for exon 7 (15 cycles of 94 °C 20 s, 60 °C 10 s, 72 °C 20 s then 25 cycles of 94 °C 20 s, 56 °C 15 s, 72 °C 20 s), exon 9 (94 °C 20s, 56 °C 20s, 72 °C 20 s), and exon 23 (5 cycles of 94 °C 20 s, 57 °C 20 s, 72 °C 20 s then 30 cycles of 94 °C 20 s, 53 °C 20 s, 72 °C 20 s). Exon 11 was analysed in nine overlapping PCR fragments. PCR products were verified by electrophoresis agarose gel containing Gel Red (Interchim, France, less toxic than ethidium bromide) and visualized by exposure to ultraviolet light. The amplified products were purified by Exo-Sap enzymatic digestion (GE Healthcare., USA), according to the manufacturer's instructions. Sequence reactions were performed on ExoSap-purified PCR products using BigDye.v3.1 reagents (Applied Biosystems, primers available on request) and purified on Sephadex™ G-50 Fine (GE Healthcare). Cycle sequencing consisted of an initial denaturation step at 94 °C for 11 min, followed by 25 cycles of 94 °C for 10 s, 52 °C for 5 s and 70 °C for 3 min. Sequencing was done using a 3130XL capillary electrophoresis system (Applied Biosystems). Alignment to the reference sequences was performed using Seqman software (DNA Star Inc, Madison, WI, USA). All mutations were confirmed on an independent second amplification and a second DNA sample where possible.

All sequence variants were named and are referred to in the manuscript according to the nomenclature used by Human Genome Variation Society (HGVS) (http://www.hgvs.org) recommendation guidelines, using the A of the ATG translation initiation codon as nucleotide +1. Mutations are also provided using the Breast Cancer Information Core (BIC) (http://research.nhgri.nih.gov/bic) nomenclature.

In silico prediction

Potential clinical effect of variants with unknown significance (UVs) was evaluated by analyses of the severity of the amino acid changes and their conservation across species. These analyses were performed using prediction analysis web tools Alignment-Grantham variation Grantham deviation (Align GVGD; http://agvgd.iarc.fr/agvgd_input.php) [16], Polymorphism Phenotyping-2 (Poly-Phen-2; http://genetics.bwh.harvard.edu/pph2/) [17], and Sorting Intolerant From Tolerant (SIFT; http://blocks.fhcrc.org/sift/SIFT.html) [18] scores.

Statistical analysis

Statistical analysis used the chi-squared test, with $P < 0.05$ taken as the threshold for significant difference.

Results

Six deleterious mutations were identified among the 19 cases with positive family history (31.6 %) and one mutation among 102 early-onset sporadic cases (1 %). Most of the carriers of pathogenic mutations are early onset patients (6 out 7 patients, 86 %); the age at early onset is ≤ 45 years. The pathogenic mutations included two frame-shift mutations (c.798_799delTT, c.1016dupA), one missense mutation (c.5095C>T), and one nonsense mutation (c.4942A>T) (Table 1). Ten missense variants of UV and other rare polymorphisms were also identified; none were predicted to affect splicing.

The c.798_799delTT (p.Ser267LysfsX19) mutation, located in exon 11, was identified in two unrelated cases (3371-01A and 3432-01A). Both carriers showed a family history of BC. The index case 3371-01A and one sister presented bilateral BC below the age of 40. The c.798_799delTT is frame-shift mutation including two small deletions, two bases (TT) deletion, that cause truncated protein signal at codon 285.

The c.1016dupA (p.Val340LysfsX6) mutation was detected in two unrelated cases (3430-01B, 3450-01A), both with strong family history suggestive of genetic predisposition to BC and/or OC. The two families had different phenotypes: one contained four BC cases but no OC, and the other three OC and two BC cases. The family history of the two cases is consistent with this mutation being highly penetrant for both BC and OC. The index case 3450-01BA also presented one variant of unknown significance. The c.1016dupA is a frame-shift mutation due to the insertion of an A at nucleotide acid 1135 of codon 340 in exon 11, which is predicted to lead to a premature stop codon 345 and a truncated protein.

The c.5095C>T (p.Arg1699Trp) mutation, located in exon 18, was identified in two unrelated cases, both with strong family history of cancer. The mutation leads to a non-conservative change from arginine to tryptophan at the highly conserved position 1699 in the N-terminal BRCT domain of the *BRCA1* gene.

