

## Characterization of the pathogenic mechanism of a novel BRCA2 variant in a Chinese family

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**Abstract** *Introduction:* Germline mutations of BRCA1 and BRCA2 account for the majority of hereditary breast cancers, many of which are classified as variants of unknown significance (VUS). We report the identification of a novel BRCA2 variant (c.7806-9T > G) in a Chinese family with multiple breast cancers and document it as a pathogenic mutation. *Methods:* The proband in this family was diagnosed with breast cancer at age 50 with a strong family history of breast cancer. DNA and RNA were

extracted from the blood of the proband and her family, and was used for BRCA gene mutation/deletion screening and RNA splicing analysis. *Results:* BRCA2 c.7806-9T > G was identified in the proband, which was suggestive of a variant. This change was also found in two sisters of the proband with a history of breast cancer, as well as from the proband's maternal gastric cancer. The only sibling free of breast cancer did not carry the BRCA2 variant, thus demonstrating that the mutation segregates with the clinical phenotype in this family. RNA analysis on the proband blood sample revealed three aberrant splicing variants: c.7806\_7874del, c.7806\_7976del, and c.7806-8\_7806-1ins. The latter causes a frameshift and creates a truncated protein, whilst the other two splicing variants resulted in shorter forms of the protein. *Conclusions:* The identified BRCA2 c.7806-9T > G [Genbank: DQ889340] was found to be pathogenic, based on aberrant splicing events resulting in the formation of truncated protein products. Thus, better understanding and classification of BRCA variants as neutral or disease causing has important implications for genetic counseling so that appropriate management can be given.

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**Keywords** BRCA1 · BRCA2 · Chinese breast cancer ·  
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### Abbreviations

DCIS	Ductal carcinoma in situ
MLPA	Multiplex ligation-dependent probe amplification
PAGE	Polacrylamide gel
RT-PCR	Reverse-transcription polymerase chain reaction
VUS	Variant of unknown significance

## Introduction

Germline mutations in the human breast cancer susceptibility genes BRCA1 [1] and BRCA2 [2] are responsible for 30–70% of hereditary breast and ovarian cancer [3], and 5–10% of all breast and ovarian cancers [4] in heterogeneous Caucasian populations. BRCA1 and BRCA2 mutations confer greatly increased risk of breast and ovarian cancer [5, 6]. A large number of distinct mutations in the BRCA1 and BRCA2 genes have been reported worldwide, but population-specific variation in the distribution of BRCA1 and BRCA2 mutations is well recognized [7–12]. The contribution of these genes to breast cancer remains relatively unexplored among the Chinese and Asian populations. To date, only a small number of investigations into the role of BRCA1 and BRCA2 in Chinese and Asian populations have been reported. Some reports suggest that some BRCA1 and BRCA2 mutations appear to be unique to specific populations [13–17].

Most reported disease-associated alleles of BRCA1 and BRCA2 have been attributed to frameshift, nonsense, insertions, deletions, or splice site alterations that lead to truncation of BRCA1 or BRCA2 proteins. Besides clear pathogenic mutations, many variants (variant of unknown significance, VUS) are also found [18]. VUS account for approximately half of all unique variants detected [18, 19]. Identifications of VUS are being increasingly reported as more studies on different ethnic populations are being performed [14, 20–22]. These observations suggest that the spectrum of mutations in different ethnic populations will differ from what is known from Caucasian populations. While VUS in different ethnic populations such as the Chinese appear at higher frequency and may well be innocuous, the presence of rare VUS that are pathogenic cannot be excluded.

In this study, we report a novel BRCA2 variant in a family of Chinese ancestry with multiple breast cancers and classify it as pathogenic.

## Materials and methods

### Samples

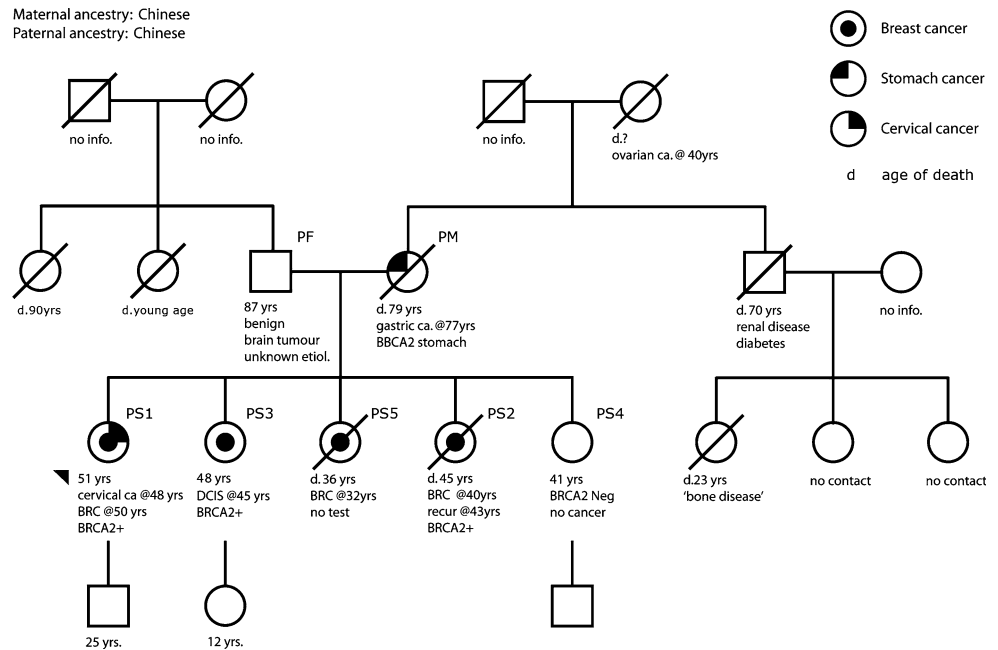
The proband (PS1), aged 51, of Chinese ancestry and living in Hong Kong, was diagnosed with stage 1b cervical cancer at age 48 and stage IIb invasive ductal carcinoma of the breast at age 50. She reported three sisters with breast cancers. Two sisters were diagnosed with invasive breast carcinoma at the ages of 32 (PS5) and 40 (PS2) and died of metastatic breast cancer at ages of 36 and 43, respectively. A third sister (PS3) was diagnosed with ductal carcinoma in situ (DCIS) at the age of 45. The proband's mother (PM)

