Case Report

Familial genetic cancer risk assessment in a male patient with pancreatic and breast cancer with a silent *BRCA2* mutation

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ABSTRACT

Male breast cancer (MBC) is relatively rare, accounting for <1% of cancers in men. Hereditary MBC is mainly attributed to BRCA1/2 germline mutations. Accordingly, National Comprehensive Cancer Network guidelines advise genetic counseling and testing for all cases of MBCs and their unaffected family members. In this report, we present an uncommon case of a male patient in his late 50s primarily diagnosed with pancreatic cancer who later developed asynchronous bilateral hormone-positive breast cancer. Genetic testing revealed a "pathogenic/likely pathogenic" BRCA2 variant (c.8754G >A, pE2918E) in the proband. Cascade testing of 34 family members identified 5 of the family members as carriers of this BRCA2 variant. Among them, two members were breast cancer affected and three were unaffected healthy carriers. Our study demonstrates the clinical relevance of this silent BRCA2 mutation and emphasizes the need for further experimental studies to elucidate its functional role in breast cancer pathology.

Key words: BRCA2, Breast cancer, Familial breast cancer, Genetics, Hereditary breast and ovarian cancer

Male breast cancer (MBC) is a rare disease accounting for 1% of the total breast cancers in the world [1]. The lifetime risk of a man developing breast cancer is 1:1000 (100 times less than that in a woman) [2]. Given the rarity of the disease and lack of large cohorts, the etiology, recommendations, and clinical management of the disease today are mainly based on the data extrapolated from female breast cancer studies. Demographic, environmental, epidemiologic, and genetic factors are risk factors known to influence the onset of MBC [3]. Genetic testing for breast cancer affected male and his first-degree relatives has been recommended by the National Comprehensive Cancer Network (NCCN) guidelines for hereditary breast, ovarian, prostate, and pancreatic cancers.

In this report, we present an uncommon case of a male patient (hereafter referred to as proband), initially diagnosed with pancreatic cancer and subsequently diagnosed with asynchronous bilateral breast cancer. Genetic testing during clinical management identified the proband as a carrier of a BRCA2 "pathogenic/likely pathogenic" variant (c.8754G>A, pE2918E). We present the clinical management and genetic screening approach for familial genetic risk assessment in the proband and his family.

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CASE REPORT

A male in his late 50s reported to our clinic with a lump in his right breast. A positron emission tomography (PET) scan and subsequent biopsy revealed a Grade II Infiltrating Ductal Carcinoma of the right breast.

The immunohistochemical analysis identified the mass as estrogen receptor (ER)/progesterone receptor (PR) positive (ER – 80%, PR – 70%), human epidermal growth factor receptor (Her2-) negative. Modified radical mastectomy was performed post-primary diagnosis. The axillary excision biopsy revealed two out of 15 pathologically positive axillary lymph nodes, suggesting tumor metastases. The patient was treated with six cycles of 5-fluorouracil; epirubicin; and cyclophosphamide adjuvant chemotherapy post-surgery. Subsequently, Linear accelerator-based IMRT/3DCRT radiotherapy was administered to the right chest wall and right supraclavicular region. Hormonal therapy (Tamoxifen) was prescribed for 5 years after the completion of the treatment.

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The patient had a previous medical history of pancreatic cancer and had presented with computed tomography obstructive jaundice with periampullary mass/nodule measuring 1.4 × 2.2 cm at one of our partner clinics. The mass was confirmed to be pancreatic cancer by duodenoscopy-guided biopsy as a well-differentiated adenocarcinoma of the periampullary region of the pancreas. Whipple pancreaticoduodenectomy surgery had been performed as part of clinical management.

Four years post the breast cancer diagnosis, the patient presented with a bloody discharge from the left nipple. On targeted sonomammography, two dilated ducts were seen at the 6 "o"clock position of the left breast and internal echoes, increased periductal and internal vascularity was also noted. The larger lesion was 2.7 mm in size. Following malignancy in cytology reports of nipple discharge, the biopsy findings and histological examinations characterized the left breast tumor as invasive ductal carcinoma Grade I, ER/PR positive. The PET scan revealed a distant metastatic lesion in the left anterior abdominal wall measuring 61.5 × 29.9 mm. The patient was treated with neoadjuvant hormone therapy (Anastrozole) and GnRH analog which was followed by modified radical mastectomy.