The nonsense mutation c.4942A>T (p.Lys1648X), located in exon 16, was found in an early onset case without family history of BC and/or OC. The c.4942A>T mutation was an adenine for thymine substitution on nucleotide 4942 and leading to prem-

ature stop codon in position 1648.

Additionally, *BRCA1* UVs were identified in 13 (10.7 %) out of 121 analyzed cases. Altogether, ten missense UVs were observed, 8 of them in cases with positive family history of BC (Table 2). The most frequently detected UV was c.5117G>C (p.Gly1706Ala), found in four cases. The other UVs were observed only once and one, c.1941T>G (p.Ser647Arg), occurred in a case with a deleterious mutation (c.1016dupA). Align-GVGD categorized 1/10 (10 %) as C55, 0/10 as C65, C45, C35 or C25, and 9/10 (90 %) as C0. The functional effects of these ten UVs were also predicted using the bioinformatics tools Poly-Phen-2 and SIFT (Table 2).

The mean age at diagnosis of BC was 44 ± 1.9 years (95 % CI, 43.5 - 44.5) in *BRCA1* carriers, and 42.2 ± 5.1 years (95 % CI, 41.8 – 42.5) in those without del-

eterious mutations (p > 0.05). BC cases ranged from 23 to 59 years and 94 (79 %) were diagnosed before the age of 45. There were 86 (70 %) patients with unilateral BC and 5 (4.1 %) patients with bilateral BC. A family history of BC and/or OC was recorded in 19 cases, and 12 (63.2 %) had a first degree relative diagnosed with BC and/or OC. Most cases were diagnosed with infiltrating ductal carcinoma. Scarff-Bloom-Richardson grade II and III were predominant (41 % and 44 % of cases, respectively). Axillary lymph nodes contained metastasis (N+) in 26 (21 %) cases. *BRCA1* mutations were significantly associated with ER negativity (p < 0.001) and PR negativity (p < 0.001) and HER2 negativity (p < 0.001). *BRCA1* mutations were more frequent in triple-negative BC compare to non-triple-negative breast tumors (p < 0.001).

Table 1: Clinico-pathological features of Moroccan BC cases with deleterious *BRCA1* germline mutations

case	age at diagnosis	exon	genetic variant	consequence	familial or sporadic	muta-tion type	histology	pathologic stage	ER	PR	HER	menopau-sal status
3371-01A	44	11	c.798_799delTT	p.Ser267LysfsX19	familial	FS	IDC	III	_	_	_	pre
3432-01A	40	11	c.798_799delTT	p.Ser267LysfsX19	familial	FS	IDC	III	_	_	_	pre
3430-01B	44	11	c.1016dupA	p.Val340LysfsX6	familial	FS	IDC	III	_	_	_	pre
3450-01A	46	11	c.1016dupA	p.Lys1698X	familial	FS	IDC	II	_	_	_	post
3393-01A	45	16	c.4942A>T	p.Lys1648X	sporadic	NS	bifocal IDC	II	_	_	_	pre
4051-01A	44	18	c.5095C>T	p.Arg1699Trp	familial	MS	IDC	III	_	_	_	pre
4051-01A	45	18	c.5095C>T	p.Arg1699Trp	familial	MS	IDC, N+	IV	_	_	_	pre

FS: frameshift, NS: nonsense, MS: missense, IDC: invasive ductal carcinoma, N+: lymph node metastasis.

Table 2: Predicted effect of unclassified missense variants of *BRCA1*

genetic variant	consequence	GV	GD	Align-GVGD class	Polyphen	SIFT
c.196A>T	p.Asn66Tyr	163.23	25.33	C0	0.996 (probably damaging)	0.04 (not tolerated)
c.666A>T	p.Gln222Asn	272.33	0	C0	0.651 (possibly damaging)	0.22 (tolerated)
c.1417A>T	p.Asn473Tyr	134.97	35	C0	0.979 (probably damaging)	0.07 (tolerated)
c.1941T>G	p.Ser647Arg	95.08	28.37	C0	0.991 (probably damaging)	0.09 (tolerated)
c.2251A>C	p.Met751Leu	240.36	0	C0	0.00 (benign)	1.00 (tolerated)
c.2869C>A	p.Gln957Lys	241.77	26.66	C0	0.883 (possibly damaging)	1.00 (tolerated)
c.2925A>T	p.Gln975H	261.51	0	C0	0.489 (possibly damaging)	0.05 (tolerated)
c.3115G>T	p.Ala1039Ser	235.38	0	C0	0.074 (benign)	0.60 (tolerated)
c.4776C>A	p.Asn1592Lys	231.18	34.45	C0	0.00 (benign)	0.31 (tolerated)
c.5117G>C	p.Gly1706Ala	0	60	C55	-	0.00 (not tolerated)

