BRCA1 and BRCA2 germline mutations screening in Algerian breast/ovarian cancer families

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Abstract. Background: Breast cancer is the leading cause of cancer death in women in Algeria. The contribution of BRCA1 and BRCA2 mutations to hereditary breast/ovarian cancer in Algerian population is largely unknown. Here, we describe analysis of BRCA1 and BRCA2 genes in 86 individuals from 70 families from an Algerian cohort with a personal and family history suggestive of genetic predisposition to breast cancer.

Methods: The approach used is based on BRCA1 and BRCA2 mutations screening by High-Resolution Melting (HRM) curve analysis followed by direct sequencing. All samples for which no pathogenic mutation was found were analyzed by MLPA for large deletions or duplications.

Results: Three distinct pathogenic mutations c.83_84delTG, c.181T>G, c.798_799delTT and two large rearrangements involving deletion of exon 2 and exon 8 respectively, were detected in BRCA1 gene. Moreover 17 unclassified variants and polymorphisms were detected in BRCA1 gene (6 described for the first time). Two pathogenic mutations, c.1310_1313delAAGA and c.5722_5723delCT and 40 unclassified variants and polymorphisms (14 never described before) were identified in BRCA2 gene.

Conclusions: For the first time, we used HRM and MLPA to identify BRCA1 and BRCA2 mutations in Algerian patients with a personal and family history suggestive of genetic predisposition to breast cancer. The implications of these new findings in regard to genetic testing and counseling are substantial for the Algerian population.

Keywords: Algeria, BRCA1, BRCA2, hereditary breast cancer, mutation analysis

Abbreviations

BIC Breast Cancer Information Core
HRM High-Resolution Melting curve analysis
MLPA Multiplex Ligation-dependent Probe Amplification
PSIC Position-Specific Independent Counts
SSCP Single Strand Conformation Polymorphism
UVs Unclassified variants

1. Introduction

The three commonest cancers in the Algerian population (females, all ages) are cancers of the breast, cervix uteri, and colon/rectum in diminishing order of incidence [1]. Breast cancer is currently the lead-
ing cause of cancer morbidity and mortality among Algerian women [1]. Age standardized incidence per 100,000 for breast cancer in 2002 was 23.5 in Algeria [1]. Moreover, like in Asian populations, the mean age of breast cancer cases in North African populations is generally younger than the mean age of breast cancer in North America and Europe. About 11% of breast cancer cases in Algeria occur in women $\leq 35$ years old, and 55% of cases at $\leq 50$ years [1]. Because of the strong association between age of onset and genetic susceptibility, a high proportion of breast cancer cases in Algeria may be attributable to BRCA1 and BRCA2.

The factor with the strongest breast cancer risk association is a family history of breast and/or ovarian cancer, the associated risk being even higher for family history of early onset disease ($< age$ 40). Two major breast cancer susceptibility genes BRCA1 and BRCA2 were localized by linkage analysis on long arms of chromosome 17 [2] and 13 [4]. Mutations in the BRCA1 and BRCA2 genes were first reported in conjunction with their identification in 1994 by Miki et al. [3] and in 1995 by Wooster et al. [4]. Germ line mutations in the BRCA1 and BRCA2 genes predispose individuals to breast and ovarian cancer. The life time risk of breast cancer in female carriers of a BRCA1 mutation is 60–80% while that of ovarian cancer is 20–40%. Women who carry BRCA1 and BRCA2 mutations have a significantly increased risk of developing breast cancer before the age of 50 years [5–9]. To date, very few reports have been published about the spectrum of BRCA1 and BRCA2 mutations in the Algerian women [10]. There is no information on the prevalence or frequency of BRCA1 and BRCA2 mutations among Algerian population. A pilot study of screening of exon 2 and 11 of BRCA1 gene by using SSCP and direct sequencing in 15 familial breast cancer patients identified 3 new unknown variants in exon 2 and one new polymorphism in exon 11 respectively [10]. The knowledge about the contribution of BRCA1 and BRCA2 mutations in Algerian familial breast/ovarian cancer will lead to better understanding of genetic risk factors of this disease.