In the same year, the younger sister of the proband was diagnosed with ER/PR-positive breast cancer (left). A few months later, her daughter (niece of proband) was diagnosed with ER/PR-positive breast cancer (right). In parallel, the proband was diagnosed with lung metastases and was treated with chemotherapy.

Considering the case history (i.e., pancreatic cancer followed by asynchronous bilateral breast cancer) was indicative of hereditary breast and ovarian cancer (HBOC) syndrome, the proband and family members were advised genetic testing. After written informed consent, the proband underwent a comprehensive multi-gene panel test covering 30 genes associated with HBOC. A heterozygous "pathogenic/likely pathogenic" variant (c.8754G>A) in exon 21 of the BRCA2 gene was detected in the index patient. The detected variant predicted to cause a synonymous substitution in the BRCA2 protein (p.E2918E) is thus a silent mutation.

Further, variant (c.8754G>A) resides 1 base upstream of the splice donor site within exon 21 of the BRCA2 gene. Splicing prediction software programs such as NNSPLICE and splice port revealed that this variant is likely to affect splicing at the junction of exon 21 and intron 21 of the BRCA2 gene (i.e., spliceogenic). At the protein level, this identified silent mutation (p.E2918E) resides in the OB2 (oligosaccharide binding fold module) region, which is contained within the DBD (DNA binding domain) of the BRCA2 gene (Fig. 1). As per NCCN guidelines, after pre-test genetic counseling, other 34 family members (affected and unaffected) were tested for the previously identified BRCA2 mutation to identify the risk of HBOC, pancreatic cancer, and prostate cancer (Fig. 2). Among the 34 family members of the proband, six members were carriers for the identified BRCA2 variant (c.8754G>A). Among the six carriers, three developed the disease, namely, the proband, his elder sister, and niece, while three were unaffected (Fig. 3).

All individuals tested were also given post-test genetic counseling to help them understand the implications of the results. For all members, medical advice wherever applicable regarding surveillance and prophylactic surgeries was also provided.

DISCUSSION

Pathogenic germline mutations in the high penetrance BRCA1 and BRCA2 genes, which act as tumor suppressors, have been long-established as the key mediators of predisposition to HBOCs [4]. Recent reports indicate that BRCA1/2 mutations also

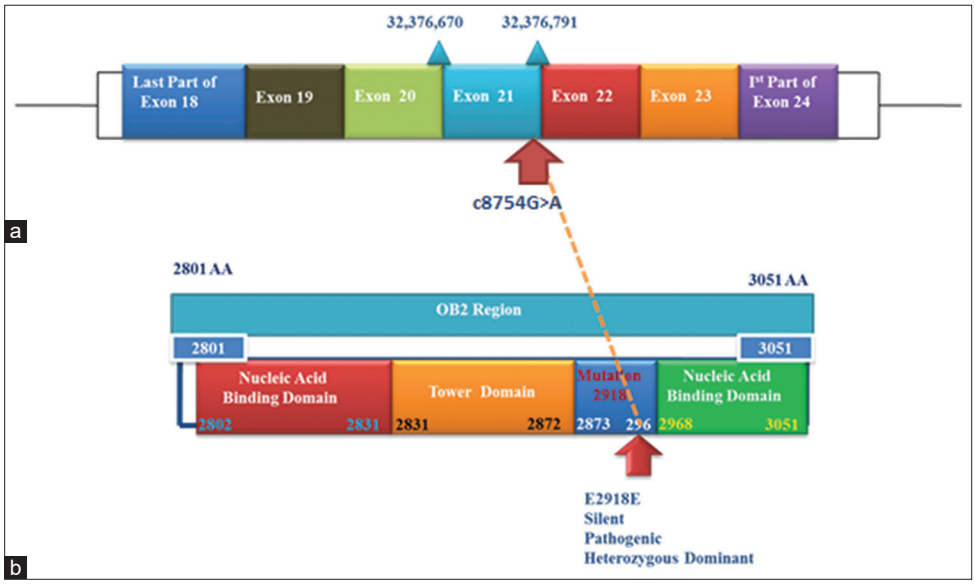

Figure 1: Mapping of the BRCA2 Silent Mutation (c.8754 G>A, p.E2918E). (a) The variant (c.8754G>A) is located 1 base upstream of the splice donor site within exon 21 of the BRCA2 gene; (b) diagram representing the structure and domains of the OB2 region, contained within the DNA binding domain of the BRCA2 gene. This region harbors the identified silent mutation (p.E2918E). Arrows indicate the position of the identified variant