Align-GVGD was used to further assess the functional effect of missense UVs, with alignment to 13 *BRCA1* and 12 *BRCA2* ortholog sequences down to sea urchin (http://agvgd.iarc.fr/alignments.php). Align-GVGD, Align Grantham Variation Grantham Deviation; GV: Grantham Variation score, GD: Grantham Deviation score, PolyPhen, Polymorphism Phenotyping; SIFT, Sorting Intolerant from Tolerant.

Discussion

Although genetic linkage analysis suggest that the prevalence of *BRCA* gene mutation in familial BC and/or OC is about 45–90 % [19,20], the frequency of *BRCA1* mutation in familial breast cancer varies from 1 to 35 % worldwide [21,22]. Although Miki et al. [23] had reported about 62 %, the sample size was extremely low. In the present, *BRCA1* mutations were identified in 31.6 % (6/19) of cases with familial BC. This is in contrast 10.3 % of French hereditary BC and/or OC families exhibiting a *BRCA1* mutation. The frequency of *BRCA1* mutations among Algerian families was 36.4 % (4/11) [24]. The prevalence of *BRCA1* mutations reported in a recent study conducted on Tunisian families (37.5 %) is approximately 2.5 times higher than that reported initially (15.6 %) [25].

Changes in the reproductive behaviours of women from North Africa were closely related to the women's educational level and socio-economic status. Urbanisation and technological evolution play a part in these changes. Changes in living standards and lifestyles have affected age at first pregnancy and numbers of children produced. Penetrance of *BRCA* mutations may be modified by other risk or protective genes or environmental factors, most notably reproductive history and diet. The effect of lifestyle on this penetrance is significant, as studies of western populations show that carriers born after 1940 have much higher BC incidence and earlier onset than carriers born before 1940 [26].

Single cases are not generally accepted for genetic testing for hereditary BC genes without a strong implication of hereditary factors, such as young age at diagnosis (< 35 years), multifocal or bilateral tumors, and/or medullar histology. In most western populations such testing is not cost-effective, with only 2.6 % of 2-case families in Finland being positive for a *BRCA* mutation [27], and very few sporadic cases being positive in the US. Other studies, however, suggest that testing of 2-case families or single cases before the age of 36 can be efficient in certain populations [28,29]. This study showed a lower prevalence of *BRCA1* mutations (1 %) in BC cases diagnosed before the age of 45 and unselected for family history of the disease. In Algerians, 9.8 % (5/51) of early-onset sporadic cases (< 38 years) was found to be associated with *BRCA1* mutations [24]. At least two explanations may contribute to this observation: the reported family histories of BC patients enrolled in this study may not have been accurate, especially in terms of paternal familial history. This could have resulted in the misclassification of familial cases as sporadic cases, which would confuse estimations of the prevalence of germline mutations in sporadic cancer. Furthermore, a different population structure in Algeria, with a relatively low incidence of BC revealing the greater contribution of genetic factors [24].

Deleterious mutations in the BRCA1 gene in Morocco

Recently, Tazzite et al [15] selected 40 patients from 39 breast and/or ovarian cancer families who were diagnosed with invasive breast or epithelial ovarian cancer. Heredity forms involve four *BRCA1* mutations (c.798_799delTT, c.3279delC/3398delC, c.2805delA/2924delA, c.5062–5064delGTT) in families with site-specific BC, and one (c.181T>G) in a breast and ovarian cancer family. In addition, 22 *BRCA1* variants including distinct polymorphisms and UVs were identified [15]. In our study, we identified six deleterious mutations among 19 cases with positive family history and one mutation among 102 early-onset sporadic cases. The pathogenic mutations included c.798_799delTT, c.1016dupA c.5095C>T among familial cases, and c.4942A>T mutations sporadic cases.

