

Research Paper

BRCA1 mutations in Algerian breast cancer patients: high frequency in young, sporadic cases

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Breast cancer rates and median age of onset differ between Western Europe and North Africa. In Western populations, 5 to 10 % of breast cancer cases can be attributed to major genetic factors such as *BRCA1* and *BRCA2*, while this attribution is not yet well defined among Africans. To help determine the contribution of *BRCA1* mutations to breast cancer in a North African population, we analysed genomic DNA from breast cancer cases ascertained in Algiers.

Both familial cases (at least three breast cancers in the same familial branch, or two with one bilateral or diagnosed before age 40) and sporadic cases less than 38 years of age were studied. Complete sequencing plus quantitative analysis of the *BRCA1* gene was performed. 9.8 % (5/51) of early-onset sporadic and 36.4 % (4/11) of familial cases were found to be associated with *BRCA1* mutations. This is in contrast 10.3 % of French HBOC families exhibiting a *BRCA1* mutation. One mutation, c.798_799delTT, was observed in two Algerian families and in two families from Tunisia, suggesting a North African founder allele. Algerian non-*BRCA1* tumors were of significantly higher grade than French non-BRCA tumors, and the age at diagnosis for Algerian familial cases was much younger than that for French non-BRCA familial cases. In conclusion, we observed a much higher frequency of *BRCA1* mutations among young breast cancer patients than observed in Europe, suggesting biological differences and that the inclusion criteria for analysis in Western Europe may not be applicable for the Northern African population.

Key words: breast cancer, familial cancer syndromes, BRCA1 mutation

Introduction

BRCA1 mutations are responsible for a significant proportion of hereditary breast and ovarian cancer (HBOC) families. *BRCA1* is responsible for more than 50 % of HBOC families with at least four cancer cases [1], and of ~15 % of families overall. In Western populations, an inherited mutation of this gene confers a lifetime risk of breast cancer of up to 80 %, with up to 40 % of carriers developing breast cancer by the age of 50 [2]. Penetrance may be modified by other risk or protective genes or environmental factors, most notably reproductive history and diet. The effect of lifestyle on penetrance of BRCA mutations is significant, as studies of western populations show that carriers born after 1940 have much higher breast

cancer incidence and earlier onset than carriers born before 1940 [3].

Studies of breast cancer in the Maghreb (including Morocco, Algeria, Tunisia, Lybia and Mauritania) have shown striking differences in breast cancer patterns. Age-standardized incidence per 100,000 for breast cancer in 2002 was 23.5 in Algeria versus 91.9 in France [4]. The size and grade of breast tumors in the Maghreb are increased, while the median age of onset (48) is more than ten years younger than the European/North American median of 61 [5]. About 11 % of breast cancer cases in Algeria occur in women \leq 35 years old, and 55 % of cases at \leq 50 years. These differences may be due to differences in exposure to female hormones, diet, physical activity, or other factors.

The combination of lower incidence and lower age of onset of breast cancer in the Maghreb suggests that the contribution of genetic factors such as mutation of *BRCA1* may contribute to a larger proportion of breast cancer overall. We therefore set out to determine the contribution of mutations in the *BRCA1* gene to breast cancer in Algeria. This was a pilot study to determine inclusion criteria for more widespread molecular diagnostic analysis of at-risk women in the Maghreb. We used the same criteria to define 'familial' cases that we use in our molecular diagnostic laboratory in France, and then added early-onset sporadic cases. All exons and splice-junctions of *BRCA1* were amplified and sequenced. The MLPA method was used to detect larger deletions and duplications of the gene.

Methods

Breast cancer cases were identified at the Pierre and Marie Curie Hospital in Algiers and chosen according to the following criteria: age at diagnosis \leq 38 years for sporadic cases; two or more first degree relatives with breast or ovarian cancer for familial cases. No families with ovarian cancer were ascertained. Approval was obtained from local institutional review boards, and each patient gave written informed consent. Peripheral blood was drawn from 51 early onset sporadic cases (average age at diagnosis 31.5 ± 4.5 , range 15 to 38) and 13 cases from 11 breast cancer families (average age at diagnosis 37.2 ± 6.8 , range 28 to 52).

