

RESEARCH ARTICLE

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BRCA1/BRCA2 Mutations Shaped by Ancient Consanguinity Practice in Southern Mediterranean Populations

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Abstract

The aim of this study is to investigate the involvement of consanguinity on BRCA1/2 mutation incidence in Southern Mediterranean populations and to confirm their low penetrance by comparison of their recurrence in sporadic and familial breast cancer in a context of ancient consanguinity practice. Our study comprises of two parts: First, a comparison of the consanguinity rates of the South Mediterranean countries in a relationship with the frequency of BRCA1 deleterious mutations in breast cancer families and the recurrence of these mutations. Second, we investigated 23 patients with a family history of breast cancer, 51 patients without a family history of breast cancer using next-generation sequencing of BRCA2 and then confirmed by Sanger sequencing for the novel mutation. As results, we clearly show a strong relationship between the frequency of BRCA1 deleterious mutations in breast cancer families and rate of consanguinity, since they are significantly inversely correlated. Four deleterious mutations were found in BRCA2 gene including a novel frame-shift mutation c.9382_9383dup in a patient with familial breast cancer and three other frame-shift mutations c.6591_6592del, c.1310_1313del and c.7654dup in patients with sporadic breast cancer. These results are discussed in a context of selective pressure of ancient consanguinity practice. In conclusion, the study of BRCA1/2 gene in Southern Mediterranean countries revealed low penetrance recurrent mutations in sporadic and familial breast cancer. These mutations have been selected in a context of ancient consanguinity practice along with protective genetic and environmental factors.

Keywords: Breast cancer- BRCA2/1 Mutation analysis- consanguinity- recurrence- penetrance

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Introduction

Breast cancer is the most frequent cancer in women around the world. The incidence of the disease is high in industrial countries (about 90 new cases per 100,000) and intermediate in developing countries (around 30 new cases per 100,000). For example, in Tunisia, the incidence of the disease is 27 new cases per 100,000. Basically, breast cancer is classified into two forms: A sporadic form that represents 90 to 95% and a familial form which represents 5 to 10% of breast cancers diagnosed in women (Andres et al., 2014).

The presence at the germinal level of deleterious mutations to the major breast cancer predisposition genes BRCA1 and BRCA2 are shown in familial breast cancer (Aghmesheh et al., 2015). Both BRCA1 and BRCA2 are considered as tumor suppressor genes, involved in the maintenance of genome stability and double-strand DNA repair, in association with the RAD51 recombinase which

functions in DNA repair.

In Occidental populations, the families with breast cancer present multiple cases and association to ovarian cancer is often observed. Contribution of BRCA1 and BRCA2 to familial forms of the breast cancer conducted by the "Breast Cancer Linkage Consortium" (BCLC) and studies on families with multiple breast and ovarian cancers cases showed that 80% are due to mutations in BRCA1, 15% in BRCA2 and 5% in minor predisposition genes (Ford et al., 1998). In the case of deleterious mutations BRCA1 and/or BRCA2, the risk of breast cancer is 10 times higher, that of ovarian cancer is 40 times higher compared to the general population (Kessenich et al., 2014). Thus compared to the 8% risk that a woman in the general population has to develop a breast cancer during her lifetime, this cumulative risk would be 56 to 87% in the presence of a BRCA1 or BRCA2 mutation (Shannon and Chittenden, 2012). The cumulative risk of developing ovarian cancer

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(very rare in the general population) would be 44 to 63% with a BRCA1 mutation. BRCA2 is most often responsible for cases of breast cancer family where men are affected (77%) (Wooster et al., 1994; Ford et al., 1998).

In North Africa, families with breast cancer do not display more than 4 or 5 members with the disease and association with ovarian cancer is not frequent: 30% among Tunisian breast cancer families according to Troudi et al. 2007 (Troudi et al., 2007) while most families (64%) present only female breast cancer cases. According to results obtained by Troudi et al. 2007, in Tunisian population, the rate of BRCA1 and BRCA2 deleterious mutations in families with breast cancer is low as compared to occidental families. This could be in agreement with a modest number of patients in the Tunisian breast cancer families. Indeed, it has been shown in European populations that the proportion of families for which a mutation on the BRCA1 gene was found, was much lower in the families of modest size. In such families with 4 to 5 cases of breast cancer without ovarian presentation, the contribution of these two genes is much lower: 32% for BRCA1, BRCA2 9% and 59% for the other genes (Ford et al., 1998).

On 164 Tunisian families analyzed, only 16% have a deleterious mutation in the BRCA1 gene. On nine mutations described in population four are recurrent, indicating a founder effect in Tunisia or in North Africa. These four recurrent mutations explain 81% of BRCA1-related families whose two (c.211dupA and c.5266dupC) very frequent (Troudi et al., 2008; Mahfoudh et al., 2012; Riahi et al., 2015). Tunisian families analyzed for BRCA2, around 6% display a deleterious mutation in BRCA2: eight frame-shift changes with a clear deleterious effect, among which three mutations are recurrent (c.1310_1313del, c.1313dupT* and c.7654dupT) and explain 90% of families BRCA2-related (Riahi et al., 2015).