died of gastric cancer at age 79. She also reported a maternal grandmother who may have died of ovarian cancer in her forties and a female cousin who died at the age of 23 due to metastatic bone disease of unknown primary. The proband's father (PF), age 87, has a history of benign brain tumor of unknown etiology. A paternal aunt was reported to have deceased at a young age and a second aunt was reported to have six sons and deceased at age 90 (Fig. 1).

Peripheral blood samples were collected from the proband (PS1) and her family for BRCA mutation screening, as they were suspected to have a pathogenic mutation due to the strong family history and the young age of presentation of breast cancer in the family.

### BRCA1 and BRCA2 mutation screening

BRCA1 and BRCA2 mutation detection was performed on genomic DNA extracted from peripheral blood samples. DNA was extracted using a QIAGEN DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Mutation analysis was performed by direct sequencing of all coding exons of BRCA1 and BRCA2 and partial flanking intronic sequences. PCR amplification was performed as previously described. PCR primers were designed according to the Intronic primer database of the Breast Cancer Information Core of the National Institutes of Health. A total of 35 and 37 PCR amplifications were performed to cover all the coding exons of BRCA1 and BRCA2 genes. Each PCR reaction consisted of 15–20 ng purified genomic DNA, 2U AmpliTag Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA), 1.5 mM MgCl<sub>2</sub>, 0.2 mM nucleotides mix, and 0.5 μM primers in a final volume of 50 μl. The cycling conditions were 94°C for 10 min, 35 cycles of 94°C for 30 s, 50°C (55°C for BRCA1 exons 1, 4, 10, 22, and 23) for 1 min and 72°C for 1 min, and final extension at 72°C for 7 min. Amplicons were purified using a QIAquick PCR purification kit (QIAGEN). Direct nucleotide sequencing using forward and reverse primers was performed using a BigDye Terminator v3.1 cycle sequencing kit and analyzed on an ABI 3130xl genetic analyzer (Applied Biosystems). Sequencing results were compared with the reference DNA sequences of the BRCA1 and BRCA2 gene using SeqScape software (Applied Biosystems) and then reviewed manually. Blood samples donated from 50 healthy Chinese female individuals were used to perform direct sequencing for the genetic variant detected in the BRCA genes to ascertain the likelihood of the variant being a mutation or common polymorphism. This sample size gave a power of >95% in detecting a single nucleotide variant occurring at an allele frequency of >3%.

**Fig. 1** Family pedigree


### BRCA1 and BRCA2 deletion or amplification by MLPA

The multiplex ligation-dependent probe amplification (MLPA) kit P002, P087 and P045 (MRC-Holland, Amsterdam, Netherlands) was used for BRCA1 and BRCA2 gene copy number determination, respectively [23–26]. DNA samples were extracted from peripheral blood sample and processed according to the manufacturer's protocol. Each MLPA analysis was carried out with five normal control samples. Amplification products were electrophoresed on an ABI 3130xl genetic analyzer and interpreted using GeneMapper software (Applied Biosystems). Results were exported to the Coffalyser program, a Microsoft Excel-based macro software (MRC-Holland) in which BRCA gene copy gain or loss was evaluated. After normalization, the peak areas of the tested samples were compared to that of the wild-type control samples. An increase or decrease by 30–55% indicated a gain or loss in copy number of the gene, respectively.

### RNA analysis of BRCA2 gene

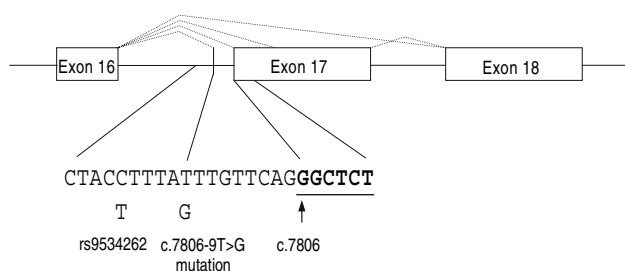
Total RNA was extracted using a QIAGEN RNA Mini Kit (QIAGEN) according to the manufacturer's instructions. One microgram of total RNA was used in cDNA synthesis reaction, which consisted of 1× reaction buffer, 0.5 mM dNTP, 2 ng random hexamer, 1× DTT, and 50 units of Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA). PCR amplification using primers flanking exons 16 and 17 were designed [BRCA2-exon15-F2-M13