DNA was extracted from 0.2 ml of peripheral blood using the QIAamp DNA Blood Mini kit (Qiagen, Courtaboeuf, France). All exons and \geq 50 bp of each flanking intron were amplified in 15 μ l with 50ng DNA, 1x reaction buffer, 0.3 mM dNTPs, 1 nM primers, and 0.5 units Taq polymerase (primers from MWG Biotech, Ebersberg, Germany; all other reagents from Applied Biosystems, Courtaboeuf, France). Sequences available on request. PCR was performed in an MWG Bioblock thermocycler with initial denaturation at 94°C for 2min, followed by 30 to 35 cycles of (94°C 20s, 54°C 20s, 72°C 20s), except for exons 7 (15 cycles of 94°C 20s, 60°C 10s, 72°C 20s then 25 cycles of 94°C 20s, 56°C 15s, 72°C 20s) and 23 (5 cycles of 94°C 20s, 57°C 20s, 72°C 20s then 30 cycles of 94°C 20s, 53°C 20s, 72°C 20s). Exon 11 was analysed in nine overlapping PCR fragments. PCR products were purified by membrane retention (Multiscreen PCR, Millipore, Molsheim, France) and resuspended in 25 μ l of water; 3 μ l was then sequenced in a total of 8 μ l using 1 nM primer and 3 μ l of Big Dye v3 reagents (Applied Biosystems, Courtaboeuf, France), purified over sephadex (Amersham Biosciences, Orsay,

France), 10 μ l of deionized formamide (Applied Biosystems, Courtaboeuf, France) added, and then resolved on a 3100 sequencer (Applied Biosystems, Courtaboeuf, France).

Sequences were compared to the *BRCA1* genomic and cDNA reference sequences (Accession N°s L78833.1 and U14680 respectively) using Seqman software (Lasergene, Madison WI, USA). All mutations were confirmed on an independent second amplification and a second DNA sample where possible. Nucleotide numbering of all mutations and polymorphisms is in reference to the coding sequence, with the A of the initiating ATG = 1.

Samples for which no point mutation was found were analysed by MLPA for large deletions or duplications according to the manufacturer's protocol (MRC Holland) (protocol available on request).

Haplotypes were determined at the following loci: D17S1321, D17S855, D17S1322, D17S1323, and D17S1327 (references and primer sequences NCBI) using PCR with fluorescent forward primers and analysis with Genescan software (Applied Biosystems, Courtaboeuf, France) as well as at biallelic polymorphisms in the coding sequence.

Statistical analysis used the chi-squared test, with $p \leq 0.05$ taken as the threshold for significant difference.

Results

Five deleterious mutations among the 51 early-onset sporadic cases were observed, and four mutations among the 11 families (Table 1). Two non-conservative missense variants, c.425C>A (p.Pro142His) and c.4072G>A (p.Gly1358Lys), and an intronic transversion with weak potential to affect splicing of exon 24, c.5467-10C>A (IVS23-10C>A), were observed in three sporadic cases: it is not currently known if these are deleterious mutations or rare polymorphisms. Other rare polymorphisms or conservative missense variants of unknown effect were also observed in some sporadic cases; none were predicted to affect splicing.

The c.798_799delTT mutation observed in families 1351 and 1612 was also observed in two families from Tunisia (data not shown). Microsatellite markers in and flanking the *BRCA1* locus showed a common haplotype in all c.798_799delTT carriers.

Complete sequencing also provided data on snps in the coding sequence, allowing the construction of haplotypes. For a core of nine snps, 18 different haplotypes were observed for the 128 chromosomes studied (Table 2). The most common, observed 74 times, corresponded to the major canonical haplotype, H1, found by Judkins et al [6]. Additional snps

dividing this canonical haplotype into three others were not informative in our study. Two other haplotypes described in that study of a North American / European population were also observed, H7 and H10, as well as a total of four copies of three haplotypes described in the Tunisian population [7]. The remaining 46 Algerian chromosomes carried 12 different haplotypes not described in either previous study; one of these was the second most frequent haplotype observed, at 18 copies. Six haplotypes were limited to single homozygous individuals. Quantitative analysis of BRCA1 exons did not suggest any large deletions that could confound homozygosity with hemizyosity.

