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BRIEF COMMUNICATION

The Novel Pathogenic Mutation c.849dupT in *BRCA2* Contributes to the Nonsense-Mediated mRNA Decay of *BRCA2* in Familial Breast Cancer

Sanrong Li*, Jing Ma[1,*], Caiying Hu, Xing Zhang, Deyong Xiao[2,3], Lili Hao[2,3], Wenjun Xia[2,3], Jichun Yang[2,3], Ling Hu, Xiaowei Liu, Minghui Dong, Duan Ma[2,3], Rensheng Liu

Wuhan Red Cross Hospital, Wuhan; [1]Shanghai Key Laboratory of Birth Defect, Children's Hospital of Fudan University, Shanghai; [2]Key Laboratory of Metabolism and Molecular Medicine, Ministry of Education, and [3]Department of Biochemistry and Molecular Biology, Collaborative Innovation Center of Genetics and Development, Institutes of Biomedical Sciences, School of Basic Medical Sciences, Fudan University, Shanghai, China

In this study, we used next-generation sequencing methods to screen 300 individuals for *BRCA1* and *BRCA2*. A novel mutation (c.849dupT) in *BRCA2* was identified in a female patient and her unaffected brothers. This mutation leads to the truncation of BRCA2 functional domains. Moreover, *BRCA2* mRNA expression levels in mutation carriers are significantly reduced compared to noncarriers. Immunofluorescence and western blot assays showed that this mutation resulted in reduced BRCA2 protein expression. Thus, we identified a novel mutation that damaged the function and expression of *BRCA2* in a family with breast cancer history. The pedigree analysis suggested that this mutation is strongly associated with familial breast cancer. Genetic counsellors suggest that mutation carriers in this family undergo routine screening for breast cancer, as well as other malignancies, such as prostate and ovarian cancer. The effects of this *BRCA2* mutation on drug resistance should be taken into consideration during treatment.

Key Words: *BRCA2 genes, Breast neoplasms, High-throughput nucleotide sequencing, Mutation, Nonsense mediated mRNA decay*

Breast cancer has become one of the most common female malignancies worldwide, accounting for 25% of cancer diagnoses among women [1]. Although male breast cancer is rare, accounting for approximately 1% of all breast cancers, the incidence of male breast cancer is also rising by 1.1% per year [2]. The etiology of breast cancer is still largely unknown. It is widely accepted that clinical disorders, obesity, liver disease, and genetic factors represent risk factors for breast cancer. A large number of genetic studies on breast cancer have supported early prevention and personalized treatment.

Mutations in several genes, including *BRCA1*, *BRCA2*, *TP53*, *CHEK2*, *PALB2*, and *BRIP1*, are reported to increase the risk of breast cancer [3-8]. Importantly, mutations in *BRCA1* and *BRCA2* contribute to approximately 5% of breast cancer cases [9]. Moreover, up to 25% of familial breast cancer cases are reported to result from pathogenic mutations in *BRCA1* and *BRCA2* [10]. Deleterious mutations in *BRCA1* or *BRCA2* not only lead to breast cancer, but also increase the risk of secondary malignancies, such as prostate cancer, pancreatic cancer, and gastrointestinal cancer [11]. Therefore, genetic testing of *BRCA1* and *BRCA2* has proven to be a powerful tool to support early prevention and appropriate personalized treatment for breast cancer in the clinic.

In this study, genetics screening of *BRCA1* and *BRCA2* was performed in patients with breast cancer. All genetic studies were approved by the ethics committee of the Wuhan Red Cross Hospital (approve number: WHSYYY2017003). All patients provided written informed consent in accordance to the Declaration of Helsinki.

Through our screening program, pathogenic mutations were identified in patients with breast cancer using next-generation sequencing methods. However, most of these mutations have been previously reported. Interestingly, a novel heterozygous mutation in *BRCA2* (c.849dupT, p.G284Wfs*11) was detected in one family with a history of breast cancer (Figure 1A). This mutation was then confirmed by Sanger sequencing, as shown in Figure 1B. In this family, the female proband (III:6) and her two brothers (III:3, III:7) were carriers, while other members did not carry the mutation (II:1,

Correspondence to: **Rensheng Liu**
Wuhan Red Cross Hospital, 392 Hongkong Road, Wuhan 430000, China
Tel: +86-27-65600558, Fax: +86-27-65600558
E-mail: 1451727512@qq.com

*These authors contributed equally to this work.