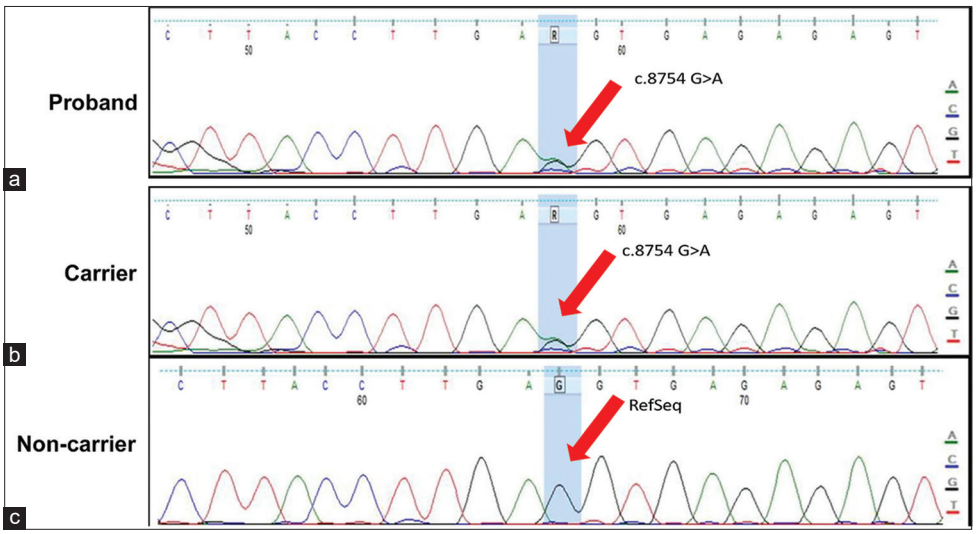

Figure 2: Cascade testing; (a) Sanger sequencing data of the proband harboring the detected variant (c.8754G>A). A heterozygous nucleotide change "G>A" at position c.8754 in the BRCA2 gene, clearly outlined on the electropherogram; (b) Sanger sequencing data revealing the individual was "positive" (carrier) for the tested variant (c.8754G>A). The electropherogram clearly displays a heterozygous nucleotide change "G>A" at position c.8754 in the BRCA2 gene; (c) Sanger sequencing data revealing the individual was 'negative' for the tested variant (c.8754G>A). The electropherogram clearly displays no change, with reference nucleotide "G" being preserved at position c.8754 in the BRCA2 gene similar to the RefSeq

