BRCA1 and BRCA2 unclassified variants and missense polymorphisms in Algerian breast/ovarian cancer families

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Abstract. Background: BRCA1 and BRCA2 germline mutations predispose heterozygous carriers to hereditary breast/ovarian cancer. However, unclassified variants (UVs) (variants with unknown clinical significance) and missense polymorphisms in BRCA1 and BRCA2 genes pose a problem in genetic counseling, as their impact on risk of breast and ovarian cancer is still unclear. The objective of our study was to identify UVs and missense polymorphisms in Algerian breast/ovarian cancer patients and relatives tested previously for BRCA1 and BRCA2 genes germline mutations analysis.

Methods: We analyzed 101 DNA samples from 79 breast/ovarian cancer families. The approach used is based on BRCA1 and BRCA2 sequence variants screening by SSCP or High-Resolution Melting (HRM) curve analysis followed by direct sequencing. In silico analyses have been performed using different bioinformatics programs to individualize genetics variations that can disrupt the BRCA1 and BRCA2 genes function.

Results: Among 80 UVs and polymorphisms detected in BRCA1/2 genes (33 BRCA1 and 47 BRCA2), 31 were new UVs (10 BRCA1 and 21 BRCA2), 7 were rare UVs (4 BRCA1 and 3 BRCA2), and 42 were polymorphic variants (19 BRCA1 and 23 BRCA2). Moreover, 8 new missense UVs identified in this study: two BRCA1 (c.4066C>A/p.Gln1356Lys, c.4901G>T/p.Arg1634Met) located respectively in exons 11 and 16, and six BRCA2 (c.1099G>A/p.Asp367Asn, c.2636C>A/p.Ser879Tyr, c.3868T>A/p.Cys1290Ser, c.5428G>T/p.Val1810Phe, c.6346C>G/p.Ser2116Asp and c.9256G>A/p.Gly3086Arg) located respectively in exons 10, 11 and 24, show a damaging PSIC score yielded by PolyPhen2 program and could be pathogenic. In addition, 5 new BRCA2 missense UVs out of six that were found to be damaging by PolyPhen2 program, also were deleterious according to SIFT program. The rare BRCA1 UV c.5332G>A/p.Asp1778Asn was found here for the first time in co-occurrence in trans with the deleterious BRCA1 mutation c.798\textsuperscript{−}799delTT/p.Ser267LysfsX19 in young breast cancer patient. Moreover, 10 new identified intronic variants with unknown clinical significance (3 BRCA1 and 7 BRCA2) in the present study, could be considered as benign, because GeneSPLICer, SpliceSiteFinder and MaxEntScan prediction programs show no splice site alteration for these variants. Several missense polymorphisms of BRCA1 c.2612C>T/p.Pro871Leu, c.3548A>G/p.Lys1183Arg, c.4837A>G/p.Ser1613Gly and BRCA2 c.865A>C/p.Asn289His, c.1114A>C/p.Asn372His, c.2971A>G/p.Asn991Asp, c.7150C>A/p.Gly2384Lys have been identified with high frequency in patients who were tested negative for BRCA1 and BRCA2 mutations. These missense polymorphisms could have a role as susceptibility breast cancer markers in Algerian breast/ovarian cancer families where pathological BRCA1 and BRCA2 mutations were not present.

Conclusions: For the first time, UVs and missense polymorphisms in BRCA1 and BRCA2 genes have been identified in Algerian breast/ovarian cancer families. Evaluation of breast/ovarian cancer risk induced by the eight new missense UVs and common polymorphisms detected in our present work is on going in a larger study.

Keywords: Algeria, BRCA1, BRCA2, breast/ovarian cancer, HRM, polymorphisms, SNP, UVs

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1. Introduction

Germline mutations in BRCA1 and BRCA2 genes predispose women to breast and ovarian cancer [1, 2]. The screenings of index cases with hereditary breast/ovarian cancer have detected other BRCA1 and BRCA2 sequence variants called variants with unknown clinical significance or unclassified variants (UVs) and missense polymorphisms. To date, up to 10–20% of patients screened are found to carry UVs [3]. UVs are mainly missense mutations, but also include a number of silent variants, intronic variants and in-frame deletions and insertions [3]. The classification of the BRCA1 and BRCA2 UVs as pathogenic or neutral pose a problem, because it is not known whether these subtle changes alter the function of the proteins sufficiently to predispose to breast and/or ovarian cancer [4]. Classification of these UVs as neutral or disease causing is important for genetic counseling. Different criteria have been used to help classify these UVs. These include evaluation of co-segregation of the variant with disease in families, observed co-occurrence of UVs in trans phase with known pathogenic mutations, evaluation of the frequency of UVs in healthy controls, analyses of the severity of the amino acid change and its conservation across species [5]. In addition, the contribution of BRCA1 and BRCA2 missense polymorphisms to breast and ovarian cancer risk remains largely unclear and pose a problem in genetic counseling [6]. Interestingly, several of the BRCA1 and BRCA2 genes missense polymorphisms are located in functional domains of BRCA1 and BRCA2 proteins known to be interaction sites for key partner proteins and many of the amino acids concerned are conserved across many species.