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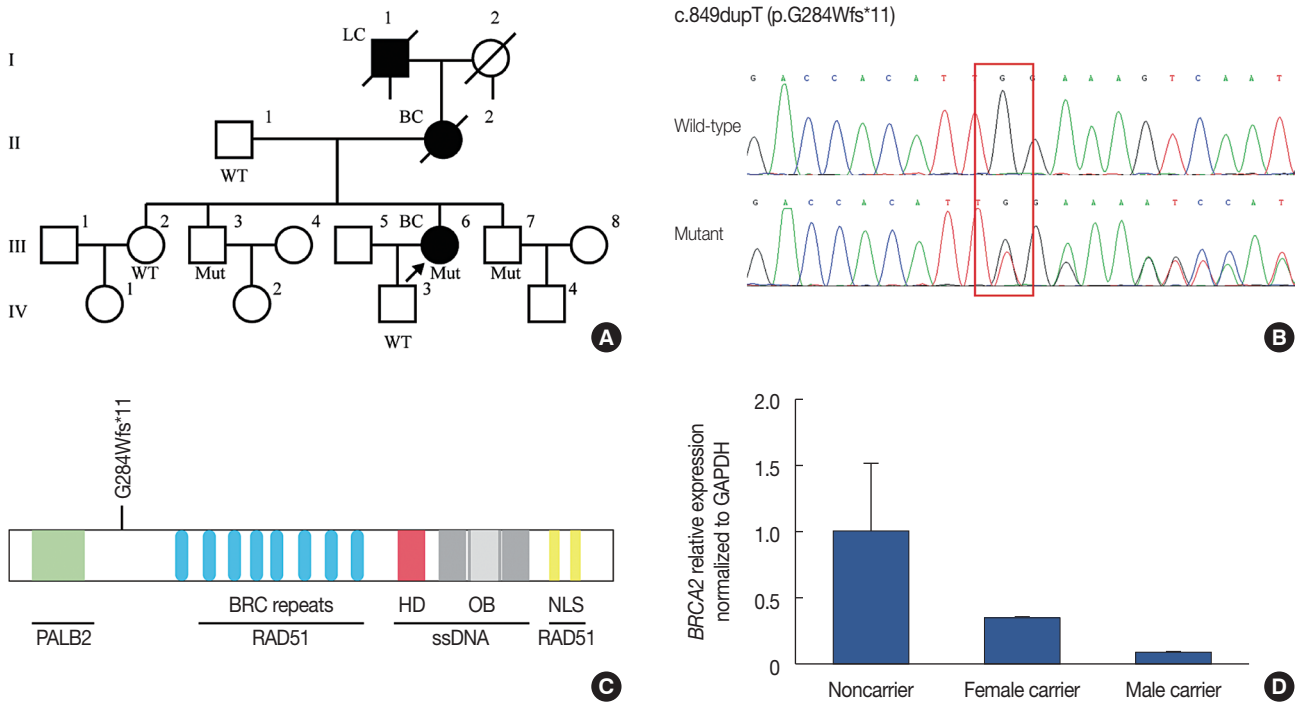

Figure 1. A novel mutation in *BRCA2*. (A) Pedigree of the family with *BRCA2* mutation. The family member II:1, III:2, III:3, III:6, III:7, and IV:3 were screened for the *BRCA1* and *BRCA2* gene, and III:3, III:6, III:7 carry the novel mutation. (B) The mutation (c.849dupT, p.G284Wfs*11) in *BRCA2* identified in this family was confirmed by Sanger sequencing. (C) The diagram of human BRCA2 protein with the location of its variant identified. The PALB2 binding domain in the N-terminal is represented by green box. The blue boxes represent the Breast Cancer (BRC) repeats, which form the RAD51 binding domain. The α-helix domain (HD) is indicated by a red box. The gray boxes denote the oligonucleotide binding (OB) domain. HD and OB domains are important for single strand DNA (ssDNA) binding. The yellow boxes indicate the nuclear localization signal (NLS). NLS is also essential for RAD51 binding. The mutation (G284Wfs*11) contributed to the truncation of BRC repeats, HD domain, OB domain and NLS. (D) The real-time quantitative polymerase chain reaction demonstrated that *BRCA2* mRNA expression in female and male carriers was significantly lower than that in noncarriers.
LC = lung cancer; BC = breast cancer; WT = wild-type; Mut = mutant; PALB2 = partner and localizer of BRCA2; GAPDH = glyceraldehyde-3-phosphate dehydrogenase.