(forward) 5'-TGT AAA ACG ACG GCC AGT TGC AAA AAC ATC CAC TCT GC-3' and BRCA2-exon18R (reverse) 5'-GCT GTG TCA TCC CTT TCC AT-3'] and performed on the synthesized cDNA. The forward primer had the M13(-21) universal sequence tail at the 5' end. The thermal reaction was performed similarly as described [27]. The 20 μl PCR reaction consisted of 1× gold buffer (Applied Biosystems), 2 mM MgCl<sub>2</sub>, 0.2 mM dNTP, 0.2 μM forward, reverse, and 6-FAM labeled M13(-21) primer each, and 1 unit AmpliTaq gold DNA polymerase (Applied Biosystems). Thermal cycle reaction of 95°C for 5 min, 30 cycles of 95°C for 15 s, 56°C for 30 s and 72°C for 45 s, followed by eight cycles of 95°C for 15 s, 53°C for 30 s and 72°C for 45 s and final extension of 72°C for 5 min was performed. The amplified products were subjected to electrophoresis on an ABI 3130xl genetic analyzer and interpreted using GeneMapper (Applied Biosystems). In order to examine the sequence of the aberrant spliced transcript products, the similar thermal cycle reaction was carried out, but replacing the forward primer with BRCA2-exon15-F2 (forward) 5'-TGC AAA AAC ATC CAC TCT GC-3', which did not contain the M13(-21) sequence tail. The amplified products were separated on 8% (29:1) polyacrylamide gel (PAGE), stained with 1× SYBR gold fluorescent dye (Molecular Probes, Eugene, OR, USA), and then visualized under ultraviolet (UV) transilluminator as described [28]. The bands that appeared on the gel were excised and subjected to direct sequencing using BRCA2-exon15-F2 and BRCA2-exon 18R. The same gel-purified products were further cloned using a pCR2.1 TOPO TA cloning kit (Invitrogen). At least five positive clones were

selected for sequencing using M13 forward and M13 reverse primers.

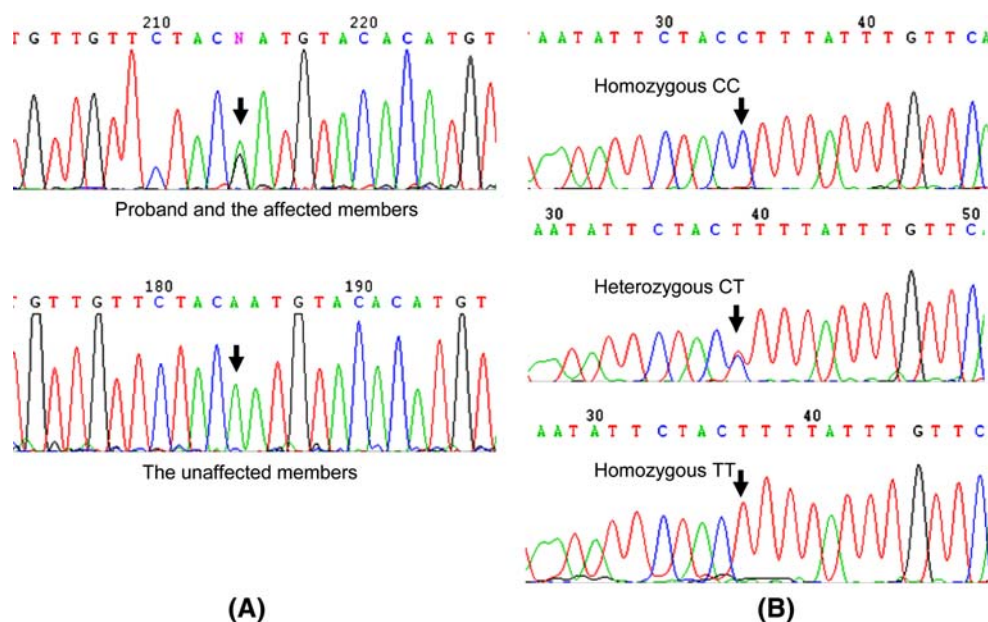
## Results

Full gene DNA sequencing and MLPA were performed on the proband's (PS1) (Fig. 1) germline DNA. A single base-pair substitution (c.7806-9T > G; RefSeq: NM\_000059.2, Genebank: DQ889340) was first identified at the BRCA2 intron 16: a noncoding intervening sequence (IVS) occurring 9 bp upstream of the exon 17 (Fig. 2). Computational analyses using a splice-site prediction program NNSPLICE [29] showed that this change might give rise to a potential novel cryptic splice site for alternative RNA splicing. This change was also found in two other sisters of the proband, one of whom was deceased (PS2) but DNA had been