In North African countries (Algeria, Morocco, and Tunisia) pathogenic mutations were detected in BRCA1 gene in 17.43% (34/195) of analyzed breast/ovarian cancer families and in BRCA2 in 7.53% (11/146) breast cancer families. One common mutation c.798_799delTT was identified in the BRCA1 gene and a common BRCA2 pathogenic mutation c.7235_7236insG was detected in Algerian and Moroccan families (Cherbal et al., 2012).

In a previous study, we have shown that consanguinity is protective against breast cancer (Medimegh et al., 2015). Consanguinity is an ancient social practice in some region of the world in particular in the Southern Mediterranean and Middle Eastern countries. As already known, consanguineous marriages are responsible for increasing rates of recessive genetic diseases. However, according to the number of consanguineous unions in these countries, this rate of patients with such pathologies is lesser than expected. This is due to selection against deleterious mutations. Indeed, a significant number of recessive genetic diseases are lethal; which does not allow their carriers to give descendent, leading to counter-selection of such mutations in populations with the ancient and strong practice of consanguinity. It has already been shown that BRCA1/BRCA2 deleterious mutations are lethal at homozygous status. Hence, all patients with

BRCA1/BRCA2 germ line mutation are heterozygous, and according to Knudson (Knudson, 1993), a second mutation or alteration happens at somatic level leading to inactivation of both alleles in the tumor.

We have revealed in our previous study that BRCA1 gene SNP haplotypes also are upon a strong effect of consanguinity (Medimegh et al., 2015). In the present paper, our aim is to investigate the role of consanguinity on the counter selection of BRCA1 and BRCA2 deleterious mutations in Southern Mediterranean populations, relating epidemiologic features that characterize breast cancer in North African populations including its effect on penetrance and recurrence of these mutations.

Our study will comprise of

First, an analysis comparing the level of consanguinity of the South Mediterranean countries which have a relationship with the frequency of BRCA1 deleterious mutations in breast cancer families and the recurrence of these mutations.

Second, it includes new results on BRCA2 mutations and UV in sporadic and familial breast cancer in Tunisia. Results of the sequence of BRCA2 at germ line level, in affected women with familial/sporadic breast cancer, are discussed according to the recurrence and the penetrance of deleterious mutations in a context of ancient consanguinity practice, from the point of view of genetics population.

Materials and Methods

Population and data involved in the BRCA1 analysis

In order to confront independent data established on one hand for BRCA1 mutations and on the other hand for consanguinity rates, we have selected two papers that themselves present comparative results between the same Southern Mediterranean countries: Morocco, Algeria, Tunisia, Egypt, and Lebanon. For BRCA1 mutations we focused on families who are positive of such mutations, among those analyzed according to (Laraqui et al., 2015). For comparison with European and Occidental population, we referred to (Ford et al., 1998).

For consanguinity, the rate varies according to the group studied in a given country and in articles the minimum and maximum values are given in each population. We have used the maximum values according to (Bener and Mohammad, 2017).

Patients and selection criteria

The genetic study involved 79 patients with breast cancer, among whom 51 are sporadic cases, 23 have the familial form of breast cancer and 5 are undefined cases. All of them were women, recruited from Salah Azaiz Institute in Tunis, thanks to specialized physicians. We also selected a control population composed of 75 healthy females with no cancer from the Rabta hospital in Tunis.

The Medical and Chemotherapy departments are responsible for providing the clinical record for each patient that contains the clinical and pathological data.

Some data lacking in the files are requested directly from the patient during a questionnaire about her personal and family history of breast cancer for the genetic survey,

a way of life of the patient. . . .

Based on these data, a familial pedigree is established and the patient classified into sporadic or familial breast cancer group.

For each patient, we have established a specific code and files are treated anonymously. All studies were conducted with the informed consent of the patients.

DNA purification and PCR amplification

All blood samples (5ml) were collected with an anticoagulant (EDTA). The samples are centrifuged at 4000 rpm for 10 min at 4 ° C and the cell pellet is frozen at -20 ° C for further use.

The extraction of the genomic DNA from the peripheral blood was performed by the phenol-chloroform method and the concentration and purity of DNA were determined by Nanodrop.

Two BRCA2 regions corresponding to a novel mutation or containing the c.-26G>A SNP were PCR amplified, each in a total reaction volume of 50 µl containing 10 µl (10*) buffer, 2µldNTPs, 1 µl of each primer, 5 µl of genomic DNA (100 ng) and 1 U of Taq polymerase. The PCR cycling program comprised an initial denaturation at 94°C for 10 min, followed by 30 cycles including one minute at 94°C, annealing at specific temperatures for each primer pair and extension at 72°C for 10 min. The sizes of the amplicons were 213 pb and 154 pb respectively. PCR products were verified on agarose gel visualized with ethidium bromide.