To date, few molecular genetics studies of BRCA1 and BRCA2 sequence variants screening have been reported in the Algerian population [7, 8]. The aim of our present study is to identify UVs and polymorphic variants in BRCA1 and BRCA2 genes in Algerian breast/ovarian cancer families tested previously for BRCA1 and BRCA2 germline mutations screening.

2. Materials and methods

This study was performed to identify UVs and polymorphic variants in the BRCA1 and BRCA2 genes in Algerian breast/ovarian cancer patients and their relatives. The approach used is based on BRCA1 and BRCA2 sequence variants screening by SSCP or High-Resolution Melting (HRM) curve analysis followed by direct sequencing. In silico analyses have been performed using different bioinformatics programs to individualize genetics variations that can disrupt the BRCA1 and BRCA2 genes function.

2.1. Patients

The patients and their families were referred through the Anti Cancer Center of Blida, the Central Hospital of Algiers, and five 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 Tables 1 and 2. Prior collecting blood sample, all selected patients and relatives were informed about the objectives of our study and that their DNA samples would be analyzed for mutations in genes associated with hereditary breast/ovarian 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 PromegaWizard Genomic DNA Purification Kit, (Promega, Madison, MI, USA) (Cat. # A1120) and in accordance with the manufacturer’s protocols.

2.3. Sequence variants analysis

2.3.1. SSCP analysis

We analyzed BRCA1 exons 2 and 11 of 15 individuals from 9 breast/ovarian cancer families by PCR-SSCP technique and direct sequencing. The PCR and SSCP assays were performed as described elsewhere [9].
2.3.3. DNA sequencing on request. primers and PCR-HRM assay conditions are available (Roche Diagnostics, Manheim, Germany). The PCR reported [10] using the LightCycler 480 II Instrument PCR and HRM assays were performed as previouslymitted to prescreening with HRM curve analysis. The Manheim, Germany). All coding exons of Resolution Melting Master Kit (Roche Diagnostics, BRCA2 including flanking intronic regions were sub-

missional nomenclature follows the rule where the nu-
cleotide +1 is the A of the ATG translation initiation codon.

2.3.4. Sequence variation nomenclature

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 description of nucleotide sequence variants is in accordance with HGVS (Human Genome Variation Society) nomenclature (www.hgvs.org/mutnomen). The HGVS approved systematic nomenclature follows the rule where the nucleotide +1 is the A of the ATG translation initiation codon.

2.3.2. High-Resolution Melting (HRM) curve analysis

Complete screening of BRCA1 and BRCA2 sequence variants were performed in 86 individuals from 70 breast/ovarian cancer families by PCR-HRM followed by direct sequencing. PCR reactions were performed in a 20 µl final volume using Light Cycler-480 High Resolution Melting Master Kit (Roche Diagnostics, Manheim, 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 previously reported [10] using the LightCycler 480 II Instrument (Roche Diagnostics, Manheim, Germany). The PCR primers and PCR-HRM assay conditions are available on request.

2.3.5. In silico analyses

The software Alamut 1.4 (http://www.interactive-biosoftware.com/alamut.html) has been used for the interpretation of the new sequence variants and for the detection of splicing aberrations caused by the new unclassified variants detected in our present study. This software includes three bioinformatics programs: Genesplicer (http://www.tigr.org/tdb/GeneSplicer), Max EntScan (http://genes.mit.edu/burgelab/maxent/ Xmax entscan_scoreseq.html) and SpliceSiteFinder (http:// violin.genet.sickkids.on.ca/~ali/splicesitefinder.html) for prediction of donor and acceptor site. To identify no synonymous amino acid changes likely to disrupt BRCA1 and BRCA2 genes function, we used two bioinformatics programs, Polymorphism Phenotyping 2 (http://genetics.bwh.harvard.edu/pph2) and SIFT program (http://sift.jcvi.org).

PolyPhen2 is a tool which predicts possible impact of an amino acid substitution on the structure and function of a human protein using straightforward physical and comparative considerations. SIFT is a sequence homology-based tool that sorts intolerant from tolerant amino acid substitutions and predicts whether an amino acid substitution in a protein will have a phenotypic effect.

Evolutionary conservation of BRCA1 and BRCA2 sites of amino acid changes was evaluated across 13 species among the following: human, chimpanzee, gorilla, orangutan, macaque, mouse, dog, cow, opossum, chicken, frog, tetraodon, rat, rabbit, cat, and armadillo.