2. Material and Methods

This study was performed to identify germ line mutations in the BRCA1 and BRCA2 genes in Algerian women with familial breast/ovarian cancer. The approach used is based on BRCA1 and BRCA2 mutations screening by High-Resolution Melting (HRM) curve analysis followed by direct sequencing. Samples for which no pathogenic mutation was found were analyzed by MLPA.

2.1. Patients

The patients and their families were referred through the Anti Cancer Center of Blida, the Central Hospital of Algiers, and three private medical clinics which provide oncology services throughout Algeria. The following selection criteria of patients and affected family members were used: (a) women with a history of two or more relatives on the same side of the family with breast and/or ovarian cancer and male relatives with prostate cancer along three generations at any age (b) two or more cases of breast and/or ovarian cancer in first degree relatives, (c) cases of bilateral breast cancer, (d) breast or ovarian cancer before the age of 40, (e) male relatives with breast cancer. Clinical characteristics of study population are presented in Table 1. Prior collecting blood, all selected patients and relatives were informed about the objectives of our study and their DNA samples would be analyzed for mutations in genes associated with hereditary breast cancer. All patients and the relatives signed informed consent and ethical approval was obtained from appropriate institutions.

2.2. DNA isolation

Genomic DNA was extracted from peripheral blood lymphocytes using a Promega Wizard® Genomic DNA Purification Kit, (Promega, Madison, WI, USA) (Cat. # A1120) and in accordance with the manufacturer’s protocols.

2.3. Mutation analysis

2.3.1. High-Resolution Melting (HRM) curve analysis

PCR reactions were performed in a 20 μl final volume using Light Cycler®-480 High Resolution Melting Master Kit (Roche Diagnostics, Mannheim, Germany). All coding exons of BRCA1 and BRCA2 including flanking intronic regions were submitted to prescreening with HRM curve analysis. The PCR and HRM assays were performed as described elsewhere [11] using the LightCycler 480 II Instrument (Roche Diagnostics, Mannheim, Germany). The PCR primers and PCR-HRM assay conditions are available on request.
Table 1
Clinical characteristics of study population: diagnosis, age at onset and family history

| Diagnosis                        | Age at Onset (years) | Family History |
|----------------------------------|----------------------|----------------|
| Breast cancer (n = 68)           | ≤ 40 (n = 49), > 40 (n = 11), > 50 (n = 8) | Yes            |
| Breast/ovarian cancer (n = 6)    | ≤ 40 (n = 3), > 40 (n = 3), > 50 (n = 3) | Yes            |
| Male breast cancer (n = 3)       | ≤ 40 (n = 2), > 50 (n = 2) | NA             |
| Ovarian cancer (n = 4)           |                      | Yes            |

NA: family history confirmed but data were not available.

2.3.2. DNA sequencing

PCR products were purified and sequenced according to the manufacturer’s protocols using Genomelab TM DTCS Quick start Master Mix (Beckman Coulter, Fullerton, CA, USA). Sequence products were analyzed using a CEQ 8000-Beckman sequencer (Beckman Coulter). Sequences analyses were performed using CEQ-8000 Software. Identified DNA sequence variants were confirmed by sequencing both DNA strands on at least two independent PCR-HRM products.

2.3.3. Multiplex ligation-dependent probe amplification (MLPA)

The principle of MLPA has been described elsewhere [12]. All samples for which no pathogenic mutation was found were analyzed by MLPA for large deletions or duplications according to the manufacturer’s protocol (MRC-Holland, Amsterdam, The Netherlands).

2.4. Nomenclature and variant analysis

All nucleotide numbers refer to the wild-type cDNA human sequence of BRCA1 (accession no. U14680; version U14680.1 GI: 555931) and BRCA2 (accession no. U43746; version U43746.1 GI: 1161383), as reported in the GenBank database. The cDNA numbering for the traditional mutation nomenclature used in the BIC (Breast Cancer Information Core) database is based on reference sequences as stated above, where the A of the ATG translation initiation codon is at position 120 of the BRCA1 mRNA and at position 229 of the BRCA2 mRNA, respectively. The HGVS (Human Genome Variation Sequence) approved systematic nomenclature follows the rule where the nucleotide +1 is the A of the ATG translation initiation codon. In the text of this article and the tables, we use the HGVS nomenclature (www.hgvs.org/mutnomen). Evaluation of the prevalence of the newly identified BRCA1 and BRCA2 UVs in a control population was performed with HRM in 80 healthy blood donors’ individuals without breast or ovarian cancer familial history.

