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SHORT COMMUNICATION

Novel pleiotropic *BRCA2* pathogenic variants in Lebanese families

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Abstract

BRCA1 and *BRCA2* associated pathogenic variants are the major cause of familial cases of early onset breast and ovarian cancers. Here we report two novel heterozygous pathogenic variants in exons 18 and 11 of the *BRCA2* gene in two Lebanese families. The double nucleotide insertion c.8052_8053dupAA was identified in a 38-year-old Lebanese woman diagnosed with a breast cancer. The patient had a family history of affected first degree relatives. The double nucleotide deletion c.4342_4343delAA was identified in a 67-year-old woman with ovarian cancer. The patient came from a family marked by the occurrence of variable cancers. Her two daughters were also found to carry the deleterious variant. Both genetic aberrations result in a framing error that leads to a premature stop codon giving rise to unstable or truncated proteins. We further discuss two non-mutually exclusive potential scenarios related to the resulting haploinsufficiency and variant-specific dominant negative phenotype that might explain, at least in part, the variable expressivity associated with *BRCA2* pathogenic variants.

Keywords BRCA2, Breast cancer, Ovarian cancer, Novel pathogenic variants, Pleiotropy.

Introduction

Breast cancer susceptibility genes, *BRCA1* and *BRCA2*, encode proteins that play a common and critical role in the homologous recombination (HR) process by which DNA double strand breaks (DSBs) are repaired [1–3]. They however intervene at different stages in the DNA repair mechanism with *BRCA1* being epistatic to *BRCA2* [2]. The common HR-mediated process is essential for tumor suppression both in inherited and sporadic breast cancers [4,5]. Recently, a new body of evidence uncovered a novel, independent, function for BRCA2 in controlling nucleolytic degradation at stalled replication forks [6,7]. Both functions are generally believed to be essential for BRCA2 tumor suppressive function. Germline pathogenic variants affecting *BRCA1* and *BRCA2* genes confer, mainly in women, a heightened susceptibility to breast cancer (BC) and increase significantly the risk of developing an ovarian cancer [8–10]. Moreover, these pathogenic variants are responsible for hereditary cases, which occur in 5% to 10% of patients with BC [11,12]. Amongst the various genetic modifications identified in *BRCA1* and *BRCA2* oncosuppressive genes, nonsense mutations, small insertions and deletions, which result in an absent or truncated protein, show the highest pathogenicity [13,14]. Here, we report two novel pleiotropic germline pathogenic variants in *BRCA2* gene in two unrelated Lebanese families with variable histories of cancers occurrence.

Materials and methods

Patients and subjects

This study included two index cases coming from two non-related Lebanese families with a history of breast and ovarian cancer. Both index cases underwent clinical evaluations and their pedigrees were assessed. Blood samples were

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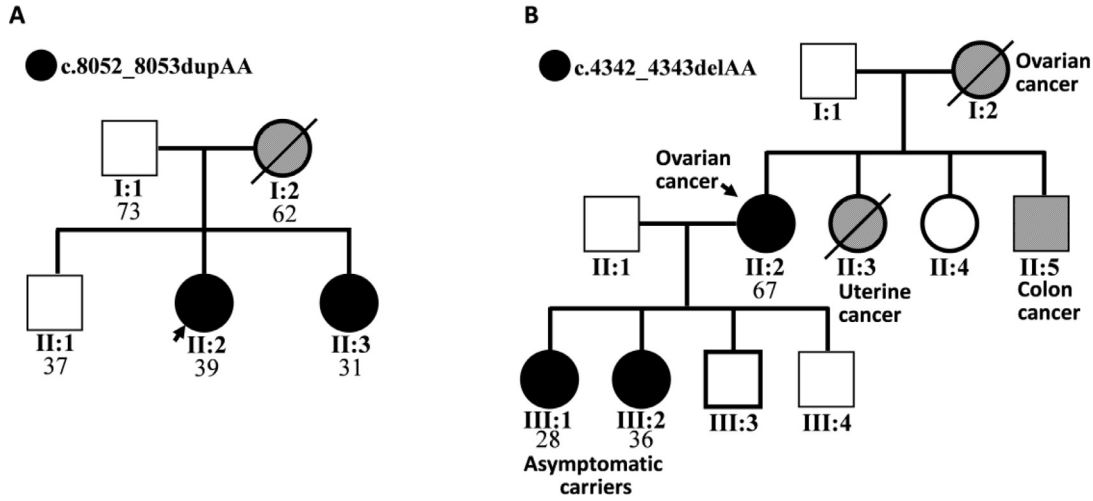

