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Specific *BRCA1* gene variations amongst young Moroccan breast cancer patients

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ABSTRACT. Germline mutations in the *BRCA1* gene are known predictive markers for the development of hereditary breast cancer. Nevertheless, no comprehensive study has been performed targeting the presence and relevance of BRCA1 mutations in Moroccan breast cancer patients. We here present an analysis of *BRCA1* gene regions (exon 2 and exon 11a/b) of 50 female Moroccan breast cancer patients with early disease onset (≤40 years) or familial disease backgrounds. Results showed that no mutation was present in either exon 2 or exon 11a of the *BRCA1* gene in any of the 50 patients analysed. However, in exon 11b, a mutation generated by a nucleotide exchange was detected in 8% of patients, most of whom were young women (≤40). This mutation leads to substitution of the amino acid glutamine by an arginine at position 356 of the polypeptide sequence (Q356R). Although this mutation was previously characterised at a lower frequency in western populations, our study is the first to describe it in a young Moroccan population.

Furthermore, another mutation was detected with a high frequency (4%) on exon 11b of the *BRCA1* gene in exclusively young patients (≤40). This mutation was silent, encoding the same threonine residue at position 327 (T327T) as the wild type. The present study is the first to describe this mutation as well, particularly in a young Moroccan population. Analysis of a larger population is required in order to highlight the relevance of the Q356R and T327T mutations in young Moroccan breast cancer patients.

Key words: Breast cancer; Clinical samples; *BRCA1* gene; Exon sequencing; Mutation

INTRODUCTION

Breast cancer is the most common cancer in women. Although epidemiological surveys in western countries represent a good overview about the incidence and the mortality rate of this disease, investigations in developing countries are very limited, despite the fact that the incidence of breast cancer has dramatically increased in recent years.

In Morocco, breast cancer is the most frequent cancer in women, accounting for 36% of all female cancers. Because there is no national cancer registry in Morocco, breast cancer cases are rarely recorded. However, in the greater area of Casablanca and Rabat, two civic centres have registered patients along with their medical histories (Benider et al., 2004; Tazi et al., 2009). Herein, an incidence of 35.4 per 100,000 persons are reported, which is higher than breast cancer rates in neighbouring states such as Algeria (28.6) and Tunisia (30.3) (Ferlay et al., 2008). Although these proportions remain far below those of developed countries, numbers are increasing exponentially.

Studies of Arabic immigrants (Chalabi et al., 2008) or within the population of Arabic countries (reviewed in Najjar and Easson, 2010) revealed that breast cancer in this population is relatively more aggressive. Although the number of cases of hereditary breast cancer is not recorded in Morocco, some studies have tried to ascertain this information. An investigation of 409 Moroccan women affected by breast cancer revealed that only 7% have a family history of breast cancer; however, one third of these patients could not be classified as either a sporadic or a familial case (Abahssain et al., 2010). Nevertheless, the relatively high incidence observed in young women suggests that family history could be an important risk factor. For example, in a Swedish study, 48% of breast cancer cases in patients younger than 40 years of age could be correlated with a family history of breast or ovarian cancer (Loman et al., 2001).

Several tumour suppressor genes are correlated with an increased risk of breast cancer onset. Among them, germline mutations in the tumour suppressor genes *BRCA1* and *BRCA2* account for a large number of hereditary breast cancer cases. Although mutations in these genes are found in only less than 10% of breast cancer patients, women carrying deleterious *BRCA1* or *BRCA2* mutations have an 80% risk of developing breast cancer during their lifetime, thereby 40% of mutation carriers will already be affected by the age of 50 (Antoniou et al., 2003).

BRCA1 and BRCA2 mutations are relatively more frequent in young breast cancer patients (De Sanjosé et al., 2003). Therefore, investigations of these genes are of special interest to Moroccan breast cancer patients who are characterised by their young age.