To identify no synonymous amino acid changes likely to disrupt BRCA1 and BRCA2 genes function, we used a comparative evolutionary bioinformatic program, Polymorphism Phenotyping (http://genetics.bswh.harvard.edu/pph). To identify the splice site alterations of new UVs occurring in intron-exon boundaries of BRCA1 and BRCA2, we used GeneSplicer program (CBCB, University of Maryland, USA). Evolutionary conservation of BRCA1 and BRCA2 sites of amino acid changes was evaluated across 13 species among the following species: human, chimpanzee, gorilla, orangutan, macaque, mouse, dog, cow, opossum, chicken, frog, tetraodon, rat, rabbit, cat, and armadillo.

3. Results

3.1. BRCA1 and BRCA2 pathogenic mutations

The analysis of DNA samples of 86 individuals revealed that nine patients (and one male relative) carried pathogenic germline mutations: eight within BRCA1 and two within BRCA2.

Five pathogenic mutations in BRCA1 gene (three were already described in the BIC database) and two within BRCA2 gene, were detected in this study. One out seven of these mutations is novel (one in BRCA1). Most of the carriers of pathogenic mutations are early onset patients (7 out 9 patients), the age at early onset is ≤ 40 years and have a personal and strong family history suggestive of genetic predisposition to breast cancer (see Table 2).

Two distinct germline frameshift mutations in BRCA1 gene leading to premature truncated proteins were identified in four cases. These mutations are located in exons 3 (c.834delTG/p.Leu28ArgfsX12) and 11 (c.798delTT/p.Ser267LysfsX19), respectively. Interestingly, the mutation c.798delTT/p.Ser267LysfsX19 was identified in two unrelated.
Table 2

| Patient ID | Affected gene | Nucleotide change | Amino acid change | Mutation type and age at onset of the proband or the tested relative | Affected family members |
|------------|----------------|-------------------|-------------------|---------------------------------------------------------------------|-------------------------|
| 2092       | BRCA1          | c.83,84delTG      | p.Leu28ArgfsX12   | FS BC, 47y                                                           | 2 sisters (M) cousin (P) |
| 2067       | BRCA1          | c.181 T>G         | p.Cys61Gly        | MS BC, 36y                                                           | 2 sisters brother niece mother, 2 sisters cousin (P) mother, 3 sisters niece cousin (P) |
| 2068*      | BRCA1          | c.181T>G          | p.Cys61Gly        | MS BC, 44y                                                           | 2 sisters uncle (P) |
| 2094       | BRCA1          | c.798,799delTT    | p.Ser267LysfsX19  | FS BBC, 33y                                                           | 2 sisters aunt (M) |
| 2095       | BRCA1          | c.798,799delTT    | p.Ser267LysfsX19  | FS BC, 30y                                                           | 2 sisters mother, sister grandmother (M) aunt (M) mother, sister grandmother (M) aunt (M) |
| 2096**     | BRCA1          | c.798,799delTT    | p.Ser267LysfsX19  | FS BC, ( ?)                                                          | 2 sisters mother, sister grandmother (M) aunt (M) mother, sister grandmother (M) aunt (M) |
| 2082       | BRCA1          | deletion of exon 2| –                 | LR BOC, 40y                                                           | 2 sisters aunt (P) |
| 20821      | BRCA1          | deletion of exon 8| –                 | LR BBC, 35y                                                           | 2 sisters aunt (M) |
| 2091       | BRCA2          | c.1310,1313delAAGA| p.Lys437IlefsX2   | FS BBC, 47y                                                           | 2 sisters mother, sister cousin (P) aunt (P) |
| 2076       | BRCA2          | c.5722,5723delCT  | p.Leu1908ArgfsX2  | FS BC, 34y                                                           | 2 sisters grandmother (P) aunt (M) |

BC: breast cancer, BBC: bilateral breast cancer, BOC: breast/ovarian cancer, OC: ovarian cancer, dx: age at diagnosis, (?): age unknown, M: maternal, P: paternal, FS: frameshift, LR: large rearrangement, MS: missense, *: brother of the patient 2067, developed breast cancer, after axillaries enlargement lymph nodes, **: sister of the patient 2095.