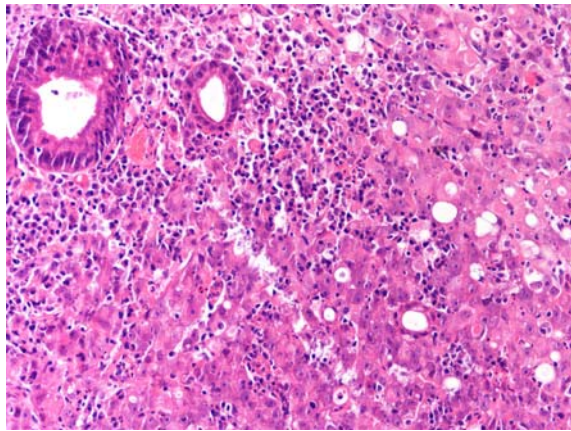
**Fig. 2** Illustration of the BRCA2 exon 16–17 and sequence flanking the c.7806-9T > G mutation. A reported SNP (rs9534262) was 5 bp upstream of the mutation. Bold and underlined text represented part of the sequence of exon 17. Dotted lines illustrated the splicing events yielding wild-type, c.7806-8\_7806-1ins, c.7806\_7874del and c.7806-7976del transcripts in the proband. Drawing not to scale

**Fig. 3** Sequencing results showing the c.440A > G variant and the c.7806-15C > T variants. (A) All c.7806-9T > G mutant carriers in the proband's family were also found to carry c.440G allele (upper panel), whereas the unaffected members (proband's father and sibling) carried the homozygous AA genotype (lower panel). (B) Representative genotyping results of the c.7806-15C > T variant (rs9534262). The c.7806-9T > G mutant carriers carried homozygous CC, whereas the unaffected members carried heterozygous CT genotype



collected with consent for genetic testing prior to her death, and a second sister, who was diagnosed with DCIS (PS3). The latter sister (PS3) resides in the United States, and was found to have the same change, which was reported as a VUS (Myriad: 00067331-BLD) as it was a first occurrence of this variant in unrelated families. Another BRCA2 variant c.440A > G (Fig. 3A) was detected in the proband (PS1); it was located at exon 5 and resulted in the substitution of arginine for glutamine at amino acid position 147 of the BRCA2 protein. This change was also reported as a VUS of which there were seven previous observations in unrelated families reported in BIC database, the majority of which were observed in individuals of nonspecific and Asian ancestry [15, 18]. This variant was not detected in donated blood samples of 50 healthy Chinese female individuals (data not shown). Finally the proband (PS1) showed homozygous CC genotype for the single nucleotide polymorphism (SNP) c.7806-15C > T, namely rs9534262 in the dbSNP database located 5 bp upstream to c.7806-9T > G (Fig. 3B). This SNP was detected with a heterozygosity rate (C > T) of 40, 48, and 52% of C- and T-allele frequency in 50 healthy Chinese female individuals' donated blood samples.

To trace the pedigree of the BRCA2 c.7806-9T > G variant, the proband's father (PF) was tested and found to be negative, whereas the proband's mother (PM) was found to harbor the same change in DNA extracted from archival gastric tumor tissue (Fig. 4). To complete the pedigree, the last surviving sister (PS4) was tested and was found to be negative. She was the only sibling who had not been diagnosed with breast cancer. The variant c.440A > G was also sought and found in the proband (PS1) the mother



**Fig. 4** Proband’s maternal archival gastric tumor tissues showing adenocarcinoma. Some cells show signet-ring formation

(PM) and the sister who was deceased (PS3). It was absent in the father (PF) and the sister without breast cancer (PS4), who showed homozygous AA genotype (Fig. 3A). Hence, both these variants appeared to co-segregate, being found in all three of the female siblings tested who were diagnosed to have breast cancer (PS1, PS2, PS3) at an early age. This co-segregation with breast cancer in the proband’s family increased the clinical suspicion of the likelihood of a novel pathogenic BRCA2 mutation c.7806-9T > G despite its initial classification as a VUS. This mutation was not found in 50 healthy female individuals (data not shown). As these control samples gave a power of >95% in detecting a variant occurring at a frequency of >3%, c.7806-9T > G, being a common polymorphism, was unlikely. As for the rs9534262 SNP, although all the c.7806-9T > G mutant carriers showed homozygous CC genotype, the unaffected members showed heterozygous CT genotype (Fig. 3B) (Table 1).

To confirm the pathogenicity of the mutation, reverse-transcription polymerase chain reaction (RT-PCR) using specific primers flanking the cryptic splice site (BRCA2 exon 15–18) and the mutation was performed on RNA extracted from the proband’s blood. Apart from the peak

(529 bp) corresponding to the wild-type BRCA2 transcript three additional peaks were observed (upper panel of Fig. 5). After PAGE gel separation and 1× SYBR gold staining (lower panel of Fig. 5), only three dominant bands were observed. The bands (Band I, II, and III) were excised from the PAGE gel and subjected to direct sequencing. The sequencing results showed that there were mixture of wild-type and aberrant BRCA2 transcripts contained in band I (Fig. 6A), whilst bands II and III corresponded to two short aberrant BRCA2 transcripts that had deletion of the first 69 bp of exon 17 (c.7806\_7874del) (Fig. 6D) and entire exon 17 (c.7806\_7976del) (Fig. 6E), respectively. Further investigation of the gel-purified products from band I using TA cloning and sequencing revealed that it contained the wild type as well as an aberrant BRCA2 transcript. The latter had an 8 bp (TTGTTTCAG) insertion between the junction of exons 16 and 17 (c.7806-8\_7806-1ins) (Fig. 6 B, C). In total, there were four different sizes of BRCA2 transcripts identified in the proband’s RNA. Sequencing results from cloning of bands II and III confirmed these direct sequencing results. The latter three aberrant transcripts were not found in the 30 control breast cancer patients (RNA extracted from tumors and nontumors), and therefore splicing events are not common. The c.7806-8\_7806-1ins transcript created a frameshift, leading to a truncated protein consisting of 2,649 amino acids and therefore potentially pathogenic. The other two short transcripts produced shorter BRCA2 protein isoforms; however, the functional significance of these mutant isoforms to disease development is not clear.