The primers used to screen for the novel mutation were as follows:

F1 5' TTCTAGGACTTGCCCCTTTTCG 3'
 R1 5' AGTGGCCCTCTTTTGGACTA 3'

The primers used to screen for the SNP were as follows:

F2 5' GAATGCATCCCTGTGTAAGTGC 3'
 R2 5' ATACCTGCTTTGTTGCAGCGT 3'

Sequencing

Sequencing of the entire BRCA2 gene breast cancer cases regardless of their status was performed using the

next sequencing generation NGS (sequences designed by Centre Jean Perrin are available on demand). Sanger sequencing was performed to confirm a novel mutation detected by NGS to analyze c.-26G>A SNP already described. Regions of BRCA2 containing the studied novel mutation and SNP were PCR amplified. After the verification of the amplification product by electrophoresis on agarose gel, amplicons are sequenced using direct sequencing according to the manufacturer's instructions.

Bioinformatics and statistics

BRCA2 mutations detected in our cohort were searched in Clinvar database. Pathogenic prediction of novel SNV mutations was performed according to HCI (Breast Cancer Genes Prior Probabilities).

Allelic and genotypic frequencies in cases and controls were compared, using a statistical test method (Epi Info 7). P values were considered statistically significant if it's less than 0.05.

Pearson correlation has been established according to SPSS statistics 19.

Results

The rate of Breast cancer families with BRCA1 germ line deleterious mutations decreases with consanguinity

We investigated the relationship between consanguinity rate in Southern Mediterranean countries and the rate of families with BRCA1 germ line deleterious mutations. According to (Bener and Mohammad, 2017), the maximum rates are given in Table 1.

The consanguinity rate seems inversely correlated with that of families with BRCA1 germ line deleterious mutations (Figure 1), with Person correlation test. The correlation coefficient was - 0,813 with p= 0,049. This correlation is significant. One has to notice that consanguinity rates are very low in Occidental societies (less than 9%) and this fact seems associated to the high percentage of families with breast cancer that displays deleterious mutation in BRCA1 gene. This percentage usually is over 50% compared to 12 % in Southern Mediterranean countries taken as a whole. Among these

Table1. Percentage of Families with BRCA1 Deleterious Mutation among Breast Cancer Families among Countries and According to Consanguinity Rate

Countries	Egypt	Lebanon	Algeria	Tunisia	Morocco	Europe North America
Number of studied families with Breast cancer	90 families	72 families	150 families	98 families	53 families	237 families
Number of studied families with BRCA1 mutations	5 families	5 families	15 families	19 families	12 families	123 families
% of families with BRCA1 mutations	5,5	7	10	19	22,6	52
Consanguinity rate(MAX)	70**	48**	34**	29**	20**	9*

** Bener and Mohammad, 2017

Table 2. Recurrent BRCA1 Mutations in North African Populations

Country	Egypt	Tunisia	Algeria	Morocco
Number of recurrent mutations	3/5	4/8	4/8	5/8
% of of recurrent mutations	60%	50%	50%	62.5%

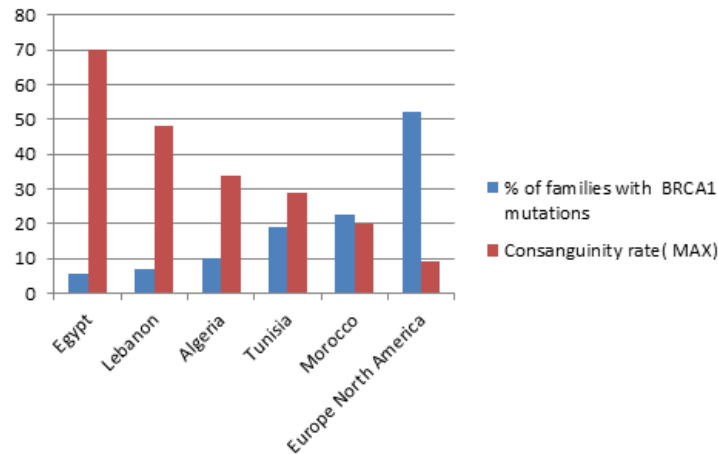


Figure 1. Percentage of Families with BRCA1 Mutations Increases as Consanguinity Rate Decreases in Populations

