Received: 18 April 2020 | Revised: 2 June 2020 | Accepted: 29 June 2020

DOI: 10.1002/mgg3.1411

ORIGINAL ARTICLE

Molecular Genetics & Genomic Medicine Open Access WILEY

BRCA2 c.8827C>T pathogenic mutation in a consanguineous Chinese family with hereditary breast cancer

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Abstract

Background: Mutations in the BRCA2 DNA repair associated gene (*BRCA2*) are associated with the development of breast cancer, with different ethnic mutations at different sites. Based on different types of *BRCA2* variants, the underlying mechanism remains still elusive.

Methods: Next-generation sequencing (NGS) was performed to detect germ line mutations in *BRCA2*. The expressions of BRCA2 mRNA and BRCA2 protein were detected by Real-time PCR and Western blot, respectively.

Results: In a consanguineous Chinese family with hereditary breast cancer, one woman had unilateral breast cancer, two women had bilateral asynchronous breast cancer, and one man had prostate cancer. We identified a mutation site (NM_000059.4: c.8827C>T, NP_ 000050.3: p.(Gln2943*)) in *BRCA2* gene, which was a nonsense mutation that predicted disrupting peptide chain synthesis and limiting BRCA2 protein production, validated by the decreased expressions of both BRCA2 mRNA and BRCA2 protein.

Conclusion: In this study, we identified a *BRCA2* c.8827C>T nonsense mutation with a truncated BRCA2 protein in a consanguineous Chinese Han family, suggesting individuals with this mutation should be regularly screened for malignancies such as breast, prostate, and ovarian cancer. Our study verified the function of this *BRCA2* mutation site and provided a new target for the precise treatment of such patients.

KEYWORDS

BRCA2, hereditary breast cancer, nonsense mutation

1 | INTRODUCTION

In recent years, the incidence of breast cancer has increased, particularly in younger individuals, which seriously affects physical and mental health. Among all breast cancer patients, approximately 5%–10% have genetic characteristics and 15%–20% have familial aggregations (Mavaddat et al., 2020). Breast cancer morbidity and mortality can be reduced by detecting breast cancer susceptibility genes, identifying and screening high-risk groups, and taking preventive measures (Nusbaum & Isaacs, 2007). The BRCA1 DNA repair associated gene (*BRCA1* [OMIM 113705]) and the BRCA2 DNA

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repair associated gene (*BRCA2* [OMIM 600185]) genes were the first breast cancer susceptibility genes to be identified with high penetrance, which were related to the occurrence of hereditary breast cancer (Li et al., 2016).

The lifetime risk of breast cancer can reach 69% for individuals with *BRCA2* mutations (Milne & Antoniou, 2016). Therefore, identifying the mutational status of *BRCA2* can optimize the clinical management and treatment strategies for mutation carriers. *BRCA2* is located at 13q12–q13, which consists of 27 exons and encodes 3,418 amino acids (Wooster et al., 1994). Many *BRCA2* gene mutations have been reported and recorded in the Breast Cancer Information Core (BIC) database and in the ClinVar database (Diop et al., 2019). BRCA2 protein includes a DNA binding domain, breast cancer repeats, and C-terminal testicular receptor 2. The main domain involved in DNA repair is the Rad51 binding region. The main function of BRCA2 is homologous recombination based on DNA strand repair, and it also plays roles in cell proliferation and DNA damage monitoring (Shamoo, 2003; Yang et al., 2002). Some researchers have proposed that the loss of the nuclear localization signals (NLSs) at the C-terminal prevents BRCA2 from entering the nucleus, leading to dysfunction and tumorigenesis (Yoshikawa et al., 2005). Most *BRCA2* gene mutations are scattered among individuals, while relatively concentrated *BRCA2* gene mutations have not been found in the Han Chinese population.

In this study, we identified a *BRCA2* (NM_000059.4: c.8827C>T, NP_000050.3: p.(Gln2943*)) nonsense mutation with a truncated BRCA2 protein in a consanguineous Chinese Han family through the next-generation sequencing (NGS) platform. We also verified the function of this *BRCA2* mutation site with Real-time PCR and Western blot analysis.