The specificity and frequency of BRCA1 and BRCA2 mutations vary among different geographical regions and ethnicities. In some populations, the mutation frequency is particularly high, and therefore represents a founder mutation (see Fackenthal and Olopade, 2007, for a review). The most well known founder mutation is BRCA1 185delAG (located in exon 2), which has been observed in 1% of the unselected Ashkenazi Jewish population, and in 41% of high-risk families of that population (Fodor et al., 1998).

To date, little is known about BRCA1 mutations in Moroccan breast cancer patients. With respect to the founder mutation 185delAG, populations other than the Ashkenazi Jewish population have been investigated, including the Moroccan Jewish population, which shows the same mutation rate, therefore opening the discussion about the origin of this particular mutation (Bar-Sade et al., 1998; Kreiss et al., 2000). The relevance of this mutation in the non-Jewish Moroccan population remains unknown, although two such familial cases have already been described (Laarabi et al., 2011).

No study has yet been carefully performed to identify specific BRCA1 mutations in Moroccan families affected by breast cancer, or in the general Moroccan population. However, studies in other neighbouring countries have proposed new founder mutations in the Arabic population, namely c798-799delTT in Algeria (Uhrhammer et al., 2008; Cherbal et al., 2010) and c.211dupA in Tunisia (Troudi et al., 2007, 2008).

In the current study, we present a sequence analysis of regions of the *BRCA1* gene from 50 Moroccan breast cancer patients, including 22 familial cases and 28 patients with early-onset breast cancer. We sequenced exon 2 and exon 11a/b, with special interest in the 185delAG mutation. Our major aim was to analyse the complete exon 11 sequence, which is the largest exon of BRCA1, and where the most mutations have been detected so far (BIC database).

MATERIAL AND METHODS

Patient samples

The patients were recruited from the Oncology Center of the University Hospital Ibn Rochd of Casablanca, following these selection criteria:

a. More than three first- or second-degree relatives with breast cancer on the same side of the family.

b. Early-onset breast cancer (≤40 years).

Twenty-two patients were classified with familial breast cancer and 28 patients were classified as early-onset breast cancer cases. Forty-six patients had unilateral breast cancer and four patients had bilateral breast cancer.

DNA sequencing

Peripheral blood was drawn from the patients and DNA was extracted using the salting-out method. Polymerase chain reaction was performed for exons 2, 11a, and 11b as described by Friedman et al. (1994). The amplified DNA was bi-directionally sequenced for the three different regions of BRCA1 with the same primer used for all amplifications. The sequencing was performed by Secugen, Madrid (Spain).

Analysis

Sequencing results were analysed using the Applied Biosystems Sequence Scanner 1.0 software and BLAST alignment, and were manually reviewed.

The nucleotide and code numbering for BRCA1 were used in this analysis as in the BIC database, i.e., according to GenBank accession No. U14680.1 with numbering starting at the first nucleotide.

RESULTS AND DISCUSSION

Fifty Moroccan breast cancer patients with a mean age of 39 years were investigated in this study. Forty-six patients had unilateral breast cancer and four patients had bilateral breast cancer. Patients were interviewed about their familial disease histories. Twenty-two patients were familial breast cancer cases, whereas 28 patients had unknown familial disease backgrounds. These 28 patients showed early-onset breast cancer (≤40 years). However, since this classification was only done by interview, these cases cannot be precisely defined as sporadic, and due to their age, were most likely correlated with a family history (Loman et al., 2001).

Successful sequences were obtained for both exon 2 (patients 4 and 5 excluded) and exon 11a (patients 8 and 45 excluded) of the *BRCA1* gene in 48 patients, while exon 11b was successfully sequenced for 49 patients (patient 50 excluded).