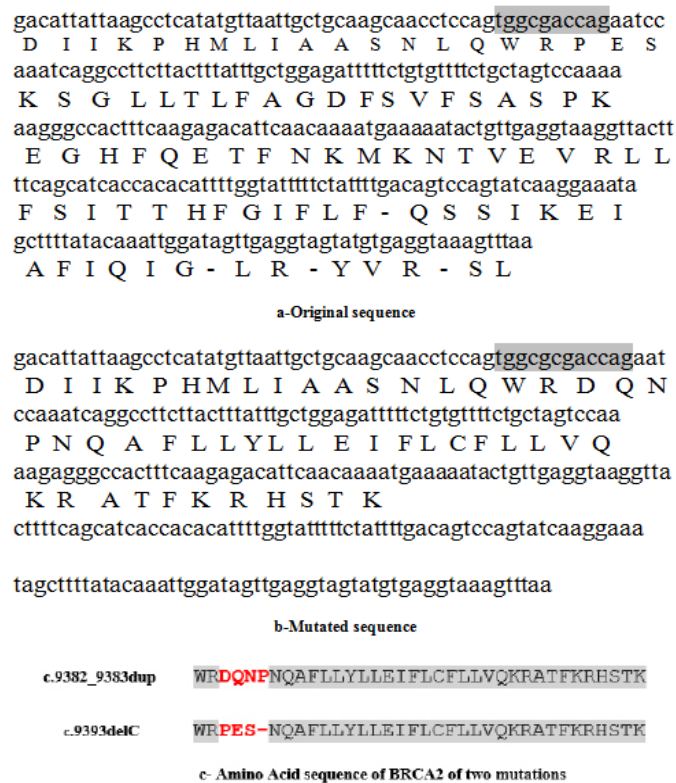


Figure 2. Amino Acid Sequence of BRCA2 Exon 25, a- sequence Encoded by Wild Type Allele. b- sequence encoded by mutated allele with a duplication CG (c.9382_9383dup) leading to a p.Pro3129Aspfs*35 change c- comparaison showing homology between two deleterious mutation leading to a truncated protein with the same position of the premature stop codon and the same 30 last AA.

countries, two main groups could be considered: that with very high consanguinity rates (48 to 70%) and very low frequency of deleterious mutation in BRCA1 gene (6% of breast cancer families are positive for such mutations) in Eastern Mediterranean and that of Maghreb region with intermediate level of consanguinity (20 to 34%) and 10 to 22% of breast cancer families with BRCA1 deleterious mutation. Taken together, these observations are in agreement with selective pressure of consanguinity against BRCA1 deleterious mutations.

Table 3. BRCA1 Mutation Detected in Young Sporadic Cases in Algeria

Mutation brca1	BIC	CLINVAR
c.46_74 del29*	ND	ND
c.83_84 delTG	YES	YES
c.1817 delC	ND	YES
c.2745 dupT	ND	YES
c.3715 delT	ND	YES

*Also described in Algerian Breast cancer family; ND, not determined; BIC, Breast Cancer Information Core.

Recurrence and penetrance of BRCA1 mutations in

Table 4. BRCA2 Deleterious Mutations

Patient	Gene	Exon	Sequence variant	BIC traditional nomenclature	Amino acid change	class
CK5	BRCA2	ex11_16	c.6591_6592del	6819delTG	p.Glu2198Asnfs*4	3
CK27	BRCA2	ex10_02	c.1310_1313del	1537_1540delAAAAG	p.Lys437Ilefs*22	5
CK90	BRCA2	ex16	c.7654dup	7882dupA	p.Ile2552Asnfs*2	5
CK123	BRCA2	ex25	c.9382_9383dup	-	p.Pro3129Aspfs*35	5

BIC database, Breast Cancer Information Core database

Table 5. Clinical–pathological Characteristics in Breast Cancer Patients with BRCA Deleterious Mutations

Patient	Age at diagnosis (years)	Cancer type	Histology	Histological grade	HER2NEU	ER status	PR status
CK5	46	SBC	IDC	II	+	-	-
CK27	60	SBC	IDC	II	+	+	-
CK90	56	SBC	IDC	II	+	+	-
CK123	46	FBC	IDC	II	+	+	+

SBC, Sporadic breast cancer; FBC, Familial breast cancer; IDC, Infiltrative ductal carcinoma; ER, estrogen receptor; PR, progesterone receptor

Southern Mediterranean populations

We also exploited the results obtained by the same study in order to determine the rate of recurrent mutations in each North African population (S1). As observed in Table 2, at least 50% to 62.5% of BRCA1 mutations described are recurrent in the population and/or be shared by other populations.

Since recurrent mutations shared by several families and populations, could be considered as ancient mutations, one has to ask the reason for maintaining a high tradition of consanguinity. In such conditions, it could be proposed that this might be related to low penetrance of such BRCA1 mutations. In order to confirm this hypothesis, we searched for the description of BRCA1 deleterious mutations in Breast cancer sporadic cases. A study realized on Algerian population clearly shows that 9.8 % (5/51) of sporadic cases less than 38 years of age displayed BRCA1 deleterious mutations (Uhrhammer et al., 2008). We searched among the mutations described in this study, which are recurrent or unique. From the results of Table 3, all the sporadic BRCA1 described in this study are recurrent mutations since already mentioned in BIC

and or CLINVAR databases and also associated to familial breast cancer, such as the case of the c.46_74del29* which is reported in a study performed in Algeria both in familial and sporadic cases.