The c.798_799delTT mutation was reported in Algerian, Tunisian and Moroccan families [24,30,31,15], suggesting the first non-Jewish founder mutation to be described in Northern Africa. This frame-shift mutation is cited twice in the BIC database, without any ethnic origin indicated. Microsatellite markers in and flanking the *BRCA1* locus has shown a common haplotype associated with this mutation in all carriers of both Algerian cases [24]. None of the founder mutations previously observed among Middle Eastern (Iranian) or Jewish populations were found.

The c.1016dupA mutation has previously been described as one of four founder mutations originating from the Eastern population of Norway [32]. In contrast to other Norwegian founder mutations, the c.1016dupA has also been reported in other ethnic groups. In the BIC database, the c.1016dupA mutation is the 12th most common frameshift mutation occurring in *BRCA1*. It has been reported to occur in populations throughout Europe including Spain, Norway, the Netherlands, Austria, and Italy, as well as in Latin America and North America; however, allelotyping results indicated an independent origin of this mutation. The c.1016dupA mutation is a duplication of one A in a series of seven. This poly-A region may thus be a hot-spot for replication errors.

Our *BRCA* patients carrying c.5095C>T mutation had a strong family history of BC and/or OC. This variant has been described previously in BC families

[33-35] and is classified as clinically significant in the BIC database. The amino acid substitution leads to a folding defect in the BRCT domain and reduces the proteolytic stability of this domain that interacts with numerous proteins involved in transcription and DNA repair [36]. Functional data and co-segregation strongly suggest that the c.5095C>T mutation has a deleterious effect and predisposes carriers to BC and OC [36-39]. The c.5095C>T mutation thus can explain some of the BC cases in the Moroccan population.

UVs

We identified ten different *BRCA1* UVs. All UVs were missense substitutions and the most were placed in A-GVGD classes that favour neutrality. It is likely that the majority of these sequence variants have no clinical relevance, and the few that may be deleterious are unlikely to change the basic conclusions of this study. For example, we identified c.314A>G (p.Lys105Lys) in early-onset sporadic cases without family history of BC and/or OC. This variant suggested by Align-GVGD as candidate risk variant have been reported in BIC as of no clinical interest primarily based on co-occurrence in trans with deleterious mutations and lack of segregation with disease in families. The c.314A>G was also classified as neutral in the likelihood-ratio model developed by Easton et al. [40]. The *in silico* evidence for c.5117G>C is somewhat stronger, but there is still no co-segregation shown. The c.5117G>C mutation that occurred at highly conserved (GV = 0) was defined as interfering with function (A-GVGD class C55), but has been observed in conjunction with a deleterious mutation in a Tunisian family. The co-occurrence of this variant with a deleterious mutation in one Tunisian family, however, suggests that it is neutral. Interpretation of UVs still remains problematic. Prediction of the effect of UVs in functional analysis studies and by prediction software may generate controversial results. Evaluation of missense UVs should consist of the combination of several approaches based on, but not limited to co-segregation analysis of UVs with disease in a family, loss of heterozygosity in tumours, determination of the frequency of variant in unaffected controls, *in silico* prediction analysis, or functional assays [40].

Sequence variants in the BRCA1 gene in North Africa

The prevalence and spectrum of *BRCA1* mutations in North African BC and/or OC families have not yet been thoroughly studied. In Algeria, Uhrhammer et al [24] conducted a study of both sporadic cases less than 38 years of age, and familial cas-

es. DNA sequencing revealed five deleterious mutations among 51 early-onset sporadic cases, and four mutations among 11 families. Two non-conservative missense variants, c.425C>A and c.4072G>A, and an intronic variant, c.5467-10C>A, were observed in three sporadic cases, though their clinical significance is unknown. Cherbal et al [30] described analysis of *BRCA1* gene in 86 individuals from 70 families from an Algerian cohort with a personal and family history suggestive of genetic predisposition to BC. All samples for which no pathogenic mutation was found were analyzed by MLPA for large deletions or duplications. Three distinct pathogenic mutations c.83_84delTG, c.181T>G, c.798_799delTT and two large rearrangements were detected as well as 17 UVs and polymorphisms.