The third pathogenic mutation in BRCA1 gene is c.181T>G/p.Cys61Gly, a missense mutation that takes place in the 100% conserved cysteine residues of the BRCA1 C3HC4 RING domain, and was found in two members of the same family (the index case 2067 and her brother).

MLPA analysis of the BRCA1 gene revealed two germline alterations in two unrelated families among 70. A novel deletion of BRCA1 exon 2 (c.-19-?_80+?del/p.?) and a deletion of BRCA1 exon 8 (c.442-?_547+?del/p.?) were identified in two patients with breast/ovarian cancer and bilateral breast cancer, respectively.

Two germline frameshift mutations were detected in the BRCA2 gene. These mutations, already described in the BIC database, were found in two unrelated patients, and located in the exons 10 (c.1310,1313delAAGA/p.Lys437IlefsX2) and 11 (c.5722,5723delCT/p.Leu1908ArgfsX2), respectively.

3.2. BRCA1 and BRCA2 unclassified variants (UVs)

Fifty seven UVs and polymorphisms (17 BRCA1 and 40 BRCA2) were detected in 42 patients. Among the fifty seven BRCA1 and BRCA2 variants, fourteen novel UVs (4 BRCA1 and 10 BRCA2) were identified in 18 patients (see Tables 3–5). We note that the newly identified UVs occurring in intron-exon boundaries of BRCA1 and BRCA2, could be considered as benign, because the GeneSplicer prediction program shows no splice alteration site for these variants. Among the new UVs, six new missense variations (one in BRCA1 and 5 in BRCA2) were identified. In addition, four new unclassified variants identified in this study, one BRCA1 (c.4066C>A/p.Gln1356Lys) located in exon 11 and three BRCA2 (c.3868T>A/p.Cys1290Ser, c.5472T>G/p.Asn1824Lys and c.5985C>A/p.Asn1995Lys) located in exon 11, show a damaging PSIC scores yielded by PolyPhen program and could be
pathogenic (see Table 5). Furthermore, associated with the damaging PSIC scores, the four newly identified UVs were found at a frequency of <1% in the control population (see Table 5).

4. Discussion

To date, few molecular genetics studies of BRCA1 and BRCA2 germline mutations have been reported in the Algerian population [10]. North African populations have not been extensively studied; consequently knowledge of the prevalence and spectrum of BRCA1 and BRCA2 mutations in these populations is sparse. A total of 70 breast cancer families have been examined for germline mutations in the BRCA1 and BRCA2 genes. Seven pathogenic mutations (including one novel pathogenic mutation) have been identified (5 BRCA1 and 2 BRCA2) in 8/70 (11.4%) families. Our results indicate, as has been documented by others, that family history is the major determinant of the risk of breast cancer [14–18]. As shown in Table 2, in all families where a pathogenic mutation was identified, there was a family history of breast cancer. We note that four deleterious mutations in BRCA1/2 genes (3 BRCA1 and 1 BRCA2) have been found in families with bilateral breast cancer and breast/ovarian cancer (4/8 breast/ovarian cancer families) (see Tables 1–2). This result shows that pathogenic mutations in the BRCA1 and BRCA2 genes highly predispose to bilateral breast cancer and breast/ovarian cancer, as it has been previously reported [15, 22, 23]. Our findings highlight the importance to use this indication for routine testing for BRCA genes in Algerian breast/ovarian cancer families.