The deleterious BRCA2 mutation identified in sporadic and familial cases in a Tunisian cohort

In order to confirm the aspects of recurrence and penetrance observed for BRCA1 mutations in a context of consanguinity, we focused on a comparison between sporadic and familial breast cancer cases without selection for age. Our cohort of the study consisted of 79 Tunisian patients with breast cancer among whom 23 were of familial type versus 51 sporadic cases, along with 5 unclassified cases. Our program of screening BRCA2 gene mutations used the next generation sequencing. Our results revealed four carriers of deleterious mutations at heterozygous state (Table 4), with a global rate of detection of 5%. All the carrier patients presented IDC and histologic grade II (ref The Pathology of Hereditary Breast Cancer) (Table 5).

In fact, three deleterious mutations, c.6591_6592del

Table 6. Location and Pathogenicity of SNV Detected in the Cohort

SNV	Exon	Amino acid change	Pathogenicity rate	Database	Type of Breast cancer
*8755-4 A>G	22	G2919S	Moderate pathogenicity	ND	FBC
4970 A>G	11	N1657S	Low pathogenicity	Clinvar	SBC
231 T>G	3	T77T	Low pathogenicity	Clinvar	SBC
*7462 A>G	15	R2488G	Moderate pathogenicity	ND	SBC
*10135 T>C	27	Y3379H	ND	ND	FBC
9191 A>G	24	A3064G	Low pathogenicity	Clinvar	FBC
122 C>T	3	P41L	Low pathogenicity	Clinvar	SBC
7278 T>A	14	I2426I	Low pathogenicity	Clinvar	SBC
*1798 T>G	10	Y600D	Low pathogenicity	ND	FBC
7504 C>T	15	R2502C	Low pathogenicity	Clinvar	FBC
9292 T>C	25	Y3098H	Low pathogenicity	Clinvar	FBC
9634 G>C	26	G3212R	Low pathogenicity	Clinvar	UD
6500 T>C	11	L2167S	Low pathogenicity	Clinvar	SBC
5572 A>G	11	T1858A	Low pathogenicity	ND	SBC

*not described in Clinvar database

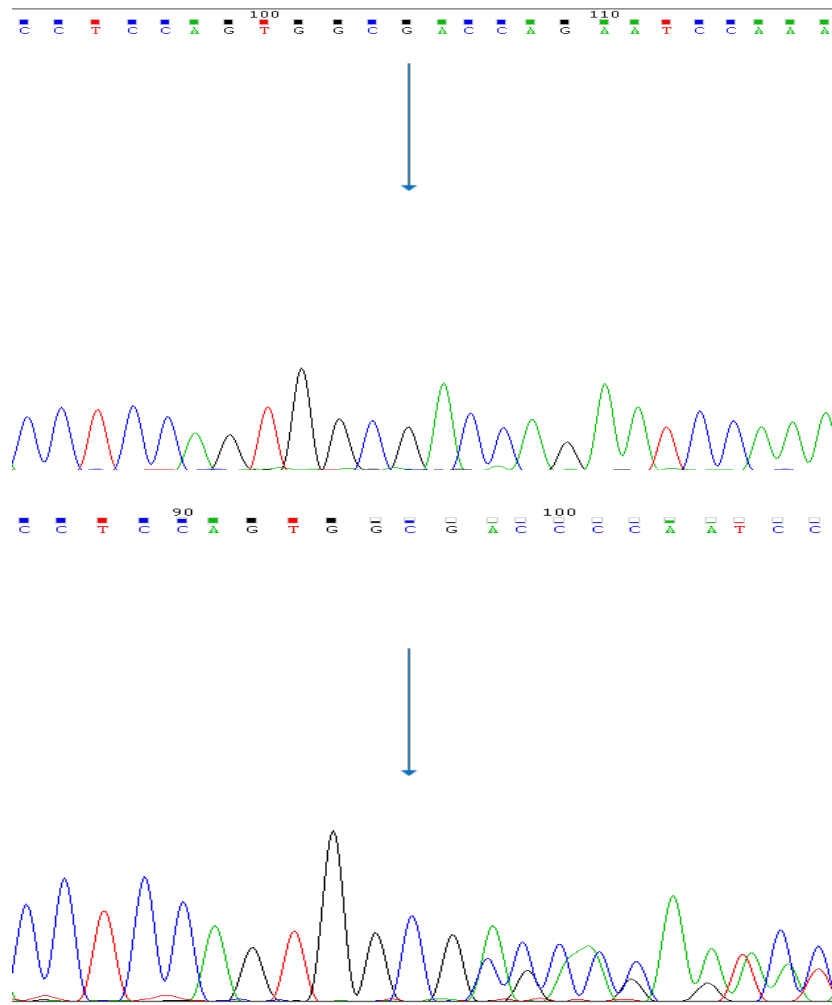


Figure 3.a. Forward Partial Sequences of the Exon 25 in BRCA2 Gene of a Healthy Control. The arrows indicate the normal sequences at the positions c.9382 and 9383. b. Forward Partial Sequences of the Exon 25 in BRCA2 Gene of Women with Familial Breast Cancer. The arrows indicate the duplication CG at the positions of c.9382 and 9383.