Two recent studies have investigated *BRCA* gene mutations in BC patients with affected relatives in Tunisia, Fifty familial cases revealed eight mutations in *BRCA1*, including c.211dupA, c.4041delAG, c.2551delG, c.798_799delTT, c.3331_3334delCAAG, c.212 + 2insG and c.5266dupC [25,31]. In addition, 13 distinct UVs were also identified. The c.798_799delTT mutation was the most commonly observed mutation in Northern Africa population. It occurred in ten unrelated family cases. The remaining mutations occurred at low frequency, and some were previously described in other populations. Thus, the cumulative mutation analysis showed that the *BRCA1* mutation spectrum is rather broad in Northern Africa (Table 3).

Overall, these preliminary data suggest that the spectrum of *BRCA1* mutations in North Africa may be large, as would be expected for extended, outbreed populations. Our results concur that family history is an important selection criterion for the identification of *BRCA1* mutation carriers. Interestingly, the major burden of BC in Morocco is due to early onset BC while it is mainly a post-menopausal disease in western population. Our findings are in agreement with previous studies which indicate that the frequency of *BRCA1* mutations among BC patients decreases as the age of cancer onset increases. This may be entirely explained by the age structure of the population. There are simply far fewer women in their 60's relative to western populations. Thus the later-onset cases are largely missing, leaving possibly the same incidence of young cases, which thus account for a higher proportion. Furthermore, *BRCA1*-associated carcinomas have been reported to have typical characteristics in that they are more frequently of the ductal invasive type, present a poorly differentiated tumor, and are ER/PR negative. In accordance with these data, all of these characteristics were also significantly more frequent in our study population.

Our study had several limitations; the major one is that we have not been able to study the role of the *BRCA2* gene. Effectively, mutations occurring in the *BRCA*2 gene may be the cause of a number of BC in our population, and we hope to investigate their role in the future. In addition, we did not evaluated large rearrangements in *BRCA1*which can be the origin of several BC cases as reported in Caucasian populations [41].

Table 3: *BRCA1* deleterious mutations in North Africa

genetic variant	consequence	age at diagnosis	familial or sporadic	BC or OC	Reference
Algerian population					
c.46_74del29	p.Asn16fs	29	sporadic	BC	24
c.46_74del29	p.Asn16fs	37+44	familial	BC	24
c.83_84delTG	p.Arg28fs	26	sporadic	BC	24
c.83_84delTG	p.Leu28Argfsx1	47	familial	BC	30
c.181T>G	p.Cys61Gly	36	familial	BC	30
c.181T>G	p.Cys61Gly	44	familial	BC	30
c.202+1G>A	Splice donor	38	familial	BC	24
_	exon 5	42	_	BC	24
c.798_799delTT	p.Val266fs	43	familial	BC	24
c.798_799delTT	p.Val266fs	32	familial	BC	24
c.798_799delTT	p.Ser267LysfsX19	33	familial	BC	30
c.798_799delTT	p.Ser267LysfsX19	30	familial	BC	30
c.798_799delTT	p.Ser267LysfsX19	n.i	familial	BC	30
c.1817delC	p.Pro606fs	37	sporadic	BC	24
c.2745dupT	p.Ser915fs	36	sporadic	BC	24
c.3715delT	p.Ser1239fs	36	sporadic	BC	24
Tunisian population					
c.211dupA	p.Arg71LysfsX80	54	familial	BOC	25
c.211dupA	p.Arg71LysfsX80	47	familial	BC	25
c.212+2insG	IVS5+2insG	n.i	familial	BC	31
c.798_799delTT	p.Ser267LysfsX19	38	familial	BC	31
c.798_799delTT	p.Ser267LysfsX19	38	familial	BC	31
c.798_799delTT	p.Ser267LysfsX19	43	familial	BC	31
c.2551delG	p.Glu851Asnfs41	45	familial	BC	25
c.3331_3334delCAAG	3450delCAAG	n.i	familial	BC	31
c.4041delAG	p.Gly1348AsnfsX6	65	familial	BOC	25
c.5266dupC	p.Asp1757LysfsX70	50	familial	BOC	25
c.5266dupC	p.Asp1757LysfsX70	n.i	familial	BC	31

FS: frameshift, NS: nonsense, MS: missense, n.i: no information

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Competing interests

The authors have declared that no competing interest exists.

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