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

All patients and healthy controls provided informed consent in accordance with the Declaration of Helsinki to participate in this study, which was approved by the Ethical Committee of Shanxi Provincial People's Hospital.

2.2 | Next-generation sequencing

The genomic DNA (gDNA) was extracted following peripheral blood sample collection from patients and healthy controls. The extracted gDNA was subsequently used for the construction of an Illumina standard library (Illumina, Inc.), and the Roche NimbleGen liquid phase hybrid capture chip was employed to perform *BRCA1/2* gene sequencing. The captured exon library was sequenced on the Illumina NextSeq 550AR platform (Annoroad Gene Technology Co. Ltd). Using the Burrows–Wheeler Alignment algorithm version 0.7.12 to compare the sequence data with the human genome (version: GRCh37), Picard version 1.115 was used to mark the polymerase chain reaction duplicates, and the quality value of the sequence alignment results was corrected by means of BaseRecalibrator in Genome Analysis Toolkit version 3.5. The MuTect2 version 3.5 software was employed for mutation detection, and all test results were annotated in the Annovar version 0722 software.

2.3 | Real-time PCR and Western blot analysis

Peripheral blood was collected from patients and healthy controls to detect BRCA2 mRNA and BRCA2 protein expression, using Real-time PCR and Western blot, respectively. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as the internal parameter. For Real-time PCR, the primer sequences were as follows: forward primer: 5′-GAAAATCAAGAAAAATCCTTAAAGGCT-3′; reverse primer: 5′-GTAATCGGCTCTAAAGAAACATGATG-3′. Total RNA was extracted from the peripheral blood of with TRIzol (Invitrogen, Inc). Reverse transcriptase-polymerase chain reactions (RT-PCR) were performed using the PrimeScript RT-PCR Kit (TaKaRa Biotechnology, Inc). Real-time PCR was performed using Platinum SYBR Green (Bio-Rad, Inc) and an MxPro Real-Time PCR System (Bio-Rad, Inc). The Real-time PCR reaction conditions were as follows: 95°C for 20 s, 63°C for 20 s, and 72°C for 45 s, for a total of 40 cycles. For Western blot, cells were lysed with RIPA lysis buffer and total protein concentration was quantified using a BCA kit (Thermo Fisher Scientific, Inc.). A total of 50 μg protein was run on SDS-PAGE and transferred to nitrocellulose membranes, which were then blocked in TBST containing 5% of skimmed milk powder for 2 hr at room temperature. Membranes were then incubated with antibody against BRCA2 (1:500; Thermo Fisher Scientific, Inc.) and β-actin (1:500; Thermo Fisher Scientific, Inc.) overnight at 4℃ before washing three times in TBST. The membranes were then incubated with secondary horseradish peroxidase antibody (1:50; Thermo Fisher Scientific, Inc.) for 1.5 hr at room temperature. Protein bands were visualized using an ECL luminescence kit (Sigma-Aldrich, Inc.), and Bandscan gel image analysis software version 5.0 was used for optical density analysis.

2.4 | Statistical analysis

GraphPad Prism version 7.0 (GraphPad Software, La Jolla California USA) was used for statistical analysis. Pairwise

comparisons were performed using an unpaired *t* test. $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Pedigree and *BRCA2* testing

The family members and their disease statuses are shown in Figure 1. We first tested the mutational status of *BRCA1* and *BRCA2* in the proband (II: 7) with breast cancer. The result showed that *BRCA2* c.8827C>T nonsense mutation appeared in this patient (Figure 2a). Then, we detected mutations in *BRCA1* and *BRCA2* genes in other members of the family. We found II: 1, II: 3, II: 5, and II: 6 carried the common mutations. Individual II: 1 was a healthy person with no evidence of any associated disease, II: 3 had prostate cancer, II: 5 and II: 6 both had bilateral breast cancer. The rest second generation of the family members were found not to carry mutations. The third generation in this family were aged under 25 years old. They were all healthy and declined to participate in this study due to personal reasons. These results suggest that the *BRCA2* mutation in these members (II: 1, II: 3, II: 5, II: 6, and II: 7) was likely inherited from their mother (I: 2), who was diagnosed with and died from breast cancer.