Fig. 1 *BRCA2* based pedigrees of two independent Lebanese families. Two novel heterozygous frameshift variants in *BRCA2* gene namely c.8052_8053dupAA (A) and c.4342_4343delAA (B) were identified in two Lebanese families. The first is characterized by a history of early onset breast cancer while the second shows a pedigree of variable cancers. Probands are indicated by an arrow. The Arabic numbers beneath the ID numbers represent the current age or the age of death. Filled and empty symbols indicate affected (BRCA2 positive) and non-affected individuals respectively. Symbols with diagonals represent individuals with a specific type of cancer of unknown molecular origin.

withdrawn after obtaining oral and written consents from all involved subjects for molecular investigations.

BRCA1 and *BRCA2* molecular analysis

Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit following the manufacturer instructions. DNA sequencing was performed using a customized target enrichment panel including all *BRCA1* and *BRCA2* exonic regions and 50 base pairs of flanking intronic sequences (SeqCap EZ choice, NimbleGen, Roche). Sequencing was carried out using illumina technology and all significant modifications were confirmed by Sanger sequencing using an automated ABI 3500 genetic analyzer (Applied Biosystems, Foster City, CA). MLPA (Multiplex Ligation-dependent Probe Amplification) technique was also used to detect potential large genomic rearrangements.

Results

Family 1

A 38-year-old Lebanese woman (proband; II:2; Fig. 1A) was found to have a unilateral breast mass during a regular examination. Immunohistochemistry analysis revealed a tubular breast cancer that is hormone receptor-positive (ER^+ and PR^+) and HER2-negative. At the request of the patient a bilateral nipple-sparing mastectomy was performed. She is currently undergoing chemotherapy treatment. Family history revealed that the proband's mother was diagnosed with a metastatic breast cancer at the age of 47 and later died at the age of 62. Following genetic counseling, DNA sequencing of *BRCA1* and *BRCA2* was performed. Analysis revealed a heterozygous double nucleotide insertion in the *BRCA2* exon 18, namely c.8052_8053dupAA (Fig. 2A–B). To our knowledge this pathogenic variant has never been reported in the literature. Because of the family history and the identification of a novel *BRCA2* pathogenic variant, we recommended genetic screening of first degree relatives. Upon their consent, blood was collected from the proband's two younger adult healthy siblings (II-1 and II-3) as well as from her father (I-1) for genetic analysis (Fig. 1A). The obtained data, which were limited to exon 18, revealed the presence of the same insertion in the female sibling while the male sibling and the father did not carry the variant.

Family 2

A post-menopausal Lebanese woman (proband; II:2; Fig. 1B) was diagnosed with a metastatic ovarian cancer at the age of 67. She underwent bilateral oophorectomy followed by chemotherapy treatment. The proband is a member of a family with strong history of cancers. Her mother (I:2) and older sister (II:3) died of ovarian and uterine cancers at ages 77 and 72 respectively, while her older brother (II:5) was treated surgically for a cancerous colon tumor at the age of 77 and passed away due to post-op complications. Further information on the types of cancers were not available. Molecular investigation of *BRCA1* and *BRCA2* genes revealed a novel heterozygous double nucleotide deletion in the *BRCA2* exon 11, namely c.4342_4343delAA (Fig. 2A–B). Considering the obtained results, genetic analyses on her adult children were recommended. Both her healthy daughters, III:1 (28 years) and III:2 (36 years), were found to carry the deleterious variant c.4342_4343delAA.