There was no significant difference in the average age of mutated sporadic cases (32.8 ± 5.0 years) versus non-mutated sporadic cases (31.3 ± 4.4 years), nor between familial mutated vs non-mutated cases ($38.4 \pm$

4.8 vs 35.7 ± 8.9 years, respectively). Algerian familial cases, regardless of BRCA status, were similar to French BRCA1 cases (37.2 ± 6.3 vs 41.2 ± 10.4 ; $p = 0.19$), younger than French BRCA2 cases (47.5 ± 14.5 ; $p = 0.015$) and much younger than French non-BRCA familial cases (50.7 ± 12 , $p = 0.00017$).

Tumor characteristics were compared between BRCA1 heterozygotes and non-heterozygotes in the two populations (Table 3). All tumors of medullary histology were observed in BRCA1 heterozygotes. BRCA1 tumors tended to be of higher grade in both populations, as expected; however, Algerian non-BRCA1 cases included a significant excess of high grade tumors ($p < 0.001$). Significantly more Algerian non-BRCA1 tumors were ER-negative and node-positive, compared to French non-BRCA1 tumors ($p < 0.001$ and $p < 0.002$, respectively), consistent with their higher grade tumors.

Table 1. Characteristics of patients with BRCA1 mutations or unclassified variants.

Case	mutation	effect	Sporadic or familial	Age at diagnosis	histology	size	grade	ER	PR	nodes	Age at menarche	parity	nursing	BMI
1357-01	c.46_74del29	p.Asn16fs	Sporadic	29	Papillary	4 cm	III	-	-	n.i	14	0	0	21.3
1490-01	c.46_74del29	p.Asn16fs	Familial	37 + 44	atypical medullar, atypical ductal	3 cm	--	n.i.	n.i.	0/1	n.i.	n.i.	n.i.	34.2
1358-01	c.83_84delTG	p.Arg28fs	Sporadic	26	Poorly differentiated ductal	1 cm	II	-	-	0/11	12	0	0	20.3
1497-01	c.202+1G>A	Splice donor	Familial	38	Atypical infiltrating ductal	9 cm	III	+	+	1/14	n.i.	n.i.	n.i.	n.i.
1497-02		exon 5		42	polymorphic infiltrating ductal	3.5 cm	III	-	-	3/10	15	6	54 m	22.7
1612-01	c.798_799delTT	p.Val266fs	Familial	43	polymorphic infiltrating ductal	0.7 cm	II	n.i.	n.i.	0/12	15	3	72 m	21.8
1351-01	c.798_799delTT	p.Val266fs	Familial	32	infiltrating ductal	6 cm	II	n.i.	n.i.	11/20	12	0	0	21.6
1351-02				33	n.i.									
1370-01	c.1817delC	p.Pro606fs	Sporadic	37	atypical infiltrating ductal	4 cm	III	n.i.	-	4/11	12	0	0	26.0
1614-01	c.2745dupT	p.Ser915fs	Sporadic	36	Sarcomatoid carcinoma	5 cm	III	n.i.	-	1/10	12	6	36 m	
1470-01	c.3715delT	p.Ser1239fs	Sporadic	36	Infiltrating	3.5 cm	II	n.i.	n.i.	0/18	12	3	72 m	22.6
Samples with unclassified variants that may be involved in breast cancer														
1620-01	c.425C>A	Pro142His	Sporadic	26	infiltrating ductal	1.5 cm	III	n.i.	+	14/18	14	0	0	23.9
1355-01	c.4072G>A	Glu1358Lys	Sporadic	35	infiltrating ductal + in situ	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
1468-01	Ivs23-10C>A	**	Sporadic	28	Atypical ductal	3 cm	III	n.i.	n.i.	1/1	12	0	0	20.9
Samples with unclassified variants that are not likely to be involved in breast cancer														
1476-01	c.981A>G	Thr327Thr	Sporadic	34	Polymorphic ductal	0.3	II	n.i.	+	1/21	12	0	0	28.6
	c.2733G>A	Gly911Gly												
	c.3024G>A	Met1008Ile												
1494-01	c.981A>G	Thr327Thr	Sporadic	37	infiltrating galactophoric	7 cm	II	n.i.	n.i.	0/1	13	2	0	21.0
	c.2733A>G	Gly911Gly												
	c.3024G>A	Met1008Ile												
1493-01	c.4883T>C	Met1628Thr	Sporadic	38 (?)	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
1488-01	c.4956G>A	Met1652Ile	Sporadic	26	infiltrating ductal	2.5 cm	II	+	-	pos	n.i.	n.i.	n.i.	n.i.
1480-01	c.5117G>C	Gly1706Ala	Sporadic	36	infiltrating ductal + in situ	2 cm	n.i.	n.i.	n.i.	1/10	n.i.	n.i.	n.i.	n.i.
1610-01	c.5117G>C	Gly1706Ala	Familial	29	infiltrating ductal	5 cm	III	-	+	3/26	13	2	6 m	27.3
1362-01	c.5175A>G	Glu1725Glu	Sporadic	32	Micro-infiltrating ductal	5.5 cm	II	n.i.	n.i.	0/13	12	3	16 m	22.8