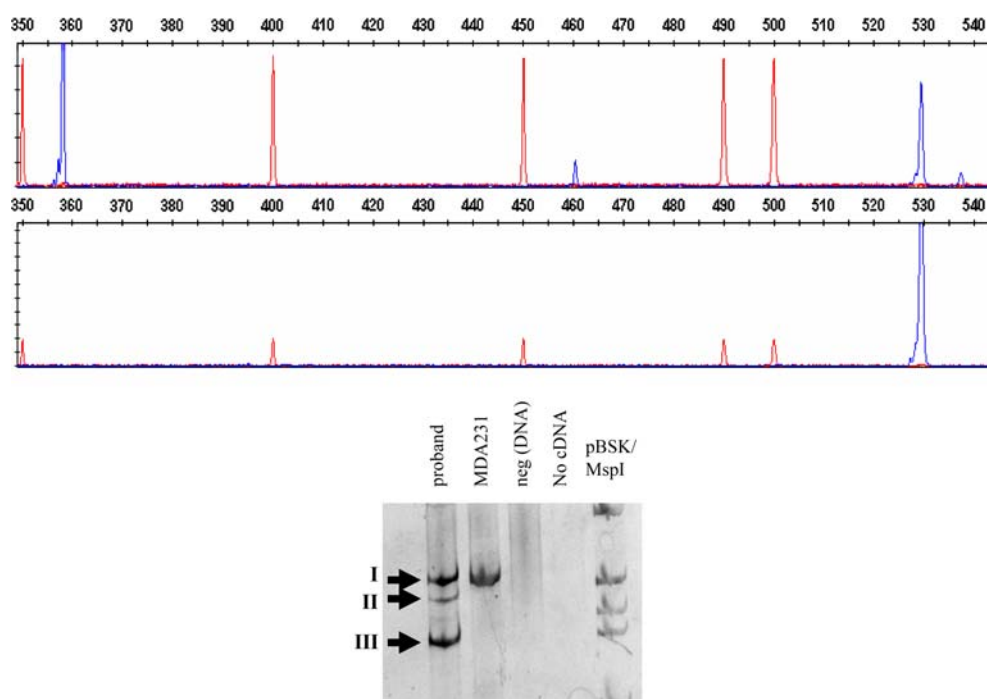
**Discussion**

Here we report a novel BRCA2 substitution point mutation c.7806-9T > G in a family of Chinese ancestry [Genbank: DQ889340] and characterize it as pathogenic. It has been reported that the prevalence of mutations in breast cancer patients with affected relatives is highly dependent on the number of affected relatives in the family and the age of

**Table 1** Summary of mutation findings

Patient	Cancer	Mutation		
		c.7806-9T > G	c.440A > G	c.7806-15C > T
Proband PS1	IDC, cervical	+	+	+
PM	Stomach	+	+	+
PF	–	–	–	–
PS2	IDC (deceased)	+	+	+
PS3	DCIS	+	+	+
PS4	No	–	–	–
PS5	IDC (deceased)	Not tested	Not tested	Not tested

IDC, invasive ductal carcinoma; DCIS, ductal carcinoma in situ



**Fig. 5** RNA analysis by RT-PCR. *Upper panel:* GeneMapper result of the RT-PCR products using primers (BRCA2-exon15-F2-M13 and BRCA2-exon 18R). Red peaks are the internal DNA ladder marker (ROX-500, Applied Biosystems). Blue peaks correspond to *c.7806-7976del*, *c.7806-7874del*, wild type, and *c.7806-8\_7806-1ins* transcripts (from left to right). *Lower panel:* PAGE (8%, 29:1) images of the RT-PCR amplified products using primers (BRCA2-exon15-F2 and BRCA2-exon 18R). *Lane 1:* RT-PCR product of BRCA2 exon