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’ in-
dividuals without breast or ovarian cancer familial history.

3. Results

In the present study, we screened 101 individuals from 79 families for UVs and common polymorphisms in BRCA1 and BRCA2 genes. To date, 86 individuals
| Patient ID | Clinical status and age at onset of the proband or the tested relative | Affected family members |
|------------|--------------------------------------------------------------------------------|-------------------------|
| 2051       | BC, 38y                                                                         | mother                  |
| 2074       | BC, 25y                                                                         | aunt (M)               |
| 2075       | BC, 41y                                                                         | mother, aunt (M)        |
|            |                                                                                | half sister             |
|            |                                                                                | grandaunt (P)          |
| 20933      | BC, 39y                                                                         | sister                  |
|            |                                                                                | aunt (P)               |
|            |                                                                                | cousin (P)             |
| 2052       | BC, 50y                                                                         | sister                  |
|            |                                                                                | 2 nieces               |
| 20671      | BC, 51y                                                                         | NA                     |
| 2093       | OC and BC, 52y                                                                  | 3 sisters              |
|            |                                                                                | sister                  |
|            |                                                                                | uncle (P)              |
| 2073       | BC, 54y                                                                         | 2 cousins (P)           |
|            |                                                                                | mother                 |
|            |                                                                                | cousin (M)             |
|            |                                                                                | cousin (P)             |
|            |                                                                                | grandaunt (P)          |
| 2085       | BC, 36y                                                                         | NA                     |
| 20816      | OC, 61y                                                                         | mother                 |
| 2086       | MBC, 74y                                                                        | NA                     |
| 20913      | MBC, 76y                                                                        |                        |
| 20670      | BC, 43y                                                                         | sister                  |
| 20825      | BBC, 36y                                                                        | 6 sisters              |
|            |                                                                                | aunt (P)               |
|            |                                                                                | cousin (P)             |
| 20824      | BBC, 33y                                                                        | 6 sisters              |
|            |                                                                                | aunt (P)               |
|            |                                                                                | cousin (P)             |
| 20925      | BC, 78y                                                                         | daughter               |
|            |                                                                                | granddaughter           |
|            |                                                                                | great-granddaughter    |
|            |                                                                                | son                    |
| 20935      | BC, 32y                                                                         | cousin                 |
|            |                                                                                | sister                 |
|            |                                                                                | sister                 |
|            |                                                                                | father                 |
| 20939      | BC, 38y                                                                         | Aunt (M)               |
| 20924      | BC, (?7y)                                                                       | 2 sisters              |
| 20810      | BC, 39y                                                                         | NA                     |
| 2081       | BC, 21y                                                                         | 2 aunts (M)            |
|            |                                                                                | grandmother (M)        |
| 2075       | BC, 41y                                                                         | mother                 |
|            |                                                                                | half sister             |
|            |                                                                                | grandaunt (P)          |
| 2074       | BC, 25y                                                                         | aunt (M)               |
| 20816      | OC, 61y                                                                         | mother                 |
| 2066       | BBC, 32y                                                                        | 3 cousins (M),         |
|            |                                                                                | grand aunt (M)         |
|            |                                                                                | cousin (M)             |
| 20817      | BC, 67y                                                                         | NA                     |
| 20937      | BC, 42y                                                                         | mother                 |
|            |                                                                                | Sister                 |
| 2092       | BC, 47y                                                                         | Cousin (M)             |
|            |                                                                                | 2 sisters              |
|            |                                                                                | cousin (M)             |
|            |                                                                                | 2 cousins (P)          |
|            |                                                                                | niece                  |
| 2092       | BC, 47y                                                                         | 2 sisters              |
|            |                                                                                | cousin (M)             |
|            |                                                                                | 2 cousins (P)          |