n.i. no information, ** slight potential to splice exon 24 eight nucleotides early (listed as an unclassified variant in the BIC by Myriad).

Table 2. BRCA1 Haplotypes among Algerian breast cancer patients

Snpl\Haplotype	H1	H7	H10	T2	T4	T17	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
c.2077A	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0
c.2082T	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
c.2311C	0	0	0	0	0	0	0	1	1	1	1	1	0	1	1	1	0	1
c.2521C	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
c.3113G	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	1
c.3119G	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
c.3548G	0	0	0	0	1	0	1	1	1	0	1	1	1	1	1	1	0	1
c.4308C	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0
c.4837G	0	0	0	1	0	1	0	0	1	0	0	1	0	1	0	0	1	0
Times appearing	74	4	2	1	2	1	2	2	2	2	5	18	2	7	1	1	1	1
% of 128 alleles	57.8	3.1	1.5	0.8	1.5	0.8	1.5	1.5	1.5	1.5	3.9	14	1.5	5.5	0.8	0.8	0.8	0.8
					*		*	*	*	*			*					

H1, H7 and H10 are described by Judkins et al [6]; T2, T4 and T17 correspond to haplotypes described by Troudi et al [7]; A1 through A12 were unique to the Algerian population. 0 indicates the nucleotide corresponding to the reference sequence; 1 to the variant nucleotide. Asterisk indicates a haplotype unique to a homozygous individual.

Table 3. Comparison of tumor characteristics from mutated and non-mutated cases, from Algeria and France.

	BRCA1, Algiers	Non-BRCA1, Algiers	BRCA1, France	Non-BRCA, France*
Age at diagnosis	36.1 ± 5.5**	31.7 ± 5.4***	41.2 ± 10.4	50.7 ± 12.2
Grade 1	1 of 10 (10 %)	1 of 43 (2 %)	2 of 39 (5 %)	32 of 115 (28 %)
Grade 2	3 of 10 (30 %)	25 of 43 (58 %)	10 of 39 (26 %)	67 of 115 (58 %)
Grade 3	6 of 10 (60 %)	17 of 43 (40 %)	27 of 39 (69 %)	16 of 115 (14 %)
Size	3.8 cm	3.4 cm	2.0 cm	2.2 cm
Medullary histology	1 of 12	0 of 48	6 of 49	0 of 139
ER, pos/tested	1/5 (20 %)	3/15 (20 %)	5/14 (36 %)	45/63 (71 %)
PR, pos/tested	2/6 (33 %)	18/32 (56 %)	4/14 (29 %)	35/64 (55 %)
Node positive	6 of 10 (60 %)	29 of 42 (69 %)	13 of 31 (42 %)	41 of 102 (40 %)

* non-BRCA cases were found to be negative for mutations in both BRCA1 and BRCA2; ** includes 5 cases selected for age ≤ 38 years; *** includes 46/52 cases selected for age ≤ 38 years.