III:2, IV:3). These results suggest that the *BRCA2* mutation in these three members (III:3, III:6, III:7) was likely inherited from their mother (II:2), who was diagnosed with and died from breast cancer.

BRCA2 is localized at 13q13.1 and the length of BRCA2 is 3,418 amino acids. Key functional domains of the BRCA2 protein include the Breast Cancer (BRC) repeats, a DNA binding domain, and the C-terminal testicular receptor 2 (TR2) domain. These domains are critical for the interaction of BRCA2 with DNA or binding partners such as PALB2 and RAD51 [12,13]. Moreover, distinct nuclear localization signals (NLSs) in the C-terminal region are essential for its nuclear localization and functions. BRCA2 is essential for DNA repair, DNA replication, telomere homeostasis and cell cycle progression [14-16]. The mutation in *BRCA2* was located on exon 7. This mutation introduces a stop codon after the 295th amino acid, leading to premature termination of translation. As shown in Figure 1C, the mutant BRCA2 protein lacks nearly all functional domains, including the BRC repeats, the α-helical domain, the oligonucleotide binding domain, and the NLS, and retains only the N-terminal region of BRCA2.

Truncations of large fragments always result in downregulation of genes at the mRNA and protein levels via nonsense mutation-mediated mRNA decay. The whole blood samples of the affected female, her younger brother and three noncarrier controls were collected for *BRCA2* mRNA expression testing. As shown in Figure 1D, *BRCA2* mRNA expression levels in the blood of the two mutation carriers were reduced by approximately 70% and 90% relative to noncarriers. Furthermore, the mutation-associated downregulation of BRCA2 protein was confirmed by immunofluorescence and western blot assays. As shown in Figure 2A, after overexpression in HEK293T cells, wild-type BRCA2 was expressed in both the nucleus and cytoplasm of cells, as detected by Flag antibody. However, mutant BRCA2 protein could not be detected in the nucleus or cytoplasm. In addition, as shown in Figure 2B,