(exon11), c.1310_1313del (exon10) and c.7654dup (exon16) have been detected among the 51 sporadic cases (5.88%), and one mutation among the 23 familial cases (4.35%). All these variations are frameshift mutations. Interestingly, the mutations detected in sporadic cases

are already known and described in the BIC database and ClinVar.

A novel mutation was revealed in a familial case that has been confirmed by direct sequencing. It consists of duplication c.9382_9383dup located in exon 25 (Figure 2), leading to p.Pro3129Aspfs*35 variation at the protein level and responsible for amino-acid sequence change from position 3129 to position 3164, associated with a premature stop codon and truncation of the protein that becomes shorter of 254 amino acids. (Figure 3).

To our best knowledge, this mutation was never reported before in the Breast Cancer Information Core database (BICD; <http://research.nhgri.nih.gov/bic/>),

Table 7. Frequency of SNV According to Breast Cancer Presentation

Type of Breast cancer	Number of SNV	Frequency of SNV
Familial (23)	6	0,26
Sporadic (51)	7	0,13
Undefined (5)	1	0,2

Table 8. Deleterious Mutations Described in BRCA2 Gene in Tunisian Familial Breast Cancer Cases

Troudi 2007	Riahi 2014-2016 (Riahi et al., 2016; Riahi et al., 2017)	Belaïba present report	Fourati et al 2014 (Fourati et al., 2014)
c. 1310_1313del4	c. 1310_1313 del4 c. 1313dupT*(X2) c. 1542_1547delAAGA		c.1310_1313del4 (X4)
c. 5682insA	c. 7654dupT *(X2) c. 7887_7888insA		
		c. 9382_9383dup	

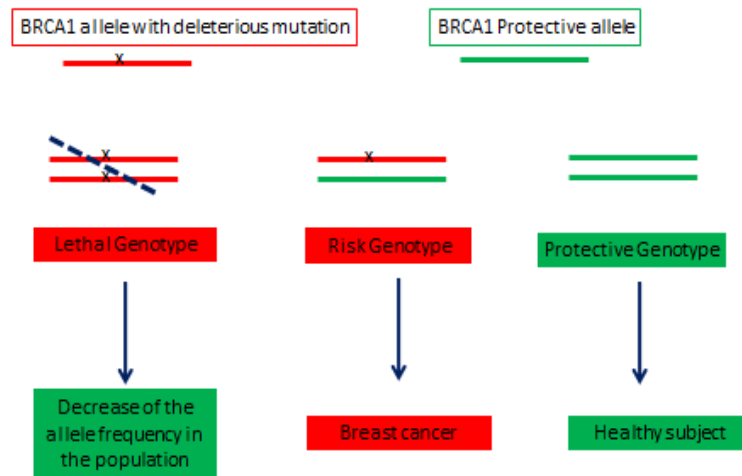


Figure 4. Effect of Consanguinity on BRCA1/2 Deleterious Versus Protective Alleles (consanguinity increases homozygous genotype)

or other resources. According to prediction using HCI (Breast Cancer Genes Prior Probabilities), this mutation was pathogenically identified as class 5 mutation. Thus, this truncated protein is dysfunctional and the deleterious c.9382_9383dup is the risk factor for breast cancer in this family.

Located in exon 25 of BRCA2 gene that comprises 27 exons, this frame-shift mutation leads to a premature stop codon but the mRNA generated might not be eliminated by NMD system, since the stop codon generated is close to the 5' terminal end of mRNA. Hence, the BRCA2 mutated protein could be produced as a truncated protein, lacking the COOH terminal region that carries functional sites, namely binding site to RAD51 and to CDK2. This truncated protein might be integrated into DNA repair complexes which would not be functional. Hence, this mutation seems to be clearly deleterious.

In fact not only the lack of the 254 last amino acids could have an impact on the activity of BRCA2 protein, the last 34 amino acids of the truncated protein are different from the normal protein due to frame shift. When we blasted this sequence on protein database, a great homology was detected with a BRCA2 mutant c.9393delC p.Lys3232AsnfsX6 described in Chinese population. This deleterious mutation also leads to a truncated protein with the same position of the premature stop codon and the same 30 last AA (Figure 2c) (Kwong et al., 2012).

To verify the presence of the c.9382_9383 dup mutation in the healthy population, we designed two primers around the mutated position and sequenced a region encompassing 246 bp in 75 Tunisian healthy women chosen at random. The indicated mutation was absent in all the sequences analyzed.