Discussion

The types of *BRCA1* mutations in this sample of the Algerian population were typical of those observed elsewhere, with six different deletions or duplications involving one to 29 nucleotides, and a novel change in the donor splice site of exon 5. Two nonconservative substitutions of amino acids were observed, as well as an intronic transversion with some potential to affect splicing of exon 24, possibly representing novel mutations in this population. Several uncommon silent or conservative sequence variants were also observed.

We observed one deleterious mutation, c.798_799delTT, in two Algerian families and also in two Tunisian breast cancer families (data not shown), suggesting the first non-Jewish founder mutation to be described in Northern Africa. This mutation is cited twice in the BIC database, without any ethnic origin indicated. Analysis of five microsatellite markers showed a common haplotype associated with this mutation in all our known carriers. None of the founder mutations previously observed among middle eastern (Iranian) or Jewish populations were found.

Haplotype analysis revealed the genetic diversity of the Algerian population. A large study of North Americans and European revealed 10 canonical haplotypes clustered around two major haplotypes

both diverged from a common ancestor [6]. Analysis of the Tunisian population revealed several new haplotypes, in concordance with the great age of this population [7]. In keeping with this, the Algerian population also exhibited several unique haplotypes as well as three in common with the Tunisian population. Three haplotypes could be considered 'common', accounting for 58, 14 and 5.5 % of observed chromosomes. All the Algerian haplotypes appeared to be derived from the major H1 chromosome described by both Judkins and Troudi; none appeared related to the other major haplotype, H2. Interestingly, several rare haplotypes occurred as homozygotes. Although we have no information on the precise geographic or tribal origin of the families, we speculate that this may reflect the insular nature of rural Algeria, where the coefficient of inbreeding is relatively high and genetic drift may establish unique regional haplotypes. No homozygosity for unclassified variants was observed.

The age at which familial Algerian cases, regardless of *BRCA1* status, developed breast cancer was similar to our *BRCA1*-positive French families, but significantly younger than French familial non-*BRCA* or *BRCA2* cases. Young age at diagnosis is an indication for referral for BRCA testing, and the older age at cancer in BRCA-negative families is common.

That familial cases from the Maghreb without *BRCA1* mutation resemble *BRCA1* families may be related to the lower age of onset and higher frequency of high grade tumors overall for breast cancer in this population.

The characteristics of *BRCA1*-related tumors were similar between Algerian and French patients, allowing for larger tumor size probably associated with later diagnosis of Algerian cases. In contrast, the non-*BRCA1* tumors from Algeria were also of significantly higher grade, presented more positive nodes and were less frequently ER-positive than French non-*BRCA* tumors. This excess of high-grade tumors in African populations has been described before, with 65 to 86 % of tumors being grade II or III [8-10]. Frequently positive nodes and negative hormone receptor status are both consistent with high-grade tumors. Low- and high-grade breast cancers may represent separate pathways of oncogenesis [11], thus the absence of low grade tumors is not explained by delay in diagnosis allowing 'progression' to a higher grade. The marked difference in distribution of breast tumor grades between Western and Middle-Eastern/African societies merits further study. One possibility is that low-grade tumors either arise infrequently or do arise but don't develop into palpable tumors initiating medical care. This relative absence of low-grade tumors may contribute to the lower incidence of breast cancer overall in African countries. It may also reflect an ascertainment bias: Western societies have instituted widespread screening programs detecting small low-grade tumors that may go undeclared in developing societies. Two arguments for the biological basis of this difference have been proposed. First, migrants from low-incidence countries gradually take on some of the risk of breast cancer of their host countries, and their descendants have a risk of breast cancer corresponding to the host country [12, 13], arguing for environmental and lifestyle factors in the difference in incidence. On the other hand, studies in the United States have shown that breast cancer in African-American women is associated with higher grade and poorer prognosis, arguing a biological difference even after socio-economic differences are controlled, and reflecting breast cancer statistics for sub-Saharan Africa [14].

