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 repair associated gene (BRCA2 [OMIM 607289]) are associated with the development of breast cancer, with different ethnic mutations at different sites. Based on different types of BRCA 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.
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′-GAAAAATCAAGAAAAATTCCCTTTAAGGCT-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°C 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: 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.
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.

ORCID
Jiayue Qin https://orcid.org/0000-0003-4721-0016

REFERENCES
de Juan Jiménez, I., García Casado, Z., Palanca Suela, S., Esteban Cardenosa, E., López Guerrero, J. A., Segura Huerta, Á., … Bolufer Gilabert, P. (2013). Novel and recurrent BRCA1/BRCA2 mutations in early onset and familial breast and ovarian cancer detected in the Program of Genetic Counseling in Cancer of Valencian Community (eastern Spain). Relationship of family phenotypes with mutation prevalence. *Familial Cancer*, 12(4), 767–777. https://doi.org/10.1007/s10689-013-9622-2
Diop, J. P. D., Diallo, R. N., Bourdon-Huguenin, V., Dem, A., Diouf, D., Dieng, M. M., Dieye, A. (2019). Novel BRCA2 pathogenic variant c.5219 T > G; p. (Leu1740Ter) in a consanguineous Senegalese family with hereditary breast cancer. *BMC Medical Genetics*, 20(1), 73. https://doi.org/10.1186/s12881-019-0814-y
Fackenthal, J. D., Zhang, J., Zhang, B., Zheng, Y., Hagos, F., Burrell, D. R., … Olopade, O. I. (2012). High prevalence of BRCA1 and BRCA2 mutations in unselected Nigerian breast cancer patients. *International Journal of Cancer*, 131(5), 1114–1123. https://doi.org/10.1002/ijc.27326
Ghadirian, P., Robidoux, A., Nassif, E., Martin, G., Potvin, C., Patocska, E., … Narod, S. A. (2014). Screening for BRCA1 and BRCA2 mutations among French-Canadian breast cancer cases attending an outpatient clinic in Montreal. *Clinical Genetics*, 85(1), 31–35. https://doi.org/10.1111/cge.12174
Lang, G.-T., Shi, J.-X., Hu, X., Zhang, C.-H., Shan, L., Song, C.-G., … Shao, Z.-M. (2017). The spectrum of BRCA mutations and characteristics of BRCA-associated breast cancers in China: Screening of 2,991 patients and 1,043 controls by next-generation sequencing. *International Journal of Cancer*, 141(1), 129–142. https://doi.org/10.1002/ijc.30692
Li, W.-F., Hu, Z., Rao, N.-Y., Song, C.-G., Zhang, B., Cao, M.-Z., … Shao, Z.-M. (2008). The prevalence of BRCA1 and BRCA2 germline mutations in high-risk breast cancer patients of Chinese Han nationality: two recurrent mutations were identified. *Breast Cancer Research and Treatment*, 110(1), 99–109. https://doi.org/10.1007/s10549-007-9708-3
Li, X., You, R., Wang, X., Liu, C., Xu, Z., Zhou, J., … Zou, Q. (2016). Effectiveness of prophylactic surgeries in BRCA1 or BRCA2 mutation carriers: A meta-analysis and systematic review. *Clinical Cancer Research*, 22(15), 3971–3981. https://doi.org/10.1158/1078-0432.CCR-15-1465
Manchanda, R., Loggenberg, K., Sanderson, S., Burnell, M., Wardle, J., Gessler, S., … Jacobs, I. (2015). Population testing for cancer predisposing BRCA1/BRCA2 mutations in the Ashkenazi-Jewish community: a randomized controlled trial. *Journal of the National Cancer Institute*, 107(1), 379. https://doi.org/10.1093/jnci/dju379
Mavaddat, N., Antoniou, A. C., Mooij, T. M., Hooning, M. J., Heemskerk-Gerritsen, B. A., Nogués, C., … Easton, D. F. (2020). Risk-reducing salpingo-oophorectomy, natural menopause, and breast cancer risk: an international prospective cohort of BRCA1 and BRCA2 mutation carriers. *Breast Cancer Research*, 22(1), 8. https://doi.org/10.1186/s13058-020-1247-4
Meisel, C., Sadowski, C. E., Kohlstedt, D., Keller, K., Stäritz, F., Grübling, N., … Kast, K. (2017). Spectrum of genetic variants of BRCA1 and BRCA2 in a German single center study. *Archives of Gynecology and Obstetrics*, 295(5), 1227–1238. https://doi.org/10.1007/s00404-017-4330-z
Milne, R. L., & Antoniou, A. C. (2011). Genetic modifiers of cancer risk for BRCA1 and BRCA2 mutation carriers. *Annals of Oncology, 22*(Suppl 1), i11–i17. https://doi.org/10.1093/annonc/mdq660