**Table 2**

Phenotypic expression in families/patients within *BRCA1* and *BRCA2* UVs and common polymorphisms.
| Patient ID | Clinical status and age at onset of the proband or the tested relative | Affected family members |
|------------|-------------------------------------------------|-------------------------|
| 2067       | BC, 36y                                         | mother, 2 sisters brother niece cousin (P) |
| 2068       | BC, 44y                                         | mother, 3 sisters niece cousin (P) |
| 20910      | BC, 18y                                         | mother sister aunt (M) cousin (M) |
| 20916      | BC, 32y                                         | 2 sisters Aunt (P) mother daughter granddaughter |
| 20920      | BC, 26y                                         | niece |
| 20926      | BC, 37y                                         | brother (P) |
| 20929      | BC, 27y                                         | sister aunt (P) |
| 20921      | BC, 35y                                         | 3 sisters aunt (M) |
| 20917      | BC, 40y                                         | Father Uncle (P) |
| 20940      | BC, 37y                                         | NA |
| 20914      | BC, 36y                                         | sister cousin (P) |
| 20911      | BBC, 50y                                        | NA |
| 20924      | BC, 50y                                         | niece |
| 20934      | BC (?), 29y                                     | NA |
| 20942      | BC, 18y                                         | sister mother aunt (M) cousin (M) |
| 20944      | BBC, 33y                                        | 2 sisters uncle (P) |
| 2095       | BC, 30y                                         | 2 aunts (M) mother sister grandmother (M) aunt (M) |
| 2096       | BC (?), 29y                                     | mother sister grandmother (M) aunt (M) |
| 2082       | BOC, 40y                                        | aunt (P) |
| 20821      | BBC, 35y                                        | sister aunt (M) aunt (P) |
| 2091       | BBC, 47y                                        | cousin (P) |
| 2097       | BC, 34y                                         | grand mother (P) |
| 20822      | MBC, 65y                                        | aunt (M) |
| 20823      | BC, 64y                                         | mother |
| 20820      | BBC, 55y                                        | mother |
| 2083       | BC, 55y                                         | 2 nieces |
| 2071       | BC, 30y                                         | sister cousin (P) |
| 20670      | BC, 43y                                         | sister |

BC: breast cancer, BBC: bilateral breast cancer, BOC: breast/ovarian cancer, MBC: male breast cancer, OC: ovarian cancer, M: maternal, P: paternal, y: years, (?): age unknown. NA: data not available.
Table 3
New unclassified variants in *BRCA1* and *BRCA2* genes within Algerian breast/ovarian cancer families/patients

| Gene | Exon | Sequence variant | Predicted effect at protein level | Number of families/patients harboring the variant | PolyPhen2* | Pathogenicity | SIFT** | Pathogenicity | Frequency in controls (%)* | N = 80 | Different species with conserved sequences |
|------|------|------------------|-----------------------------------|------------------------------------------------|-------------|---------------|--------|---------------|--------------------------|-------|----------------------------------------|
| BRCA1 | 2    | c.16C>G          | p.Leu6Val                         | 1                                              | 0.000      | Benign         | 0.00   | Deleterious   | ND                       |       | 3a,b,c,     |
| BRCA1 | 2    | c.80+56A>C       | p.?                              | 1                                              |            |               |        |               |                          |       |                         |
| BRCA1 | 7    | c.302-3C>T       | p.=?                             | 1                                              |            |               |        |               |                          |       |                         |
| BRCA1 | 11   | c.2748T>C        | p.=                              | 1                                              |            |               |        |               |                          |       |                         |
| BRCA1 | 11   | c.3114A>G        | p.=                              | 1                                              |            |               |        |               |                          |       |                         |
| BRCA1 | 11   | c.4066C>A        | p.Gln1356Lys                      | 1                                              | 0.256      | Possibly damaging | 0.50 | Tolerated     | 0/100                    |       | g,b,c,d,f,i,g,j,     |
| BRCA1 | 12   | c.4113G>A        | p.=                              | 1                                              |            |               |        |               |                          |       |                         |
| BRCA1 | 12   | c.4185+47T>C     | p.?                              | 2                                              |            |               |        |               |                          |       |                         |
| BRCA1 | 16   | c.4901G>T        | p.Arg1634Met                      | 1                                              | 0.588      | Possibly damaging | 0.20 | Tolerated     | ND                       |       | 5a,b,c,d,e,      |
| BRCA1 | 19   | c.5175A>G        | p.=                              | 2                                              |            |               |        |               |                          |       |                         |
| BRCA2 | 2    | c.67+14T>C       | p.?                              | 2                                              |            |               |        |               |                          |       |                         |
| BRCA2 | 2    | c.67+1ST>C       | p.?                              | 2                                              |            |               |        |               |                          |       |                         |
| BRCA2 | 2    | c.68-16T>A       | p.?                              | 1                                              |            |               |        |               |                          |       |                         |
| BRCA2 | 2    | c.68-21T>G       | p.?                              | 1                                              |            |               |        |               |                          |       |                         |
| BRCA2 | 3    | c.21T>G          | p.=?                             | 1                                              |            |               |        |               |                          |       |                         |
| BRCA2 | 5    | c.475+25A>G      | p.?                              | 1                                              |            |               |        |               |                          |       |                         |
| BRCA2 | 9    | c.794-5A>T       | p.?                              | 1                                              |            |               |        |               |                          |       |                         |
| BRCA2 | 10   | c.1099G>A        | p.Asp367Asn                       | 1                                              | 0.985      | Probably damaging | 0.42 | Tolerated     | ND                       |       | 7a,b,c,d,e,f,r,o,g,     |
| BRCA2 | 11   | c.2636C>A        | p.Ser879Tyr                       | 1                                              | 0.004      | Benign         | 0.01   | Deleterious   | ND                       |       | 4a,b,c,i,      |
| BRCA2 | 11   | c.2673A>G        | p.Asn886Ser                      | 1                                              | 0.995      | Probably damaging | 0.00 | Deleterious   | ND                       |       | 8a,b,c,d,e,f,m,a,o,g,     |
| BRCA2 | 11   | c.3555A>T        | p.=?                             | 1                                              |            |               |        |               |                          |       |                         |
| BRCA2 | 11   | c.3868T>A        | p.Cys1290Ser                      | 1                                              | 0.408      | Possibly damaging | 0.00 | Deleterious   | 0/100                    |       | g,b,c,d,e,f,m,a,p,     |
| BRCA2 | 11   | c.5397A>T        | p.=?                             | 1                                              |            |               |        |               |                          |       |                         |
| BRCA2 | 11   | c.5428G>T        | p.Val810Phe                       | 1                                              | 0.877      | Probably damaging | 0.00 | Deleterious   | ND                       |       | 7a,b,c,d,e,f,r,o,g,     |
| BRCA2 | 11   | c.5553C>T        | p.=?                             | 2                                              |            |               |        |               |                          |       |                         |
| BRCA2 | 11   | c.5976A>G        | p.=?                             | 1                                              |            |               |        |               |                          |       |                         |
| BRCA2 | 11   | c.6309A>C        | p.=?                             | 1                                              |            |               |        |               |                          |       |                         |
| BRCA2 | 11   | c.6346C>G        | p.His2116Asp                      | 1                                              | 0.996      | Probably damaging | 0.00 | Deleterious   | ND                       |       | 6a,b,c,d,e,f,m,a,o,g,i,     |
| BRCA2 | 19   | c.8487+19A>C     | p.?                              | 1                                              |            |               |        |               |                          |       |                         |
| BRCA2 | 24   | c.9256G>A        | p.Gly3086Arg                      | 1                                              | 0.999      | Possibly damaging | 0.00 | Deleterious   | ND                       |       | 10a,b,c,d,e,f,r,o,g,i,j,s,     |