Discussion

Germline pathogenic variants in *BRCA1* and *BRCA2* are the leading cause of breast cancer in women worldwide. These highly penetrant, autosomal dominant mutations have also been associated with ovarian, prostate, stomach, male

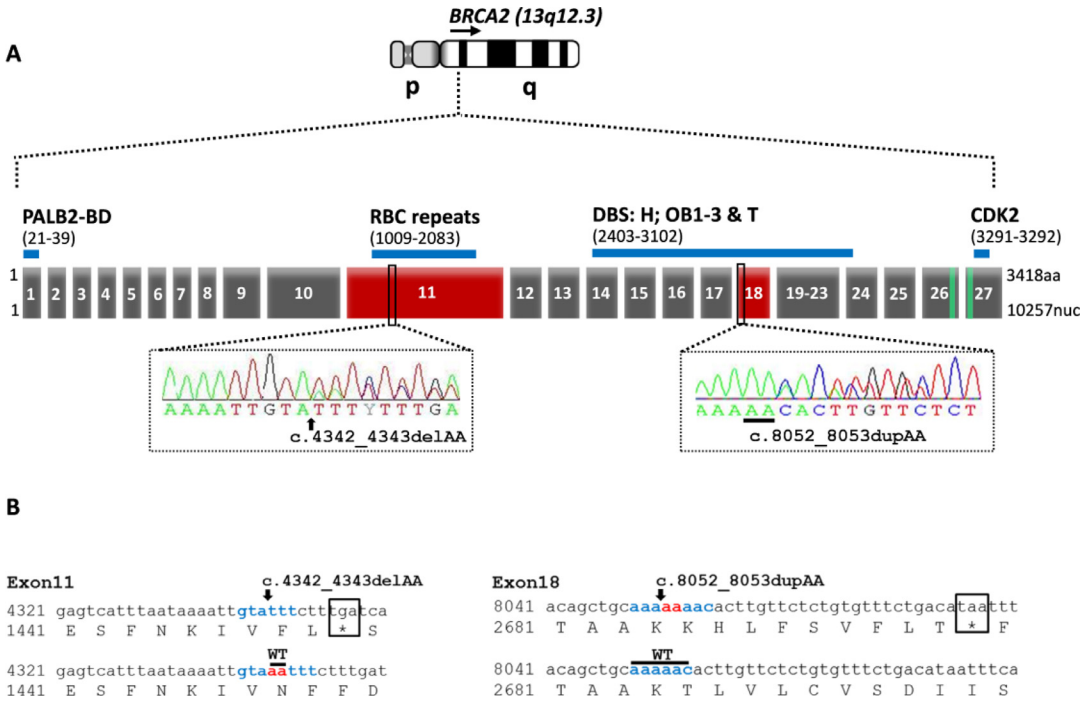

Fig. 2 Schematic representation of the BRCA2 coding sequence (CDS) and functional domains with the positioning of the novel frameshift variants. (A) Segment of electropherograms showing the two-nucleotide deletion at positions 4342–4343 and the two-nucleotide insertion at positions 8052–8053 are shown in the dotted squares beneath exons 11 and 18 respectively. BRCA2 GRCh 37(hg19) NM_000059.3 was used as reference sequence. Functional domains of the 3418 amino acids BRCA2 protein are represented by blue bars above their corresponding exonic sequences. The N-terminal PALPB2 binding domain (PALPB2-BD) binds PALB2 (Partner and localizer of BRCA2) at amino acids 21–39. This is followed by 8 BRC repeats between amino acids 1009 and 2083 essential for RAD51 recombinase binding. The C-terminal domain contains the DNA binding domain (DBD), which is made of a helical domain (H), three oligonucleotide binding folds (OB1-3) and a tower domain (T). DBD allows BRCA2 to attach to both single and double stranded DNA. The C-terminal domain contains also two nuclear localizing signals (NLS; vertical green bars) as well as a cyclin-dependent kinases (CDK2) phosphorylation site at S3291 that also binds RAD51 recombinase. (B) Overview of the premature stop codons generated by heterozygous c.4342_4343delAA and c.8052_8053dupAA variants 3 and 10 residues downstream theaffected site. A small DNA segment around pathogenic variant sites is shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

breast cancers, and familial pancreatic cancers [10,15–17]. Hundreds of different types of pathogenic variants scattered along these oncosupressive genes have so far been reported. These cover all types of mutations including single nucleotide substitutions in both coding and non-coding regions, deletions and insertions [9]. However, the highest pathogenicity of breast cancer cases is often associated with nonsense mutations, small insertions and deletions, which result in an absent or truncated protein [13,14].