Our data show also that some of the mutations identified in BRCA1 and BRCA2 genes of Algerian breast cancer patients were previously described in other populations in many countries [19]. We note that the BRCA1 pathogenic mutation c.181T>G described in this study for the first time in North African populations, is one of the most frequent founder BRCA1 mutations identified in many European countries, Poland, Czech, Germany, Hungary [19]. Contrary to the BRCA1 exon 2 deletion reported here in one Algerian breast/ovarian cancer family which seems never described before, the deletion of exon 8 in BRCA1 gene found in a patient with bilateral breast cancer has been recently reported for the first time in two German patients with hereditary breast cancer [13]. But interestingly, we found by using Long Range PCR technique that our patient with the BRCA1 exon 8 deletion is heterozygous for a 2.6 kb deletion (data not shown). This deletion is different with the 5.7 kb and 3.5 kb deletions identified respectively in the two German patients [13]. Investigations aimed at determining the genomic breakpoint of the BRCA1 exon 2 and exon 8 deletions described in our study are on going.
### Table 4
Phenotypic expression in Algerian breast/ovarian patients with newly identified BRCA2 UVs and polymorphisms

| Patient ID | Clinical status and age at onset of the proband or the tested relative | Affected family members | Sequence variation (cDNA/protein) | Interpretation |
|------------|---------------------------------------------------------------------|-------------------------|----------------------------------|----------------|
| 20670      | BC, dx 43y                                                          | sister                  | c.67+14T>G/p.?                   | UV             |
| 20825      | BBC, dx 36y                                                         | 6 sisters aunt (P)      | c.67+14T>G/p.?                   | UV             |
| 20670      | BC, dx 43y                                                          | sister                  | c.67+15T>G/p.?                   | UV             |
| 20825      | BBC, dx 36y                                                         | 6 sisters aunt (P)      | c.67+15T>G/p.?                   | UV             |
| 20824*     | BBC, dx 33y                                                         | 6 sisters aunt (P)      | c.68-14T>A/p.?                   | UV             |
| 20824*     | BBC, dx 33y                                                         | 6 sisters aunt (P)      | c.68-21T>G/p.?                   | UV             |
| 20824*     | BBC, dx 33y                                                         | 6 sisters aunt (P)      | c.231T>G/p.=                     | SP             |
| 20810      | BC, dx 39                                                          | Data not available      | c.3555A>T/p.=                   | SP             |
| 2081      | BC, dx 21y                                                         | 2 aunts (M) grandmother (M) | c.3555A>T/p.=                 | SP             |
| 2075      | BC, dx 41y                                                         | half sister grandaunt (P) | mother                          | SP             |
| 2074      | BC, dx 25y                                                         | aunt (M)                | c.3868T>A/p.Cys1290Ser          | UV             |
| 20816      | OC, dx 61y                                                         | mother                  | c.5553C>T/p.=                   | SP             |
| 20819      | OC, dx 54                                                          | Data not available      | c.5472T>G/p.Asp1824Lys          | UV             |
| 2074      | BC, dx 25y                                                         | 3 cousins (M), grand aunt (M) | c.5976A>G/p.=                 | UV             |
| 2066      | BBC, dx 32y                                                         | 3 cousins (M), grand aunt (M) | c.5985C>A/p.Asn1995Lys         | UV             |
| 20670      | BC, dx 43y                                                         | sister                  | c.5985C>A/p.Asn1995Lys          | UV             |
| 2074      | BC, dx 25y                                                         | aunt (M)                | c.5985C>A/p.Asn1995Lys          | UV             |
| 20810      | BC, dx 39                                                          | Data not available      | c.5985C>A/p.Asn1995Lys          | UV             |
| 20817      | BC, dx 67y                                                         | Data not available      | c.8487+19A>C/p.?                | UV             |

BC: breast cancer, BBC: bilateral breast cancer, OC: ovarian cancer, dx: age at diagnosis, M: maternal, P: paternal, SP: synonymous polymorphism, UV: unclassified variant, p.? : protein has not been analyzed, unknown effect at protein level, p.= : no amino acid change, *: sister of the patient 20825.