p.? : protein has not been analyzed, unknown effect at protein level, p. = : no amino acid change, *PSIC score difference: >0.15: possibly damaging substitution; >0.85: probably damaging substitution, **SIFT score: ranges from 0 to 1. The amino acid substitution is predicted damaging (not tolerated) if the score is <=0.05, and tolerated if the score is >0.05. 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, z = zebrafish. ND: not determined.
from 70 Algerian breast/ovarian cancer families have been tested previously for complete BRCA1 and BRCA2 germline mutations screening [8]. According to classification in BIC database and by using Alamut 1.4 software for the interpretation of the new sequence variants, we identified 80 UVs and polymorphisms in BRCA1 and BRCA2 genes (33 BRCA1 and 47 BRCA2). (Tables 3, 4 and 5).

3.1. BRCA1 and BRCA2 UVs

We detected 31 new UVs (10 BRCA1 and 21 BRCA2) and 7 rare UVs (4 BRCA1 and 3 BRCA2) in BRCA1 and BRCA2 genes of 47 families (Tables 3 and 4). We note that 10 new identified intronic variants with unknown clinical significance (3 BRCA1 and 7 BRCA2) could be considered as benign, because GeneSplicer, MaxEntScan and SpliceSiteFinder prediction programs show no splice alteration site for these variants. In addition, GeneSplicer, MaxEntScan and SpliceSiteFinder prediction programs show no splicing aberrations for new BRCA1 and BRCA2 missense variants identified in our present study. Interestingly, for the two new silent variants with unknown clinical significance $\text{BRCA1 (c.4113G>A)}$ located in exon12 and $\text{BRCA2 (c.6309A>C)}$ located in exon11, the 3 prediction programs for donor or acceptor site show the creation of new donor splice site in exonic region for both. Studies by using RNA analyses are necessary to determine or confirm use of these new donor splice sites and defining if aberrant transcripts are associated with these two new silent variants $\text{BRCA1 (c.4113G>A)}$ and $\text{BRCA2 (c.6309A>C)}$ with unknown clinical significance.

Three new BRCA1 missense variants were identified respectively in exon 2 (c.16C>G/p.Leu6Val), exon 11 (c.4066C>A/p.Gln1356Lys) and exon 16 (c.4901G>T/p.Arg1634Met) (Table 3). The c.4066C>A/p.Gln1356Lys and c.4901G>T/p.Arg1634Met BRCA1 missense substitutions, identified respectively in two unrelated patients with early onset breast cancer, are possibly damaging because PolyPhen2 yielded a damaging PSIC scores of 0.256 and 0.588 respectively (Table 3). However, these two new $\text{BRCA1}$ UVs were observed to be not deleterious by the SIFT program, having a tolerance index score $> 0.05$ (Table 3).