BRCA2 SNV identified in our cohort

The analysis by the NGS for the 79 women in search of the mutations allowed the discovery of 14 SNV at different positions of the BRCA2 gene: 6 were found in 23 familial cases and 7 in 51 sporadic cases, the remaining SNV being detected among 5 undefined breast cancer cases (Table 6). All the SNV are single substitutions leading most of them

to amino acid change. All were found once in our cohort where they are present in the heterozygous state. One has to notice that the frequency of SNV is twice in FBC as compared with SBC (Table 7).

Among the 14 SNV detected in our cohort, five were not found in Clinvar database. Most of these point mutations lead to amino acid change. Three of these novel SNVs are shown in FBC cases. Interestingly, two of these mutations are predicted to display moderate pathogenicity and one namely 8755-4 A>G with a probability of 0.34, leads to a damage at the wild-type splice acceptor site. The last at position 10135 is located at the end of the protein which renders the difficult evaluation of its effect. However, the amino acid change generated by this substitution could have an impact since Tyrosine replacement by Histidine might have a drastic effect on the structure/ function of the region.

Discussion

Breast cancer is a disease that is often sporadic and considered as a multifactorial pathology. More rarely, it comes under a familial form with as risk factor, a mutation in BRCA1 or BRCA2 major gene. Familial breast cancer is described as due to germ line mutations in genes BRCA1 or BRCA2 which have a deep role in hereditary breast-ovarian cancer. There are no hotspot regions which were reported (Yazici et al., 2002) and the detection of mutations is still hard laborious in reason of the high size of these genes.

However, this scheme is simplistic and this pathology requires more investigation at the genetic level in association with ethnic and environmental factors in order to improve its diagnosis and prognosis.

In this study we were interested into BRCA1/BRCA2 genes in sporadic and familial breast cancer, investigating the role of consanguinity and its relationship with recurrence and penetrance of the mutations. We show that rate of families with BRCA1 mutation decreases when consanguinity rate increases in Southern Mediterranean populations. This could be in agreement with the report

of Denic and Al-Gazali (2002) indicating a trend of decreasing breast cancer incidence with an increasing consanguinity rate. The BRCA1/2 mutation carrier rates are significantly lower in consanguineous than in non-consanguineous populations. Consanguinity may explain part of the worldwide variation of breast cancer incidence.

In a previous work, we have shown that consanguinity is protective against breast cancer (Medimegh et al., 2015). Indeed, it leads to an increase of homozygous protective genotypes and to a counter selection of homozygous deleterious genotypes (Figure 4). Indeed, in families with deleterious mutation, consanguinity increases the probability of homozygous genotypes among descendants. Homozygote genotypes for deleterious mutations which are lethal should lead with a long time of consanguinity practice, to the disappearance of these mutations from the population. As already shown in mice, deleterious BRCA1/BRCA2 mutations are lethal in the homozygous state (Evers and Jonkers, 2006). Hence, they are observed only in the heterozygous form in human leading to a risk for cancer (Rashid et al., 2009).

With a very strong and ancient practice of consanguinity, Southern Mediterranean populations should have eliminated most of the ancient deleterious BRCA1/BRCA2 mutations.

From this point of view, deleterious mutations in such genes in breast cancer should be of recent occurrence, escaping to the selective pressure of consanguinity. This might be the case for the c.9382 and 9,383 mutations in BRCA2 described in this paper. Based on 236 Tunisian families including those analyzed in this study (Troudi et al., 2007; Mahfoudh et al., 2012; Riahi et al., 2015); only one patient with FBC presents this mutation (allelic frequency of 0,2% among FBC). We verified the presence of the mutation c.9382_9383dup, in the general population, investigating 75 healthy Tunisian women chosen at random and the mutation was absent in all the sequences analyzed and none of the 75 healthy women tested carries this novel mutation.

However, in this study, we show that around 50% of BRCA1 mutations described in breast cancer families in North Africa are recurrent. It also seems the case for BRCA2 mutations. Considering for example studies on BRCA2 mutations in Tunisian familial cases including this work, it also appears that at 3/7 (43%) of BRCA2 deleterious mutations are recurrent (Table 8). The three recurrent mutations (c.1310_1313del, c.1542_1547delAAGA and c.7887_7888insA) would explain 90% of families BRCA2-related (Riahi et al., 2017) in Tunisian population. Moreover, four recurrent mutations would explain 80% of families BRCA1-related. These important data should be taken into account for genetic counseling in this country.

This recurrence is the testimony of the very ancient occurrence of these mutations. Under selective pressure of consanguinity, these mutations should have disappeared. Their absence at the homozygous status in all the cases analyzed in Southern Mediterranean countries though very high consanguinity rates is a strong argument toward their lethality. Their maintenance in such consanguineous populations might be explained by penetrance and/or by

selective advantage of heterozygous. Argument toward ancient BRCA1/2 mutations maintenance would be the selective advantage given by heterozygous genotypes. This could be related to the pleiotropy of BRCA1 and BRCA2 proteins and to a dual role of these proteins (Dobbins et al., 2016). But we need more specialized research in order to demonstrate such a selective advantage.