Analysis of exon 2 sequence

After obtaining the exon 2 sequence, we performed a thorough analysis of the whole sequence in order to identify any mutations present. No mutation was detected in exon 2 of the *BRCA1* gene for the entire analysed sample of 48 patients. This result was not surprising since in both the Ashkenazi and Moroccan Jewish populations, the well-established founder mutation 185delAG was only expected with a low frequency of 1% (Bar-Sade et al., 1998; Kreiss et al., 2000; Antoniou et al., 2003). Therefore, a larger patient population is required to investigate the role of the 185delAG mutation within the Moroccan population.

Recently, Laarabi et al. (2011) described the 185delAG mutation in two unrelated Moroccan breast cancer patients. The presence of this mutation was also tested in healthy family members to assess their individual risks of hereditary breast cancer. While this mutation was absent in one of the examined families, two women in the other family were found to carry this mutation and therefore live with an increased breast cancer risk. This finding emphasises the importance of further screening for this mutation in the Moroccan population.

Analysis of the exon 11a sequence

As was the case for exon 2, we did not detect any mutation in the sequenced exon 11 of the *BRCA1* gene for the 48 patients examined. Notably, a mutation in this region has previously been identified in two Algerian and two Tunisian families, and is considered as a potential North African founder mutation (Uhrhammer et al., 2008). Therefore, this finding made this region of particular interest in terms of the identification of an Arabic, or at least a North African, founder mutation. Nevertheless, both our current, and the above-described

studies have analysed less than 100 patients, and are therefore too limited to accurately test the hypothesis of a founder mutation at this site.

Analysis of the exon 11b sequence

By examining the exon 11b sequence of the *BRCA1* gene, we identified two different mutations in six of the 49 patients analysed.

In four patients (1, 13, 31, and 48), a single nucleotide mutation (A>G) was detected at position 1186 of the exon 11b sequence with a frequency of 8% (Figure 1A). This missense mutation leads to the replacement of arginine by a glutamine residue (Q356R). To the best of our knowledge, the current study is the first to describe this missense mutation in a Moroccan breast cancer population. However, this same mutation was previously described in other western European populations (Friedman et al., 1995), and it was later suggested that the homozygous variant (both alleles encode for the 356R) might have a protective role, as homozygosity was found more frequently in control groups (Dunning et al., 1997). Interestingly, this mutation was not reported in the *BRCA1* gene analysis performed with populations in other North African countries, such as Algeria and Tunisia (Troudi et al., 2007, 2008, 2009; Uhrhammer et al., 2008; Cherbal et al., 2010). Further investigations are therefore needed to determine the importance of the Q356R polymorphism within the Moroccan population.

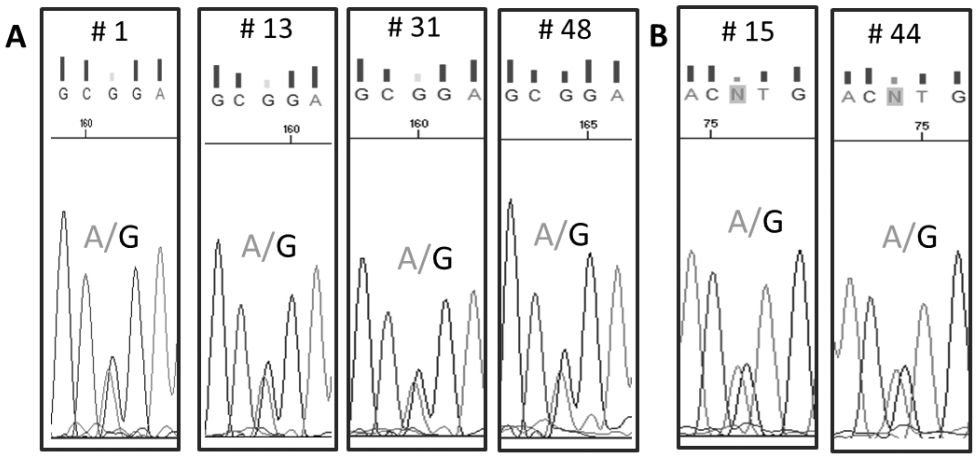

Figure 1. Results of forward sequencing of exon 11b. **A.** Identification of single nucleotide exchange A>G 1186 in patient 1, 13, 31 and 48. **B.** Single nucleotide exchange A>G 1100 in patient 15 and 44.