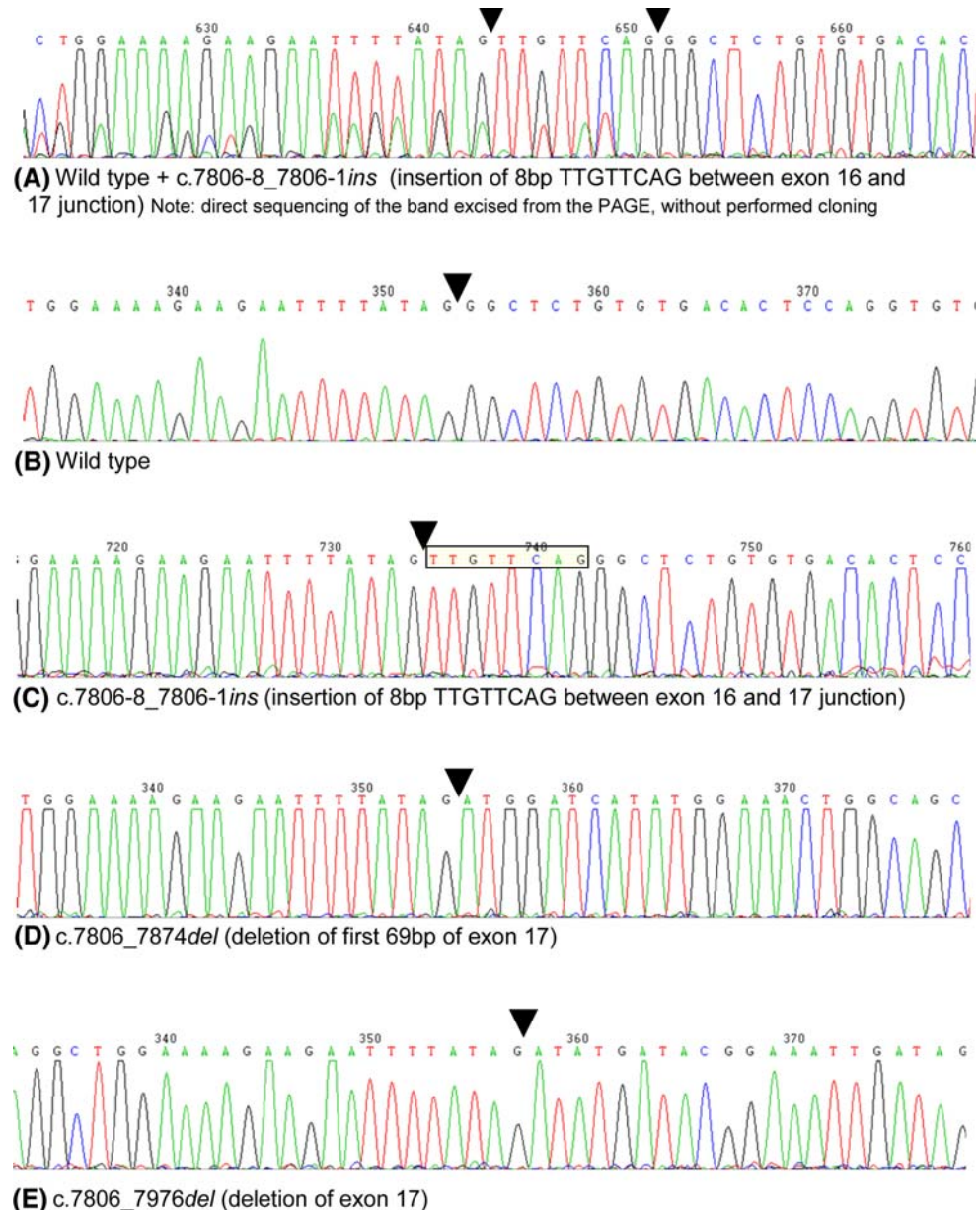
15-18 of the proband. Band I corresponded to BRCA2 wild-type transcript and *c.7806-8\_7806-1ins* transcripts. Band II corresponded to *c.7806-7874del*, and band III corresponded to *c.7806-7976del*. *Lane 2:* positive control using MDA231 breast cancer cell line RNA. *Lane 3:* negative control using MDA231 breast cancer cell line DNA. *Lanes 4 and 5:* no cDNA control and *MspI* digested pBluescript DNA marker, respectively

onset of cancer [21, 30]. The frequency and variety of BRCA1 and BRCA2 mutation carriers including VUS in women with breast cancer demonstrates considerable variation with ethnic and geographical diversity. Increasing reports of VUS may be due to a higher frequency of variants in specific ethnic populations or may be the result of the limited knowledge of the mutation spectrum in ethnic populations, with available data being largely based upon Caucasian DNA sequence. It is likely that these VUSs are still not well defined as disease-causal mutations due to their low frequency of occurrence in the Caucasian population.

The clinical relevance of missense and intron variants poses problems for risk assessment due to possible ethnic specificity [31, 32] and their low frequency. Several lines of investigation may assist in the classification of a variant into deleterious/high risk or neutral/low clinical significance [33]. The occurrence of a variant in a high-risk individual can be compared to controls of the same ethnicity to provide clues to its status. Segregation of a family affected by the disease can be a strong indicator that a variant is pathogenic [34]. The proband in this study and her family were suspected of having a pathogenic mutation

due the segregation of the disease phenotype and the young age of presentation of breast cancer in the family. Further RNA analysis confirmed that the reported BRCA2 variant from this study was in fact pathogenic. A large number of intron variants cannot be readily distinguished as either disease-associated mutations or benign polymorphisms if studied only at a genomic DNA level [31]. However, alterations occurring in the intron–exon boundaries have potential impact on mRNA splicing fidelity. A previous report suggested that changes at the splicing sites affecting mRNA processing can account for 15% of point mutations that are associated with genetic disease [35]. Approximately 5% of all BRCA1 and BRCA2 alterations are splice-site mutations [35, 36]. Large number of cancer-related genes that exhibit alternative splicing have been characterized, including BRCA variants, and these splice variants could either be causative of disease, or be involved in disease development by acting as surrogate cancer biomarkers [37–39]. Therefore, even splice variants or intronic sequence variations should raise suspicion and be considered as pathogenic in patients who are considered of high familial risk. In vitro functional experiments to demonstrate the role of the BRCA2 *c.7806-9T > G* mutation