3.2 | Expression of BRCA2 mRNA in individuals with mutant and wild-type *BRCA2*

BRCA2 mRNA expression was analyzed in the family members: average expression in II:3, II: 5, II: 6, and II: 7 (who carried the *BRCA2* mutation) was 2.92 ± 0.07, while that of II:2, II:4, and II:8 (who did not carry the *BRCA2* mutation) was 3.43 ± 0.05, which showed a statistically significant result ($p = 0.003$) between the mutated *BRCA2* and *BRCA2* wild-type groups (Figure 3a).

3.3 | Expression of BRCA2 protein in individuals with mutant and wild-type *BRCA2*

Average BRCA2 protein expression in II:3, II:5, II:6, and II:7 (who carried the *BRCA2* mutation) was 0.31 ± 0.02, while that in II:2, II:4, and II:8 (who did not carry the *BRCA2* mutation) was 0.38 ± 0.01, which revealed significant difference ($p = 0.005$) between the truncated BRCA2 protein (~323 kDa) and full-length BRCA2 protein (~384 kDa) groups (Figure 3b).

4 | DISCUSSION

BRCA1 and *BRCA2* are important tumor suppressor genes associated with breast cancer. Although the mutation rates of *BRCA1* and *BRCA2* are not high in sporadic breast cancer, women who carry *BRCA1* and *BRCA2* mutations have a higher incidence of breast cancer (Milne & Antoniou, 2011). There are different mutations in different ethnic populations (Li et al., 2008; Meisel et al., 2017; Rebbeck et al., 2015). The *BRCA2* gene was discovered by Wooster et al. (1994) and cloned in 1996 (Tavtigian et al., 1996), while there was no significant correlation of gene sequencing alignment between *BRCA2* and *BRCA1*.

In this study, we identified a nonsense mutation site of *BRCA2* in exon 22 (c.8827C>T), which becomes the termination codon after mutation and terminates the synthesis of peptide chains in advance, thus, affecting the synthesis of BRCA2 protein (Figure 2b). Some in vitro studies have shown that the NLS region of BRCA2 is closely related to its nuclear localization (Yano et al., 2000), and thus, this mutation will affect the normal structure and function of the protein. We demonstrated the effect of the mutation on the expression of BRCA2 at both the mRNA and protein level, and confirmed that it is a harmful mutation. The effect of this *BRCA2* mutation on drug resistance should also be considered in the treatment of patients.

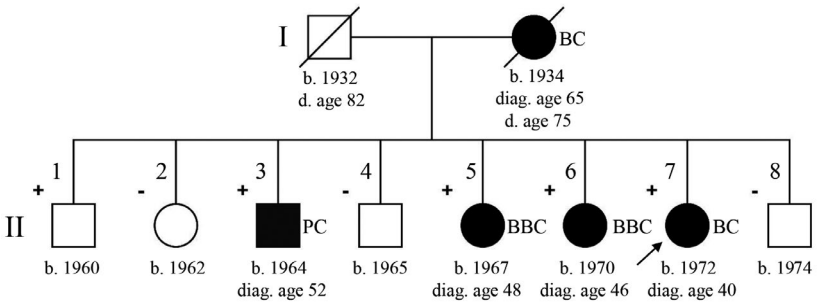

FIGURE 1 Pedigree diagram of a consanguineous Chinese family with *BRCA2* mutations. The pedigree shows two generations (I and II). Filled symbols indicate the patients, while blank symbols indicate unaffected members. Diagonal lines through each square or circle signify the deceased family members. The black arrow denotes the proband (II: 7). PC, prostate cancer; BC, breast cancer; BBC, bilateral breast cancer; +, proven *BRCA2* c.8827C>T mutation; -, proven normal at *BRCA2* c.8827C; b., year of birth; diag. age, cancer detection age; d. age, death age