We identified 7 new missense variants in the $\text{BRCA2}$ exon 10, 11 and 12 respectively (Table 3), six out seven being located in the DNA repair recombination protein domain (c.1099G>A/p.Asp367Asn, c.2636C>A/p.Ser879Tyr, c.2657A>G/p.Asn886Ser, c.3868T>A/p.Cys1290Tyr, c.5428G>T/p.Val1810Phe, c.6346C>G/p.His2116Asp) and one (c.9256G>A/p.Gly3086Arg) located in the DNA binding domain and the DNA recombination repair protein domain. By using PolyPhen2 program and SIFT program, 6 new variants out seven were identified to disrupt $\text{BRCA2}$ function and to be deleterious respectively (Table 3). We note that these 7 new $\text{BRCA2}$ missense variants have been identified in patients with a family history of breast/ovarian cancer and each variant has been found in one family. Furthermore, associated with the damaging PSIC scores, two newly identified UVs were found at a frequency of $< 1\%$ in the control population (Table 3).

3.2. Polymorphisms in BRCA1 and BRCA2 genes

In this work, we detected 42 polymorphic variants (19 $\text{BRCA1}$ and 23 $\text{BRCA2}$) (Table 5). Missense polymorphisms in $\text{BRCA1}$ gene ($\text{BRCA1}$ c.1067A>G/p.Gln356Arg, c.2612C>T/p.Pro871Leu, c.3548A>G/p.Lys1183Arg, c.4837A>G/p.Ser1613Gly) and $\text{BRCA2}$ gene ($\text{BRCA2}$ c.865A>C/p.Asn289His, c.1114A>C/p.Asn372His, c.2971A>G/p.Asn911Asp, c.7150C>A/p.Gln2384Lys) have been identified with high frequency in patients who tested negative for $\text{BRCA1}$ and $\text{BRCA2}$ mutations.

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### Table 4

| Gene  | Exon | Sequence variant | Predicted effect at protein level | Interpretation | Number of families harboring the variant | BIC* |
|-------|------|-----------------|----------------------------------|----------------|----------------------------------------|------|
| $\text{BRCA1}$ | 2    | c.19C>T         | p.Arg7Cys                        | UV             | 1                                      | 4    |
| $\text{BRCA1}$ | 2    | c.43A>C         | p.Ile15Leu                       | UV             | 1                                      | 1    |
| $\text{BRCA1}$ | 18   | c.5117G>C       | p.Gly1706Ala                     | UV             | 3                                      | 6    |
| $\text{BRCA1}$ | 21   | c.5332G>A       | p.Asp1778Asn                     | UV             | 2                                      | 1    |
| $\text{BRCA2}$ | 11   | c.3869G>A       | p.Cys1290Tyr                     | UV             | 1                                      | 2    |
| $\text{BRCA2}$ | 11   | c.5704G>A       | p.Asp1902Asn                     | UV             | 1                                      | 9    |
| $\text{BRCA2}$ | 12   | c.6892G>A       | p.Glu2299Lys                     | UV             | 1                                      | 2    |