Environmental and lifestyle factors may be largely responsible for the low incidence of breast cancer in the Maghreb. These factors are difficult to identify precisely, but their combined effect has serious consequences, as the clear increase in breast cancer incidence in American Ashkenazi *BRCA* carriers born after vs before 1940 shows [3].

Protective reproductive factors tend to diminish as societies become "westernized" and women delay and limit their families. The protective effect of pregnancy is associated with younger age at first pregnancy as well as with increasing parity, while longer breastfeeding has an independent protective effect [15, 16]. Parity levels are converging for Europe and the Maghreb, with 6.49 children per woman in Algeria and 1.87 in France in 1980-1985, but 2.53 in Algeria and 1.87 in France in 2000-2005 (<http://www.un.org/esa/population/ordering.htm>). In the present study, parity was lower among women from Algeria (1.84 ± 2.04) than from France (2.18 ± 1.52), probably because many of the women in our study were of childbearing age and had not completed their families (whereas there are many more older carriers in our french families).

It thus seems that a major protective factor in the Algerian population is rapidly disappearing; the reduction in parity is likely accompanied by increased age at first pregnancy and reduced duration of breastfeeding. This change in lifestyle may soon be reflected in increased breast cancer incidence as this cohort of women reaches the age at which breast cancer is most prevalent. Other lifestyle factors that may have contributed to breast cancer risk in western populations for multiple generations now but which have only more recently begun to affect the Maghreb include the use of oral contraceptives, less physical activity, increased use of refined foods and chemical food additives, and decreased intake of fresh fruits and vegetables.

Single cases are not generally accepted for genetic testing for hereditary breast cancer 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 [17], and very few sporadic cases being positive in the US. Other studies, however, suggest that testing of 2-case families or single cases before age 36 can be efficient in certain populations [18, 19]. The 9.8 % *BRCA1* mutation frequency we observed in young sporadic cases in Algeria is remarkable in comparison to these other populations. At least two explanations may contribute to this observation: the misclassification of familial cases, and a different population structure in Algeria, with a relatively low incidence of breast cancer revealing the greater contribution of genetic factors.

Although our sporadic cases did not signal any family history of breast or ovarian cancer, the stigma attached to cancer in this society makes it is possible

that they were not aware of a positive history. Our discussions with familial cases showed that women with breast cancer often hid this diagnosis from their close relatives. At this time, the medical structures in place, such as cancer registries, are not sufficient to ascertain family history other than by asking the index case.

The second hypothesis, that of the relatively greater contribution of genetic factors in a population where the overall incidence of breast cancer is low, would suggest a greater proportion of familial vs sporadic cases. Although we have not yet performed a population-based study to determine this ratio, the high frequency of *BRCA1* mutation in isolated cases may indicate that this is the case, especially if the penetrance of *BRCA1* mutations is lower in this population. The protective lifestyle factors discussed above may have spared the relatives of our young isolated carriers from breast cancer in spite of their carrier status. Thus the western *BRCA1* model where most mutations manifest in familial aggregations of breast and/or ovarian cancer with penetrance for breast cancer of 50 % by age 50, may be expressed differently in the Maghreb, where mutations are found in familial cancer but also in a significant proportion of isolated cases, penetrance is reduced. It remains to be seen whether recent changes in lifestyle will increase the incidence of breast cancer in carrier families.

In conclusion, our findings suggest that the norms of accepting breast cancer cases for BRCA analysis must be adapted to the population. We are extending our study to additional cases and families from Algeria, and hope to soon be able to compare these results with our analyses of the Tunisian, Lebanese and Moroccan populations. The role of *BRCA2* in breast cancer in the Maghreb is also under study.

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Conflict of Interest

The authors have declared that no conflict of interest exists.

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