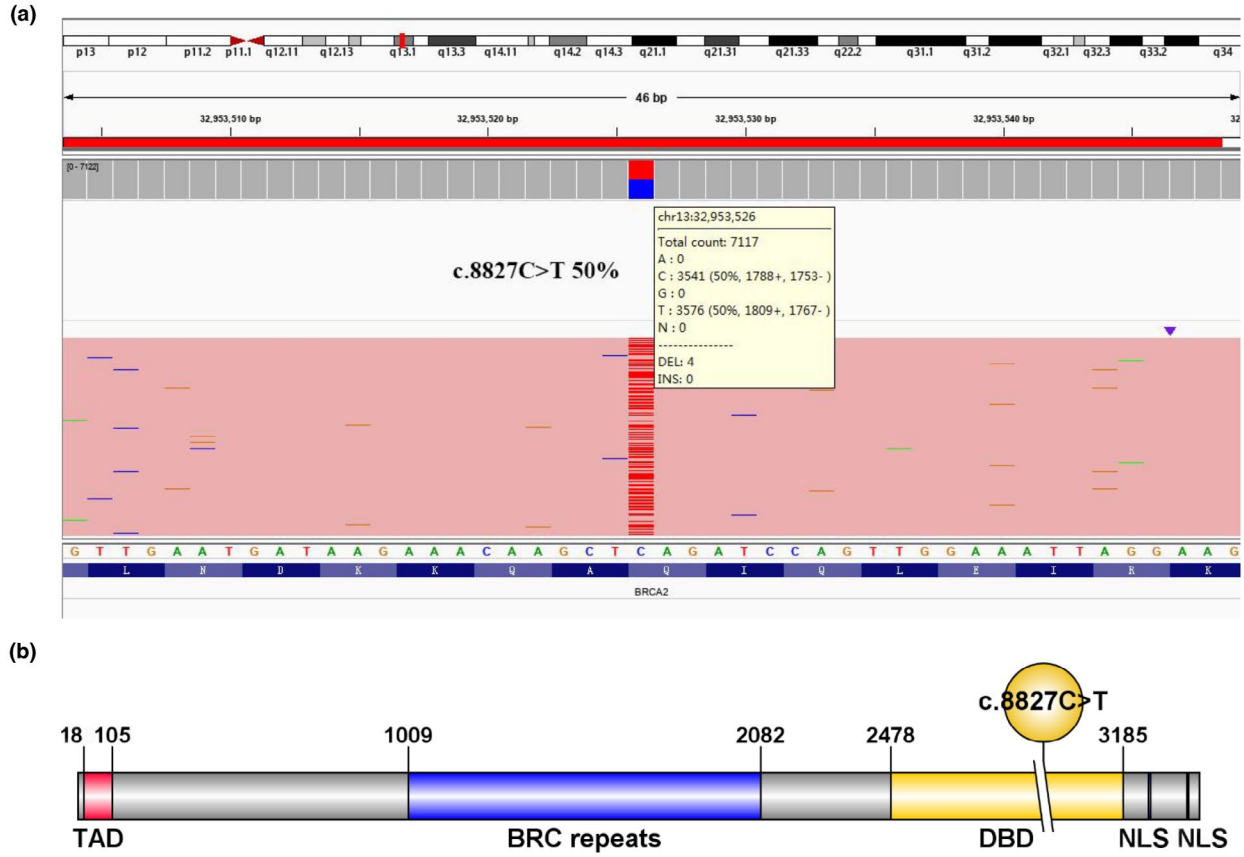

FIGURE 2 (a) The nonsense *BRCA2* c.8827C>T mutation identified in proband (II: 7) in this family through next-generation sequencing platform. (b) *BRCA2* c.8827C>T mutation locating in the DNA-binding domain of its coding protein. BRC, breast cancer; DBD, DNA-binding domain; NLS, nuclear localization signal; TAD, transactivation domain