UV: Unclassified variant, *= number of times reported in BIC database (http://research.nhgri.nih.gov/bic/index.shtml).
| Gene | Exon | Sequence variant | Predicted effect at protein level | Frequency (%) | BIC* | dbSNP** rs number | dbSNP** MAF (population) |
|------|------|------------------|----------------------------------|---------------|------|--------------------|--------------------------|
| BRCA1 | 8    | c.442-34C>T      | p.?                              | 13.95         | 1    | rs799923           | (HapMap-CEU) 0.257       |
| BRCA1 | 11   | c.1067A>G        | p.Gln356Arg                       | 9.3           | 82   | rs1799950          | (HapMap-CEU) 0.049       |
| BRCA1 | 11   | c.2077G>A        | p.Asp693Asn                       | 1.16          | 16   | rs4986850          | (HapMap-CEU) 0.079       |
| BRCA1 | 11   | c.2082C>T        | p.=?                             | 13.90         | 14   | rs1799949         | (HapMap-CEU) 0.280       |
| BRCA1 | 11   | c.2311T>C        | p.=?                             | 22.06         | 25   | rs16940            | (HapMap-CEU) 0.161       |
| BRCA1 | 11   | c.2521C>T        | p.Arg841Trp                       | 1.16          | 114  | rs1800709          | (HapMap-CEU) 0.011       |
| BRCA1 | 11   | c.2612C>T        | p.Pro871Leu                       | 30.22         | 26   | rs799917           | (HapMap-CEU) 0.336       |
| BRCA1 | 11   | c.2733A>G        | p.=?                             | 1.16          | 0    | rs1800740          | (CAU) 0                  |
| BRCA1 | 11   | c.3113A>G        | p.Glu1038Gly                      | 29.06         | 37   | rs16941            | (HapMap-CEU) 0.329       |
| BRCA1 | 11   | c.3119G>A        | p.Ser1040Asn                      | 2.32          | 45   | rs4986852          | (HapMap-CEU) 0.058       |
| BRCA1 | 11   | c.3418A>G        | p.Ser1140Gly                      | 1.16          | 28   | rs227945           | (HapMap-CEU) 0.005       |
| BRCA1 | 11   | c.3548A>G        | p.Lys1183Arg                      | 31.39         | 33   | rs16942            | (HapMap-CEU) 0.022       |
| BRCA1 | 13   | c.4308T>C        | p.=?                             | 26.74         | 249  | rs1060915          | (HapMap-CEU) 0.332       |
| BRCA1 | 16   | c.4837T>C        | p.Ser1613Gly                      | 24.41         | 247  | rs1799966          | (HapMap-CEU) 0.314       |
| BRCA2 | 2    | c.-26G>A         | p.?                              | 15            | 12   | rs1799943          | (HapMap-CEU) 0.173       |
| BRCA2 | 10   | c.865A>C         | p.Asn289His                       | 3.48          | 13   | rs766173           | (HapMap-CEU) 0.053       |
| BRCA2 | 10   | c.1114A>C        | p.Asn372His                       | 5.8           | 9    | rs144848           | (HapMap-CEU) 0.009       |
| BRCA2 | 10   | c.1365A>G        | p.=?                             | 3.4           | 7    | rs1801439          | (HapMap-CEU) 0.031       |
| BRCA2 | 10   | c.1909+22delT    | p.?                              | 1.1           | 0    | –                  | (HapMap-CEU) NFD         |
| BRCA2 | 11   | c.2971A>G        | p.Asn991Asp                      | 5.8           | 6    | rs1799944          | (HapMap-CEU) 0.031       |
| BRCA2 | 11   | c.3396A>G        | p.=?                             | 6.9           | 8    | rs1801406          | (HapMap-CEU) 0.027       |
| BRCA2 | 11   | c.3807T>C        | p.=?                             | 4.6           | 3    | rs543304           | (HapMap-CEU) 0.195       |
| BRCA2 | 11   | c.4068G>A        | p.=?                             | 3.4           | 1    | rs28897724         | (HapMap-CEU) 0.239       |
| BRCA2 | 11   | c.4563A>G        | p.=?                             | 3.4           | 2    | rs206075           | (HapMap-CEU) 0.995       |
| BRCA2 | 11   | c.5744C>T        | p.Thr1915Met                     | 1.1           | 7    | rs4987117          | (HapMap-CEU) 0.032       |
| BRCA2 | 11   | c.6513G>C        | p.=?                             | 10.40         | 1    | rs206076E          | (HapMap-CEU) 0.005       |
| BRCA2 | 14   | c.7150C>A        | p.Gln2384Lys                     | 13.90         | 31   | rs55977008         | (HapMap-CEU) 0.083       |
| BRCA2 | 14   | c.7242A>G        | p.=?                             | 3.4           | 10   | rs1799955          | (HapMap-CEU) 0.190       |
| BRCA2 | 14   | c.7397C>T        | p.Ala2466Val                     | 1.1           | 48   | rs169547           | (HapMap-CEU) 0.014       |
4. Discussion

To date, very few reports have been published about the spectrum of BRCA1 and BRCA2 sequence variants in the Algerian population [7,8]. A total of 101 individuals from 79 breast cancer families have been examined for UVs and polymorphisms in the BRCA1 and BRCA2 genes. We note that UVs were more frequent in BRCA2 (24 different UVs) than in BRCA1 (14 different UVs). In all families where UV was identified, there was a family history of breast cancer/ovarian cancer. 8 new missense UVs identified in our present study (2 BRCA1 and 6 BRCA2) show a damaging PSIC score yielded by PolyPhen2 (Table 3) and could have a functional role in breast/ovarian cancer development, which deserves to be explored further. Furthermore, 5 new missense BRCA2 UVs out six that were found to be damaging by PolyPhen2 program also were deleterious according to SIFT program (Table 3). Hence, we could infer that results obtained for new BRCA2 UVs by PolyPhen2 were in good correlation with the results found by SIFT program.