### Table 5
The amino acid properties of new unclassified variants in BRCA1 and BRCA2 genes within Algerian breast/ovarian cancer patients

| Gene     | Amino acid change | Number of families harboring the variant | PolyPhen² | Pathogenicity | Prevalence in controls (%) | Different species with conserved sequences |
|----------|-------------------|------------------------------------------|-----------|---------------|----------------------------|------------------------------------------|
| BRCA1    | p.Gln 1356 Lys    | 1                                        | 1.679     | Possibly damaging | 0                          | $g^a,b,c,d,e,f,g,h,i,j$                      |
| BRCA2    | p.Ser 1013Thr     | 1                                        | 1.323     | Benign        | ND                        | $g^a,b,c,d,e,f,g,h,i,j,m,p$                 |
| BRCA2    | p.Cys 1290 Ser    | 1                                        | 2.717     | Probably damaging | 0                          | $g^a,b,c,d,e,f,g,h,i,j,m,o,p$               |
| BRCA2    | p.Asp 1824 Lys    | 1                                        | 1.737     | Possibly damaging | 0                          | $g^a,b,c,d,e,f,g,h,i,j,m,o,p$               |
| BRCA2    | p.Asp 1864 Glu    | 1                                        | 1.245     | Benign        | ND                        | $g^a,b,c,d,e,f,g,h,i,j,m,o,p$               |
| BRCA2    | p.Asn 1995 Lys    | 4                                        | 1.702     | Possibly damaging | 0                          | $g^a,b,c,d,e,f,g,h,i,j,m,o,p$               |

*PSIC score difference: 1.5–2: possibly damaging substitution; > 2: probably damaging substitution.

a = human, b = chimpanzee, c = gorilla, d = orangutan, e = macaque, f = mouse, g = dog, h = cow, i = opossum, j = chicken, k = frog, l = tetraodon, m = rat, n = rabbit, o = cat, p = armadillo.

ND: not determined.
Furthermore, in this study we note that exons 2 and 8 deletions in \textit{BRCA1} gene have been identified in bilateral breast cancer and breast/ovarian cancer patients, respectively. Deletions of exons 1a-2, exons 2 and 3 or exon 8 in \textit{BRCA1} gene have already been reported in patients from Holland, Italy and Germany, respectively [13,22,23]. Interestingly, most of these patients developed bilateral breast cancer or breast/ovarian cancer. The exon1a-2 deletions of \textit{BRCA1} are relatively frequent in breast/ovarian cancer families [23]. Our results reinforce the idea that bilateral breast cancer and breast/ovarian cancer families tested negatively for small point mutations should be routinely screened with MLPA for large rearrangements.

We found in one family with a strong history of hereditary bilateral/ovarian cancer, a rare pathogenic mutation in \textit{BRCA1} exon 3, c.83,84delTG (cited one time in BIC database and described in one Caucasian family). Interestingly, this mutation has been found in family from Kabylia, a region located in the mountains of the north of Algeria, whose population shows genetic peculiarity due to geographical isolation. This mutation could be specific to Algerian population. The screening for this rare mutation in extended breast/ovarian cancer families from Kabylia could help in the identification of specific effect.

We note that the pathogenic mutations described in Algerian breast/ovarian cancer families in this report differ from that seen in Tunisian population despite that this study was also performed in Arabic/Berber population [20,21]. These findings could suggest a large \textit{BRCA1} and \textit{BRCA2} mutations spectrum in North African populations. The novel pathogenic germline mutation \textit{BRCA1} exon 2 deletion and the rare mutation c.83,84delTG in \textit{BRCA1} exon 3, and as well as the variety of new missense variations found in our present study, may therefore be specific for the Algerian population. In addition, it is likely that the new four UVs identified in this study (1 \textit{BRCA1} and 3 \textit{BRCA2}) with a damaging PSIC score yielded by PolyPhen (see Table 5), may have a functional role in breast cancer development, which deserves to be explored further. The accumulating knowledge about the prevalence and nature of \textit{BRCA1} and \textit{BRCA2} mutations in Algerian population will contribute to the assessment of the necessity of the preventive program for mutation carriers as part of the national public health policy in Algeria.

5. Conclusion

In this report, we attempted to delineate genetic component of hereditary breast/ovarian cancer among the Algerian population. For the first time, we used HRM and MLPA to identify \textit{BRCA1} and \textit{BRCA2} mutations in Algerian patients with a personal and family history suggestive of genetic predisposition to breast cancer. The implications of these new findings in regard to genetic testing and counseling are substantial for the Algerian population.

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