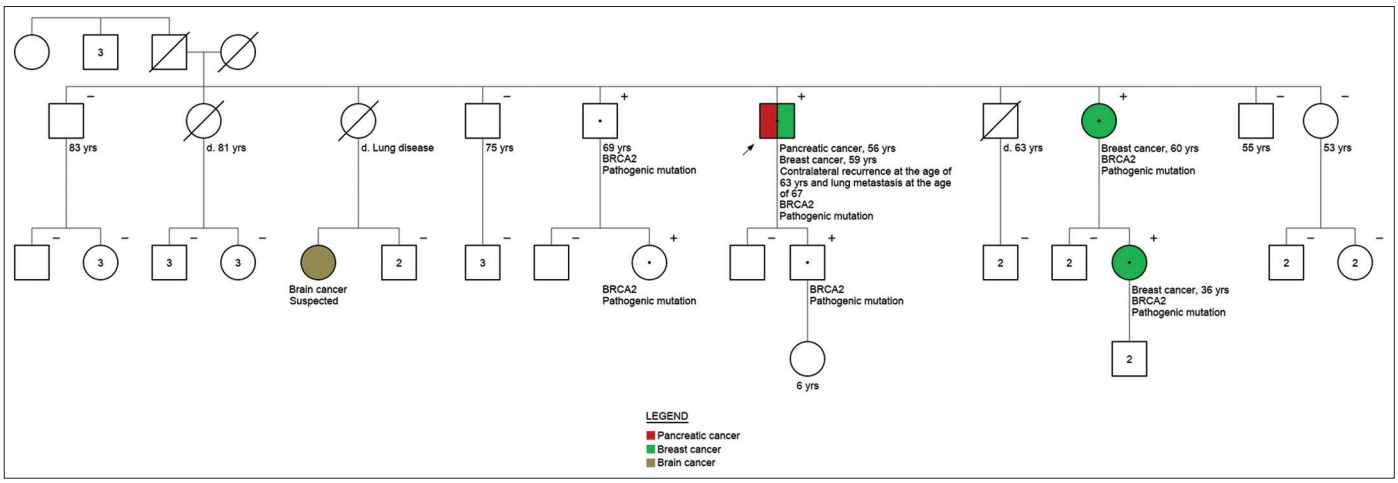

Figure 3: Family pedigree of the proband – The figure depicts the pedigree of the proband. The family pedigree was constructed using Invitae Family History Tool Case (2023 Invitae Corporation, Version:4824429094). The black arrow indicates the proband. The red symbol indicates the individuals who are carriers of the BRCA2 variant (c.8754G>A). The affected members of the family are also identified in the pedigree. Legend on the left illustrates the type of cancer and carrier status

increase the risk of developing pancreatic and prostate cancers and approximately 14% of MBCs harbor BRCA2 mutation [5]. The association of BRCA2 pathogenic mutation (i.e., genotype) with ER-positive status (i.e., phenotype) of MBC observed in our case report is consistent with previous reports [6]. Although not as widely studied as female breast cancers, a few studies have investigated the molecular basis of MBCs. Fentiman present a comprehensive review of the molecular studies on MBCs and a comparative analysis with female breast cancer cohorts. The luminal A subtype is found to be predominant in MBC with a higher BRCA2 germline mutation frequency than breast cancer in females [3]. Data from the Consortium of Investigators of Modifiers of BRCA1/2 cohort analyzing the characteristics of BRCA1/2 mutations in MBCs and female breast cancer report pathologic differences [6]. The frequency of BRCA2 mutations in MBC is found to be higher than BRCA1 in comparison to female breast cancers [6].

The spliceogenic variant identified in the current study was located at conserved splice donor and acceptor sites. Evolutionary conservation analysis indicates that the Exon21-Intron junction is highly conserved among multiple species. Spliceogenic variants are reported to induce major aberrations in the transcript, generate a premature termination codon, or cause in-frame deletion of a known functional domain [7].

Adding to the growing body of literature, in the present study, we report the inheritance of a BRCA2 silent mutation (c.8754G>A, p.E2918E) associated with male and female hormone-positive breast cancer. In the BRCA2 gene, this variant resides in the oligonucleotide binding (OB2) domain. This OB2 region is known to bind to DSS1, an evolutionarily conserved

acidic protein, which is linked to stabilizing BRCA2, promoting homologous recombination, and implicated in various other processes including development, and protein degradation [4]. In agreement with our bioinformatics analysis, the c.8754G>A variant leads to an alteration of the canonical donor site, and the splicing outcome leads to a 46-nt insertion of intron 21 resulting in a frameshift and premature appearance of a stop codon [8]. This mutation and a nearby silent mutation (c.8755) have been reported in the Latin American population [9,10]. Also referred to as the c.8982G>A variant, this variant has been previously reported in individuals afflicted with HBOC syndrome [11].

Further functional genomics studies are warranted to understand biological mechanisms that link the genotype of the BRCA2 pathogenic variant (i.e., c.8754 G>A) to the HBOC phenotype (i.e., loss of tumor suppressor function). It is likely that such functional studies will reveal newer insights into BRCA2 biology with a focus on mRNA stability associated with homologous recombination processes.

CONCLUSION

In the present study, we report the presence of a clinically relevant silent BRCA2 mutation (c.8754G.A, p.E2918E) characterized as "pathogenic/likely pathogenic" in six members of a family, three of whom presented with hereditary breast cancers. Our study underlines the importance of counseling and testing in the context of HBOC. In our study, 34 relatives of the proband underwent genetic testing. Genetic testing is yet not widely undertaken across India, attributable mainly due to the lack of awareness and the cost of testing. It is imperative that genetic counseling and testing be accessible to the population to enable early detection of high-risk individuals. In addition, elucidating the mutational prevalence of breast cancer-predisposing genes in the Indian scenario and determination of their functional relevance may help inform population-specific guidelines and thus enable better clinical management strategies.

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