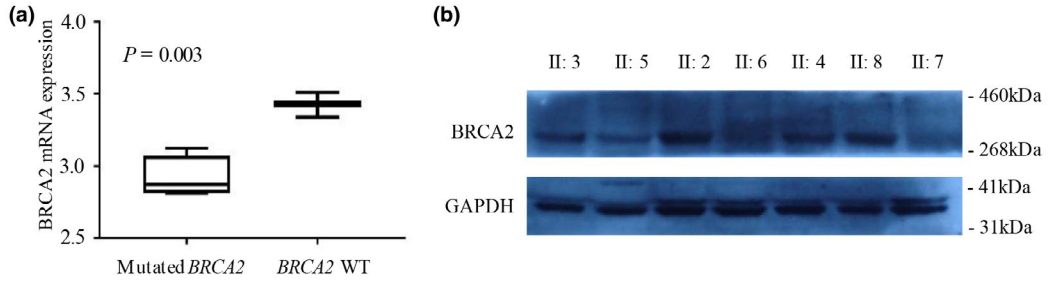

FIGURE 3 Real-time PCR result (a) and Western blot image (b) showing the expressions of *BRCA2* mRNA and BRCA2 protein in individuals with or without *BRCA2* c.8827C>T mutation, respectively. WT, wild-type

Many *BRCA2* mutations have been reported at varying frequencies in different populations and countries worldwide. Fackenthal et al. (2012) tested 434 breast cancer patients at University College Hospital in Ibadan, Nigeria, and found that the *BRCA2* mutation frequency in Nigerian breast cancer patients was 3.9%, while Troudi et al. (2007) identified two frameshift mutations in *BRCA2* (c.1309_1312del and c.5682_5683insA) in a study of 36 breast cancer patients in Tunisia. Rodriguez et al. (2008) tested germ line mutations in *BRCA1* and *BRCA2* in 336 Cuban women with breast cancer and identified eight mutations, one of which was a *BRCA2* mutation, while de Juan Jimenez et al. (2013) examined *BRCA2* mutations in 1,763 inherited breast cancer families in Valencia, Spain, and identified 155 *BRCA2* mutation carriers with a high mutation frequency of c.9026_9030del, c.3264_3265insT, and c.8978_8991del.

However, in the Chinese Han population, Zhang et al. (2012) screened 409 Chinese women with familial breast cancer in northern China for *BRCA1/2*, and the *BRCA2* mutation frequency was 6.6% (27/409), which was 1.7 times

higher than that of *BRCA1*, while Lang et al.'s research based on large numbers of breast cancer patients showed that the frequency of *BRCA2* mutation was 4.51% (135/2,991) (Lang et al., 2017).

As described above, *BRCA2* mutations are closely related to familial breast cancer, but their role in sporadic breast cancer is still unclear. To our knowledge, no primogenitor mutations have been found in the Chinese Han population. *BRCA2* mutation is quite frequent in some ethnic backgrounds, and its primogenitor effect is very significant. The most prominent of these was c.6174del among German Jews (Manchanda et al., 2015). Primordial mutations c.6174del and c.995del had also been identified in Icelandic populations (Torres-Mejia et al., 2015). Common primordial mutations c.8537_8538del and c.3170_3174del in *BRCA2* have been found in French Canadian populations (Ghadirian et al., 2014). Although carriers of these mutations were found to have a significant risk of cancer, reports vary widely and further studies are still needed. In future studies, we will continue to study the distribution of this *BRCA2* c.8827C>T mutation to explore whether it is an original mutation of the Chinese Han population. Patients with this mutation should be screened regularly for malignancies such as breast, prostate, and ovarian cancer.

In summary, we identified a *BRCA2* nonsense mutation (c.8827C>T) in five carriers of a family, three of whom were breast cancer patients, while one was a prostate cancer patient and one carrier was a healthy man. We also verified the function of this *BRCA2* c.8827C>T mutation site. Our study provided insights into role in the pathogenesis and a new target for the precise treatment of such patients.

ACKNOWLEDGMENTS

We thank Liwen Bianji, Edanz Editing China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

J.W. and Y.Z. planned the study. J.W., J.Q., and Y.Z. wrote this manuscript. J.W. performed Real-time PCR and Western blot analysis. J.Q. performed next-generation sequencing and genetic analysis. C.X. acquired clinical phenotype data. All authors approved the final version of this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Wang J, Qin J, Xi C, Zhang Y. *BRCA2* c.8827C>T pathogenic mutation in a consanguineous Chinese family with hereditary breast cancer. *Mol Genet Genomic Med*. 2020;8:e1411. https://doi.org/10.1002/mgg3.1411