Furthermore, an additional single mutation (A>G) was detected at position 1100 of the exon 11b sequence in two of the 49 examined patients (15 and 44). This nucleotide mutation was silent and does not generate any amino acid change since it encodes for the same threonine residue (T327T) as the wild type (Figure 1B). Although this same mutation has already been described in other studies, but with a low frequency (<0.5%) (Stoppa-Lyonnet et al., 1997; Malone et al., 1998), the current study is the first to describe this silent mutation within the Moroccan breast cancer population as well. Moreover, the relevance of this mutation was

considered to be minor with respect to predicting breast cancer risk since it does not affect the composition of the BRCA1 protein. However, the relatively high frequency found in our study (4%) suggests that this mutation might play a role in the genetic polymorphism of the *BRCA1* gene, and could potentially be defined as a founder mutation in the Arabic population. Indeed, the same silent mutation was found in two of 51 breast cancer patients in an Algerian study (Uhrhammer et al., 2008), therefore supporting our hypothesis of the importance of this mutation. Interestingly, both identified cases also carried two additional nucleotide substitutions: 2733G>A (G911) and 3024G>A (M1008I). Whereas the G911 variant has not been previously described, the M1008I missense mutation has been frequently (139 BIC entries) found to be associated with the disease (Durocher et al., 1996).

Together, these results suggest that if a combination of mutations displays a relevant haplotype for increased breast cancer risk, particularly in an Arabian population, analysis of the remainder of exon 11 for the two patients carrying the T327T mutation and an increased number of patient samples should be carried out.

Besides its possible role in the generation of a deleterious haplotype, the T327T mutation could also influence the function of the *BRCA1* gene, even though it does not change the amino acid composition of the protein. Indeed, various silent mutations have been described to be involved in *BRCA1* gene expression, such as allelic imbalance (Sharp et al., 2004) or exon skipping (Raponi et al., 2011). The T327T variant might play a role in exon skipping, as suggested by Anczukow et al. (2008), since the normal and mutated forms differ in their respective prevalence of exon skipping (application of EX-SKIP, freely available at http://www.dbass.org.uk; data not shown).

Patient backgrounds

Interestingly, the detected nucleotide sequence variations, Q365R and T327T, were exclusively found in young patients, under the age of 40 years (Table 1).

Table 1. Age, cancer type and family history background information of the patients with a detected nucleic acid exchange in BRCA1 exon 11b.

Patient	Mutation	Age (years)	Cancer type	Family history
1	Q356R	28	Unilateral Bresat cancer	None
13	Q356R	31	Unilateral Bresat cancer	None
31	Q356R	36	Unilateral Bresat cancer	None
48	Q356R	39	Bilateral breast cancer	None
15	T327T	36	Unilateral Bresat cancer	None
44	T327T	30	Unilateral Bresat cancer	None

For Q356R, this finding does not appear to coincide with data obtained from western countries where the frequency of this polymorphism is the same across different age groups (Dunning et al., 1997). The T327T variant was also found in high frequencies in an Algerian study (Uhrhammer et al., 2008), where it was also found in two very young patients (34 and 37 years of age), both with unknown familial backgrounds, which is comparable to the findings of the present study. This result suggests that age may influence the onset of breast cancer.

CONCLUSIONS

In the present study, 50 Moroccan breast cancer patients, either with a strong history of family disease or affected at young age, were investigated for BRCA1 mutations. Two different mutations were found exclusively in young patients (<40 years) in high frequencies: the missense mutation Q356R, and the silent mutation T327T. Further studies are needed to explore these findings in a larger number of patients.

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Conflicts of interest

The authors declare no conflict of interest.

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