Milne, R. L., & Antoniou, A. C. (2016). Modifiers of breast and ovarian cancer risks for BRCA1 and BRCA2 mutation carriers. *Endocrine-Related Cancer, 23*(10), T69–84. https://doi.org/10.1530/ERC-16-0277

Nusbaum, R., & Isaacs, C. (2007). Management updates for women with a BRCA1 or BRCA2 mutation. *Molecular Diagnosis & Therapy, 11*(3), 133–144. https://doi.org/10.1007/BF03256234

Rebbeck, T. R., Mitra, N., Wan, F., Sinilnikova, O. M., Healey, S., McGuffog, L., … Andrulis, I. (2015). Association of type and location of BRCA1 and BRCA2 mutations with risk of breast and ovarian cancer. *JAMA, 313*(13), 1347–1361. https://doi.org/10.1001/jama.2014.5985

Rodriguez, R. C., Esperon, A. A., Ropero, R., Rubio, M. C., Rodriguez, R., Ortiz, R. M., … Narod, S. A. (2008). Prevalence of BRCA1 and BRCA2 mutations in breast cancer patients from Cuba. *Familial Cancer, 7*(3), 275–279. https://doi.org/10.1007/s10689-008-9187-7

Shamoo, Y. (2003). Structural insights into BRCA2 function. *Current Opinion in Structural Biology, 13*(2), 206–211. https://doi.org/10.1016/s0959-440x(03)00033-2

Tavtigian, S. V., Simard, J., Rommens, J., Couch, F., Shattuck-Eidens, D., Neuhausen, S., … Goldgar, D. E. (1996). The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds. *Nature Genetics, 12*(3), 333–337. https://doi.org/10.1038/ng0396-333

Torres-Mejia, G., Royer, R., Llacuachaqui, M., Akbari, M. R., Giuliano, A. R., Martinez-Matsushita, L., … Narod, S. A. (2015). Recurrent BRCA1 and BRCA2 mutations in Mexican women with breast cancer. *Cancer Epidemiology, Biomarkers & Prevention, 24*(3), 498–505. https://doi.org/10.1158/1055-9965.EPI-13-0980

Troudi, W., Uhrhammer, N., Sibille, C., Dahan, C., Mahfoudh, W., Bouchlaka Souissi, C., … Ben Ammar Elgaied, A. (2007). Contribution of the BRCA1 and BRCA2 mutations to breast cancer in Tunisia. *Journal of Human Genetics, 52*(11), 915–920. https://doi.org/10.1038/sj.hg.3003195-5

Wooster, R., Neuhausen, S., Mangion, J., Quirk, Y., Ford, D., Collins, N., … Averill, D. (1994). Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. *Science, 265*(5181), 2088–2090. https://doi.org/10.1126/science.8091231

Yang, H., Jeffrey, P. D., Miller, J., Kinnucan, E., Sun, Y., Thoma, N. H., … Pavletich, N. P. (2002). BRCA2 function in DNA binding and recombination from a BRCA2-DSS1-ssDNA structure. *Science, 297*(5588), 1837–1848. https://doi.org/10.1126/science.297.5588.1837

Yano, K., Morotomi, K., Saito, H., Kato, M., Matsuo, F., & Miki, Y. (2000). Nuclear localization signals of the BRCA2 protein. *Biochemical and Biophysical Research Communications, 270*(1), 171–175. https://doi.org/10.1006/bbrc.2000.2392

Yoshikawa, Y., Morimatsu, M., Ochiai, K., Nagano, M., Yamane, Y., Tomizawa, N., … Hashizume, K. (2005). Insertion/deletion polymorphism in the BRCA2 nuclear localization signal. *Biomedical Research, 26*(3), 109–116. https://doi.org/10.2220/biomedres.26.109

Zhang, J., Pei, R., Pang, Z., Ouyang, T., Li, J., Wang, T., … Xie, Y. (2012). Prevalence and characterization of BRCA1 and BRCA2 germline mutations in Chinese women with familial breast cancer. *Breast Cancer Research and Treatment, 132*(2), 421–428. https://doi.org/10.1007/s10549-011-1596-x

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