Interestingly, the rare BRCA1 UV c.5332G>A/p. Asp1778Asn was found here for the first time in co-occurrence in trans with the deleterious BRCA1 mutation c.798_799delTT/p.Ser267LysfsX19 in young breast cancer patient with a strong breast cancer history (patient 2095, see Table 2). The rare UV c.5332G>A/p. Asp1778Asn could be neutral because co-inheritance in trans phase of two pathogenic mutations in BRCA1 or BRCA2 induces embryonic lethality or are associated with severe syndromes like Fanconi anemia [11]. In addition, the new BRCA2 UV c.6346C>G/p.His2116Asp (with both probably damaging PSIC score 0.996 and deleterious SIFT score 0.00) has been detected in breast/ovarian cancer patient (tested negative for a BRCA1 and BRCA2 mutation) but not in her sister (index case 2092, diagnosed with breast cancer) who carries the BRCA1 mutation c.83_84delITG/p.Leu28ArgfsX12 (Tables 2, 3). As compound heterozygosity for BRCA1 and BRCA2 genes deleterious mutations is a very rare finding (1/190,000) and often involve Ashkenazi founder mutations [12], the new UV c.6346C>G/p.His2116Asp could be pathogenic and evaluation of co-segregation of this variant with disease in this family is ongoing. However, the influence of the majority of the UVs on BRCA1 and BRCA2 genes function is not known [13]. Because many of these variants are very rare, the available genetic information from families carrying these variants is very limited for assessment of breast or ovarian cancer risk [14].

In the present work, 42 polymorphic variants have been characterized in BRCA1 (19 different polymorphisms) and BRCA2 (23 different polymorphisms) of individuals with breast and ovarian cancer family history (Table 5).

We detected several missense polymorphisms in BRCA1 and BRCA2 genes with high frequency in patients where pathological mutations BRCA1 and BRCA2 mutations were not present (Table 5). In addition, BRCA1 c.1067A>G/p.Gln356Arg has been identified in index case 2067 and her brother 2068; both carry the BRCA1 mutation c.181T>G/p.Cys61Gly (Table 2). Interestingly, recently it has been reported that the BRCA1 pathogenic mutation c.181T>G/p.Cys61Gly is associated with BRCA1 SNP p.Gln356Arg in 13 Slovakian breast/ovarian cancer families [16]. BRCA1 c.3113A>G/p.Glu1035Gly has been detected in breast cancer patient 2092 who carries the BRCA1 mutation c.83_84delITG/p.Leu28ArgfsX12 (Table 2). Whether these are common SNPs in BRCA1 and BRCA2 genes
modify the risk of breast and/or ovarian cancer in BRCA1 or BRCA2 mutation carriers, or are associated with risk of breast and/or ovarian cancer in patients tested negative for BRCA1 or BRCA2 mutations, remains unclear. To date, several studies have evaluated risk associated of breast and/or ovarian cancer with selected SNPs in BRCA1 (c.1067A>G/p.Gln356Arg) [15,17,18,25] and BRCA2 (c.1114A>C/p.Asn372His) [20–26]. Results from these studies showed conflicting evidence. In addition, Dombernowsky et al. [6] in a large study, evaluated risk associated of breast and/or ovarian cancer by 9 missense polymorphisms in BRCA1 c.1067A>G/p.Gln356Arg, c.2612C>T/p.Pro871Leu, c.3113A>G/p.Glu1038Gly, c.4837A>G/p.Ser1613Gly, c.4956G>A/p.Met1652Ile and BRCA2 c.865A>C/p.Asn289His, c.1114A>C/p.Asn372His, c.4258G>T/p.Asp1420Tyr, and c.5744C>T/p.Tyr1915Met. They found no association between heterozygosity or homozygosity for any of the nine polymorphisms and risk of breast and/or ovarian cancer in either study [6]. Pila- to et al. [26] studied both transmission of BRCA1 and BRCA2 pathogenic mutations and polymorphic variants in breast cancer familial members. They found that SNPs BRCA1 c.3548A>G/p.Lys1183Arg and BRCA2 c.1114A>C/p.Asn372His were more frequently present in breast cancer relatives belonging to families tested negative for BRCA1 and BRCA2 mutations.

Several missense polymorphisms detected here in our breast/ovarian cancer patients who were tested negative for BRCA1 and BRCA2 genes mutations, could have a role as susceptibility breast cancer markers in BRCA1 and BRCA2 non mutated Algerian breast/ovarian cancer families. Evaluation of risk of breast/ovarian cancer by these BRCA1 and BRCA2 missense polymorphisms is going on in breast/ovarian cancer cases and healthy controls.

5. Conclusions

In this report, for the first time, we identified UVs and missense polymorphisms in BRCA1 and BRCA2 genes in Algerian breast/ovarian cancer families. Evaluation of risk of breast/ovarian cancer induced by the eight new missense UVs and missense polymorphisms detected in our present work is going on in a larger study.

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