Penetrance is more obvious to the argument. According to Knudson (1993), germ line mutations in tumor suppressor genes such as BRCA1/2 are heterozygous and a second mutation or alteration should occur at a somatic level in order to inactivate both alleles in the tumor. This second event might not occur which explains that the cumulative risk to develop breast cancer in the presence of a BRCA1 or BRCA2 mutation should not exceed 56 to 87% (Shannon and Chittenden, 2012).

Moreover, penetrance could lead to the specific situation observed in Southern Mediterranean breast cancer families: no more than 3 or 4 cases in the family. In our study, we also investigated for the presence of BRCA1/2 deleterious mutations in sporadic Breast cancer cases. Interestingly, both for BRCA1 and for BRCA2, deleterious mutations described respectively in Algerian (Uhrhammer et al., 2008) and in Tunisian sporadic breast cancer cases, are all recurrent mutations and hence their presence in sporadic cases cannot be attributed to the de novo mutational event. In the present work our research performed on 79 Tunisian women with breast cancer, carried out 3 recurrent BRCA2 mutations (c.6591_6592del, c.1310_1313del, c.7654dup) in sporadic cases with a frequency of 5,9% versus one new mutation (c.9382_9383dup) in a familial case (with a frequency of 4,3%). The mutation c.1310_1313del has been already reported in three previous studies on Tunisian patients with familial breast cancer. The mutation c.7654dup has also been described by Riahi et al and c.6591_6592del is found in databases. Another common BRCA2 pathogenic mutation c.7235_7236insG was detected in Algerian and Moroccan families. These mutations could be considered characteristic of Northern African geographic origin. This data about origin could be taken into account to enrich the BIC database (Breast Cancer Information Core database).

This incomplete penetrance might be related to the mutations themselves which are mostly frame-shift mutation leading to premature codon stop in mRNA which in itself is eliminated by NMD system. Heterozygous genotype for such mutations should not generate truncated protein but only wild BRCA1/2 protein. Single amino acid substitution could also lead to low penetrance. In fact, most of the mutations of unknown values are substitutions, the impact of which on protein function is not established. In the present paper, the genomic analysis for the 79 women revealed several SNVs (14 SNV) at different positions of the BRCA2 gene. In this study, we reveal that each SNV is unique in our cohort but most of them are already known. Five are described for the first time among whom two are predicted of moderate pathogenicity. Moreover, SNV are two fold more frequent in FBC than in SBC, all of them being described as a heterozygous state. Their absence at homozygous state could be explained

by their low frequency in the population but also by their possible lethality in such genotype as already known for clear deleterious mutations. All these data associated to the consanguinity context suggest that the BRCA2 SNVs are involved in breast cancer. However, we need additional studies at a functional level in order to confirm these predictions.

On the other hand, penetrance could be due to other genetic or environmental protective factors that characterize Southern Mediterranean populations. Indeed as already reported, a Mediterranean diet which is healthy could be a protective factor associated with lifestyle (D'Alessandro et al., 2016). For genetic factors, a recent study revealed the interference of genotype of X fragile locus with BRCA1/2 mutations. Indeed, independently from neurological effects, the FMR1 gene plays an important role in ovarian function, as BRCA1/2 gene. Minor expansions of CGG repeats in FMR1 are associated with an increased risk for premature ovarian aging (Weghofer et al., 2012). In contrast to controls, BRCA1/2 carriers demonstrated almost a complete absence of all constitutional FMR1 genotypes except for sub-genotypes with low (CGG n<26) alleles. Women with low FMR1 sub-genotypes, therefore, should reflect increased BRCA1/2-associated cancer risks, while the remaining with high sub-genotypes, should face almost no such risks. In this case, these sub-genotypes could be protective and would explain the penetrance of BRCA1/2 mutations.

For Southern Mediterranean populations, characterized by consanguinity, recurrent BRCA1 and BRCA2 mutations with incomplete penetrance, it can be argued that co-occurrence with a high probability of BRCA1/2 mutations with protective genes is possible. It has already been shown that more than one inherited deleterious gene can affect consanguineous families (Romdhane et al., 2014) leading to comorbidity. In our case, due to a founder effect, to genetic drift or to co-evolution, BRCA1/2 mutations might have been associated in Southern Mediterranean populations with protective genes, namely in this case with high FMR1 sub-genotypes. Further investigations should be undertaken in order to confirm this hypothesis.

In conclusion, the study of BRCA1/2 gene in Southern Mediterranean countries revealed low penetrance recurrent mutations in sporadic and familial breast cancer. These mutations have been selected in a context of ancient consanguinity practice along with protective genetic and environmental factors.

Conflict of interest

The authors declare that they have no conflict of interest.

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