**Fig. 6** Showing BRCA2 wild type and three aberrant spliced transcripts. (A) Direct sequencing result (using reverse primer BRCA2-exon18R) of the gel-excised product from band I shown in Fig. 5. This band contained a mixture of both wild-type and c.7806-8\_7806-1ins transcripts. Direct sequencing results of band II (D) c.7806\_7874del and band III (E) c.7806\_7976del shown in Fig. 5. Some clones of band I were shown to have sequences of wild-type BRCA2 transcript (B) and aberrant BRCA2 transcript c.7806-8\_7806-1ins (C). The inverted black triangle indicates the splicing junctions: the sequence to its left corresponds to exon 16. The yellow shaded box indicates the 8 bp insertion



affecting splicing events leading to the production of aberrant spliced variants will be performed using a mini-gene assay. The VUS c.440A > G also co-segregated in the same family members and hence is likely to be located in the same allele as c.7806-9T > G. The contribution of this nonsynonymous change to modulating splicing events and RNA stability remains to be investigated.

Interestingly, the proband's mother, who carried the BRCA2 mutation, died of gastric cancer but has no history of breast cancer. BRCA2 mutation has been reported to be associated with an increased risk of stomach cancer [40, 41]. Very few large-scale BRCA genes mutation screening studies have been conducted on Chinese breast or ovarian cancer patients. Recently a few reports have

been published by institutions in China, mainly from Beijing and Shanghai, which consist of Northern and Eastern Chinese populations, respectively [15, 16, 42]. There are very few publication on BRCA gene mutations in breast cancer in the Southern Chinese population, the ethnicity of the majority of those who reside in Hong Kong [43]. Most of the reports on BRCA mutation in the Southern Chinese population were based on women with ovarian rather than breast cancer [44]. One of these studies identified recurrent BRCA mutations in ovarian cancer only in Southern Chinese population and not Northern Chinese population, indicating possible heterogeneity in the BRCA genotype between the Northern and Southern Chinese populations [44]. Hence, studying the genetic

contribution in the Hong Kong Chinese population would provide important information among Chinese of Southern ancestral origin, which may differ from other parts of China. Due to the heterogeneous and broad spectrum of the BRCA genes, which are increasingly reported to have ethnic diversity, more research to improve our understanding of the spectrum of BRCA mutations in the Chinese population is necessary to provide appropriate genetic counseling and guidelines for genetic testing to this population.

## Conclusions

We report a novel BRCA2 variant (c.7806-9T > G) and characterized it as pathogenic [Genbank: DQ889340]. It may be responsible for aberrant splicing events, producing three mutant transcripts (c.7806-8\_7806-1ins, c.7806\_7874del, and c.7806\_7976del). The increasing identification of novel mutations and reclassification of previously reported gene VUS in different ethnic populations such as the Chinese population suggests that some BRCA 1 and BRCA 2 mutations and pathogenicity of these mutations may be unique to specific populations. Classification of VUS as neutral or disease causing, particularly in ethnic groups where limited knowledge is available, is a challenge. Determination of the effect of a VUS on function is important for genetic counseling. Research on the spectrum of mutations in such diverse ethnic groups has important implications for genetic counseling so that a more ethnic-specific, cost-effective risk reduction management, including an increased level of breast surveillance or medical and surgical prophylactic interventions, can be given.