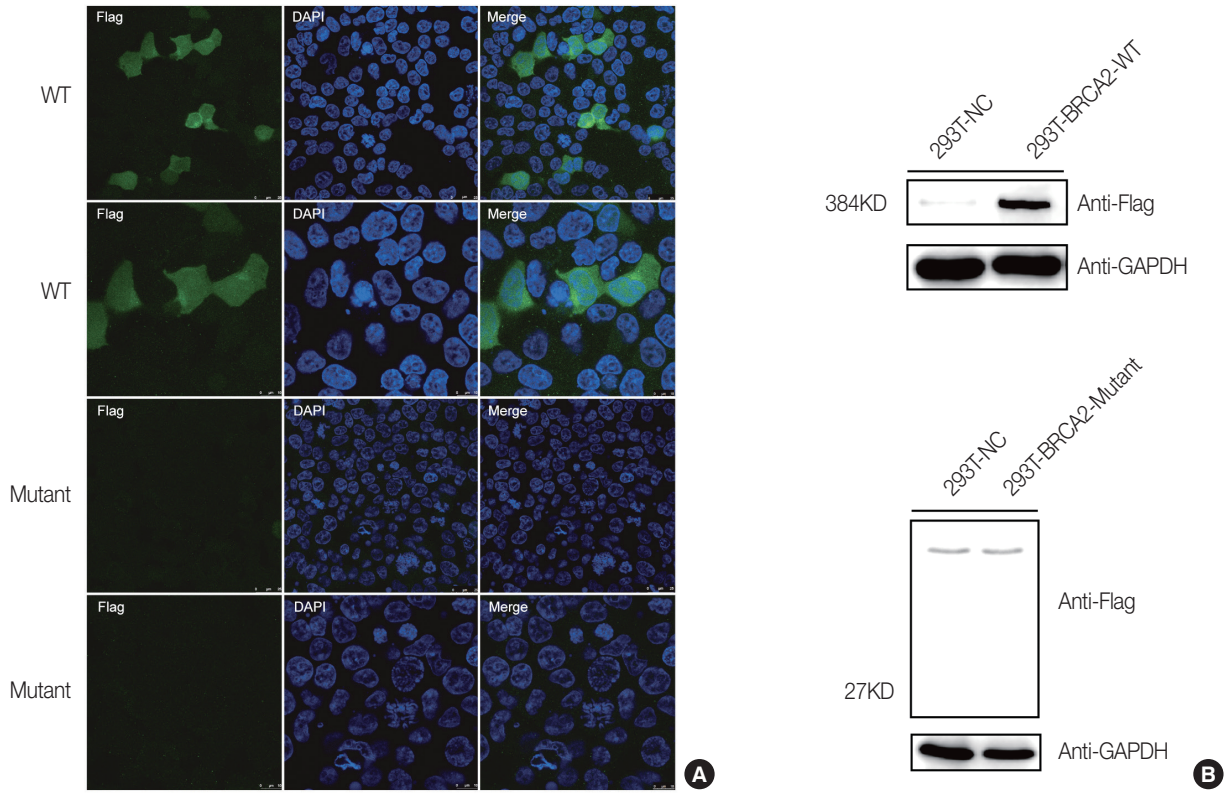

Figure 2. The abolished expression of the mutant BRCA2 *in vitro*. (A) The immunofluorescent demonstrated that wild-type (WT) of BRCA2 was over-expressed in both nucleus and cytoplasm of HEK293T cells which could be detected by anti-Flag antibody. However, mutant BRCA2 protein could not be detected in HEK293T cells, which were transiently transfected with the Flag-tagged mutant BRCA2 plasmids. (B) Western blotting also showed that wild-type of BRCA2 was successfully detected by anti-Flag antibody, and that mutant BRCA2 protein could not be detected in HEK293T cells.
DAPI = 4′,6-diamidino-2-phenylindole; NC = negative control; GAPDH = glyceraldehyde-3-phosphate dehydrogenase.

wild-type BRCA2 could be detected with an anti-Flag antibody using western blot. However, mutant BRCA2 protein could not be detected. In summary, these *in vivo* and *in vitro* assay results suggested that the mutation (c.849dupT, p.G284Wfs*11) could lead to a significant decrease of BRCA2 expression and therefore be damaging to the function of BRCA2.

By carrying the mutation in *BRCA2*, the female proband is at a greater risk for right breast cancer and other malignancies, such as ovarian cancer. Therefore, routine examinations of the right breast and other organs are indispensable. Moreover, since cells with *BRCA1/2* mutations are more sensitive to DNA cross-linking drugs, such as cisplatin and carboplatin, and less susceptible to taxanes [17,18], the effects of this mutation on drug sensitivity should be taken into consideration during treatment. Considering the positive family history and pathogenicity of this mutation, gene testing for other family members was also necessary and meaningful. Another two males were also identified as carriers of the mutation. Although the proband's two brothers had not been diagnosed with any malignancies yet, greater attention should be paid to breast and examinations for other cancers, such as prostate, colon and genitourinary cancer, as 4% to 14% of males with *BRCA2* mutations will develop breast cancer at any age [19,20], and men under 65 years old with *BRCA2* mutations have a 8.6-fold higher risk of developing prostate cancer [21]. Moreover, this mutation was likely to be inherited by the brothers' offspring, who have not yet undergone genetic testing. Therefore, we recommended that these individuals undergo gene screening as soon as possible.

In this study, we identified a novel heterozygous mutation of *BRCA2* in a family with a history of breast cancer. Pedigree analysis suggested that the novel mutation could be a highly pathogenic factor in familial breast cancer. *In vivo* and *in vitro* studies demonstrated that this frameshift mutation led to *BRCA2* mRNA decay and that it could be damaging for BRCA2 function. Our finding not only enriches the Breast Cancer Information Core database (BICD; http://research.

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nhgri.nih.gov/bic/), but also provides assistance to genetic counseling for this family. More importantly, genetic counseling based on *BRCA1/BRCA2* testing was clinically beneficial and significant for this family, as the results contributed to personalized treatments for the patients and prevention and early detection of breast cancer or other malignancies in the family members.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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