Here we report two novel heterozygous pathogenic variants in exons 11 and 18 of *BRCA2* gene in two Lebanese families. The identified variants have not been, to the best of our knowledge, documented in any report in the international literature.

A double nucleotide insertion (c.8052_8053dupAA) in the 18th exon of *BRCA2* gene was identified in a 38 year old breast cancer patient with a family history of breast cancer. Her pedigree analysis revealed the presence of the deleterious variant in an asymptomatic first degree relative.

A double nucleotide deletion in exon 11 of *BRCA2* gene (c.4342_4343delAA) was identified in another patient with reported family history of ovarian, uterine and colon cancers. Genetic aberrations in both families result in a framing error leading to a premature stop codon, 10 and 3 amino acids residues downstream the insertion and deletion sites respectively (Fig. 2A–B).

A premature termination of the growing peptide could lead to a highly unstable protein that would be subject to proteolysis. The resulting haploinsufficiency of BRCA2 activity might trigger variable genomic alterations and promote tumorigenesis [18].

Otherwise, a premature termination might give rise to a truncated protein characterized by a mutant-specific gain of function. Indeed, if not proteolyzed, a truncated protein associated with p.(Thr2685Lysfs*10) would be devoid of its double and single strand DNA binding modules, namely Oligonucleotide binding folds 1, 2 and 3 (OB1-3) and the tower domain (TD) that protrudes from OB2. Nuclear localization sequences (NLS) and the cyclin-dependent kinase (CDK) phosphorylation site will be missing as well (Fig. 2A). Furthermore, a truncated protein related to p.(Asn1448Phefs*3) would additionally lack exon 11 that harbors a series of eight BRC repeats essential for binding RAD51, which plays a key role in recombinational repair of the DNA double strand breaks [18,19]. The truncated proteins, however, conserve their N-terminal domain, which is implicated in binding PALB2

(Partner and localizer of BRCA2). The latter binds also to BRCA1 C-terminal domain providing a physical link between BRCA1 and BRCA2 [20]. Following the above-mentioned scenario, a substantial proportion of BRCA1–PALB2–BRCA2 complexes would harbor truncated BRCA2 proteins, which will not only impede BRCA1-BRCA2-mediated HR response but also generate variable dominant negative properties. Such a consequence might explain partly the pleiotropy associated with BRCA2 pathogenic variants and more importantly, the pleiotropy observed among family members harboring the same pathogenic variant. Moreover, genetic interactions could further modulate the penetrance of *BRCA2*-associated pathogenic variants and contribute to the observed clinical variability. For instance, it was shown that the occurrence of specific variants in *CHEK2*, which encodes a cell cycle checkpoint kinase, increases significantly the pathogenicity of a specific *BRCA2* low penetrant variant in a synergistic epistasis [21]. Our study did not extend to genes other than *BRCA*s, therefore possible genetic interaction in that respect could not be addressed. Such extensive investigations may be needed to have deeper insights into molecular mechanisms and observed phenotypic variability of breast and ovarian cancers and furthermore to rule out co-existence of genetic variants in other culprit genes when reporting a new variant For instance, in our second family, the occurrence of various cancers in different individuals may be as well suggestive of other familial cancer syndromes such as Lynch syndrome.

Ovarian and early-onset breast cancers are highly indicative of predisposing inherited factors. We herein, report novel pleiotropic pathogenic variants in *BRCA2* gene in two Lebanese families. Identifying and reporting genetic alterations in different ethnic groups and geographic regions will pave the way for a better definition of the complex genotype-phenotype relationship and consequently enhance our understanding of the discrepancy in *BRCA*s expressivity. Such findings may promote the development of new targeted therapies or improve the clinical applications of existing therapies. Moreover, characterization of genetic risk factors is vital for both symptomatic and asymptomatic patients. Indeed, adapted follow-up plans with multidisciplinary medical teams and available prevention and therapeutic measures have produced substantial improvements of both morbidity and mortality in high-risk individuals.

Conflict of interest

We have no conflict of interest to declare.

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