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## References

- Miki Y et al (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 266(5182):66–71
- Wooster R et al (1994) Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12–13. *Science* 265(5181):2088–2090
- Ford D, Easton DF, Peto J (1995) Estimates of the gene frequency of BRCA1 and its contribution to breast and ovarian cancer incidence. *Am J Hum Genet* 57(6):1457–1462
- Claus EB et al (1996) The genetic attributable risk of breast and ovarian cancer. *Cancer* 77(11):2318–2324
- Ford D et al (1998) Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. *Am J Hum Genet* 62(3):676–689
- Struwing JP et al (1997) The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. *N Engl J Med* 336(20):1401–1408
- Claes K et al (2004) BRCA1 and BRCA2 germline mutation spectrum and frequencies in Belgian breast/ovarian cancer families. *Br J Cancer* 90(6):1244–1251
- Frank TS et al (2002) Clinical characteristics of individuals with germline mutations in BRCA1 and BRCA2: analysis of 10,000 individuals. *J Clin Oncol* 20(6):1480–1490
- Hamann U et al (2003) Similar contributions of BRCA1 and BRCA2 germline mutations to early-onset breast cancer in Germany. *Eur J Hum Genet* 11(6):464–467
- Ikeda DM et al (2001) Development, standardization, and testing of a lexicon for reporting contrast-enhanced breast magnetic resonance imaging studies. *J Magn Reson Imaging* 13(6):889–895
- Nanda R et al (2005) Genetic testing in an ethnically diverse cohort of high-risk women: a comparative analysis of BRCA1 and BRCA2 mutations in American families of European and African ancestry. *JAMA* 294(15):1925–1933
- Panguluri RC et al (1999) BRCA1 mutations in African Americans. *Hum Genet* 105(1–2):28–31
- Tiling R et al (2001) 18F-FDG PET and 99mTc-sestamibi scintimammography for monitoring breast cancer response to neoadjuvant chemotherapy: a comparative study. *Eur J Nucl Med* 28(6):711–720
- Sng KW et al (2000) Spectrum of abnormal mammographic findings and their predictive value for malignancy in Singaporean women from a population screening trial. *Ann Acad Med Singapore* 29(4):457–462
- Suter NM et al (2004) BRCA1 and BRCA2 mutations in women from Shanghai China. *Cancer Epidemiol Biomarkers Prev* 13(2):181–189
- Zhi X et al (2002) BRCA1 and BRCA2 sequence variants in Chinese breast cancer families. *Hum Mutat* 20(6):474
- Zhou P, Gautam S, Recht A (2007) Factors affecting outcome for young women with early stage invasive breast cancer treated with breast-conserving therapy. *Breast Cancer Res Treat* 101(1):51–57
- de Barros N et al (2001) Cutaneous myiasis of the breast: mammographic and us features-report of five cases. *Radiology* 218(2):517–520
- Goldgar DE et al (2004) Integrated evaluation of DNA sequence variants of unknown clinical significance: application to BRCA1 and BRCA2. *Am J Hum Genet* 75(4):535–544
- Ng K, Yip K, Choi C (2003) A case of oral myiasis due to *Chyrsomya bezziana*. *Hong Kong Med J* 9:454–456
- Hu Z et al (2003) The analysis of BRCA1 mutations in eastern Chinese patients with early onset breast cancer and affected relatives. *Hum Mutat* 22(1):104
- Wappenschmidt B et al (2005) Strong evidence that the common variant S384F in BRCA2 has no pathogenic relevance in hereditary breast cancer. *Breast Cancer Res* 7(5):R775–R779
- Bunyan DJ et al (2004) Dosage analysis of cancer predisposition genes by multiplex ligation-dependent probe amplification. *Br J Cancer* 91(6):1155–1159
- Hogervorst FB et al (2003) Large genomic deletions and duplications in the BRCA1 gene identified by a novel quantitative method. *Cancer Res* 63(7):1449–1453
- Sellner LN, Taylor GR (2004) MLPA and MAPH: new techniques for detection of gene deletions. *Hum Mutat* 23(5):413–419
- Lazovich D et al (1999) Breast conservation therapy in the United States following the 1990 National Institutes of Health Consensus Development Conference on the treatment of patients with early stage invasive breast carcinoma. *Cancer* 86(4):628–637
- Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. *Nat Biotechnol* 18(2):233–234

28. Yau TK et al (2002) Breast conservation treatment in Hong Kong—early results of 203 patients: retrospective study. *Hong Kong Med J* 8(5):322–328
29. Reese MG et al (1997) Improved splice site detection in Genie. *J Comput Biol* 4(3):311–323
30. Drew PJ et al (2001) Evaluation of response to neoadjuvant chemoradiotherapy for locally advanced breast cancer with dynamic contrast-enhanced MRI of the breast. *Eur J Surg Oncol* 27(7):617–620
31. Campos B et al (2003) RNA analysis of eight BRCA1 and BRCA2 unclassified variants identified in breast/ovarian cancer families from Spain. *Hum Mutat* 22(4):337
32. Durocher F et al (1996) Comparison of BRCA1 polymorphisms, rare sequence variants and/or missense mutations in unaffected and breast/ovarian cancer populations. *Hum Mol Genet* 5(6): 835–842
33. Hartmann LC et al (1999) Clinical options for women at high risk for breast cancer. *Surg Clin North Am* 79(5):1189–1206
34. Phelan CM et al (2005) Classification of BRCA1 missense variants of unknown clinical significance. *J Med Genet* 42(2):138–146
35. Krawczak M, Reiss J, Cooper DN (1992) The mutational spectrum of single base-pair substitutions in mRNA splice junctions of human genes: causes and consequences. *Hum Genet* 90(1–2):41–54
36. Chen X et al (2006) Intronic alterations in BRCA1 and BRCA2: effect on mRNA splicing fidelity and expression. *Hum Mutat* 27(5):427–435
37. Bieche I, Lidereau R (1999) Increased level of exon 12 alternatively spliced BRCA2 transcripts in tumor breast tissue compared with normal tissue. *Cancer Res* 59(11):2546–2550
38. Brinkman BM (2004) Splice variants as cancer biomarkers. *Clin Biochem* 37(7):584–594
39. Lu M et al (1996) Characterization of functional messenger RNA splice variants of BRCA1 expressed in nonmalignant and tumor-derived breast cells. *Cancer Res* 56(20):4578–4581
40. The Breast Cancer Linkage Consortium (1999) Cancer risks in BRCA2 mutation carriers. *J Natl Cancer Inst* 91(15):1310–1316
41. Jakubowska A et al (2002) BRCA2 gene mutations in families with aggregations of breast and stomach cancers. *Br J Cancer* 87(8):888–891
42. Li N et al (2006) BRCA1 germline mutations in Chinese patients with hereditary breast and ovarian cancer. *Int J Gynecol Cancer* 16(Suppl 1):172–178
43. Hong CC et al (2004) Cytochrome P450 1A2 (CYP1A2) activity, mammographic density, and oxidative stress: a cross-sectional study. *Breast Cancer Res* 6(4):R338–R351
44. Cheung KL et al (2001) Palpable asymmetrical thickening of the breast: a clinical, radiological and pathological study. *Br J Radiol* 74(881):402–406