Nature of genetic variants in the *BRCA1* and *BRCA2* genes from breast cancer families in Taiwan

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Received Febrary 2, 2008

Abstract

A total of six families with two cases of breast cancer from southern Taiwan were identified, and the nature of genetic mutations was analyzed. One novel missense substitution of Gln1886Pro (A5885C), as well as one novel silent nucleotide change of A4806G at Thr1526, of the *BRCA2* gene was found in a breast cancer family. Four missense substitutions of Pro871Leu (C2731T), Glu1038Gly (A3232G), Lys1183Arg (A3667G) and Ser1613Gly (A4956G), and two silent nucleotide changes of T2430C at Leu 771 and T4427C at Ser1436 of the *BRCA1* gene, as well as one silent nucleotide change of T4035C at Val1296 of the *BRCA2* gene, were identified in five other breast cancer families. All of the *BRCA1* and *BRCA2* variations identified thus far in Taiwan are compared with those reported from China. [Life Science Journal. 2009; 6(3): 99 – 103] (ISSN: 1097 – 8135).

Keywords: *BRCA1*, *BRCA2*, variants, breast cancer, Taiwan

1. Introduction

Breast cancer is the most common malignancy among women. The *BRCA1* (MIM#113705) [Miki et al., 1994] and *BRCA2* (MIM#600185) [Wooster et al., 1995] genes are associated with inherited susceptibility to breast cancer, and the mutations in these two genes accounted for about 5-10% of all breast cancer cases [Szabo and King, 1997]. It is about twice as many have either a first-degree or a second-degree relative with breast cancer [Johnson et al., 1995]. The risk conferred by a family history of breast cancer has been assessed in both case-control and cohort studies, using volunteer and population-based samples, with generally consistent results [Pharoah et al., 1997]. Both males and females can inherit and transmit an autosomal dominant cancer predisposition. A male who inherits a cancer predisposition and shows no evidence of it can still pass the altered gene on to his sons and daughters.

BRCA1 and *BRCA2* are involved in a myriad of functions within cells including homologous DNA repair, genomic stability, transcriptional regulation and cell cycle control [Gudmundsdottir and Ashworth, 2006]. Nearly 2,000 distinct mutations and sequence variations in *BRCA1* and *BRCA2* have already been described. Approximately one in 400 to 800 individuals in the general population may carry a pathogenic mutation in *BRCA1* and *BRCA2* [Ford et al., 1995; Whittemore et al., 2004]. Our laboratory had previously reported the nature of mutations in the *BRCA1* and *BRCA2* genes from 18 breast cancer families in Taiwan [Li et al., 1999], and here we describe the nature of genetic variations in these two genes among six new breast cancer families in Taiwan.

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2. Materials and methods

Samples

A total of six new families with two breast cancer cases from southern Taiwan were identified. The younger patients from each family were analyzed for the mutations at all exons and exon-intron junctions of the *BRCA1* and *BRCA2* genes, and the identified variations were confirmed from second patient and/or other members of each family.

DNA extraction/polymerase chain reaction

Genomics DNAs were isolated from peripheral blood lymphocytes by phenol/chloroform extraction method, and the DNA fragments were amplified using the previously published 48 and 58 PCR primer pairs for the *BRCA1* and *BRCA2* genes, respectively [Li et al., 1999].

Subcloning/DNA sequencing

The normal and variant alleles from the some heterozygotes were separately isolated by subcloning into the pUC19 vector (Fermentas, MD, USA). All fragments were sequenced on both strands. The sequencing reactions were performed with a dye-labeled terminators kit (Perkin-Elmer) using the cycle sequencing method. Both separation of DNA fragments and sequence analysis were performed in an ABI Prism 377 DNA sequencer.

3. Result

The clinical information of patients and the nature of genetic variations at the *BRCA1* and *BRCA2* genes from six new breast cancer families in Taiwan are summarized in Table 1 and Table 2.

Table 1. The clinical information of patients from six new breast cancer families in Taiwan

Family	Sample ID *	Age at diagnosis	Disease $	Stage	Histological type
A	AI 1	63	BC	Stage 1	Invasive ductal carcinoma
	AII 2	40	BC	Stage 1	Invasive ductal carcinoma
B	BII 1	53	BC	Stage 0	Intraductal carcinoma
	BII 2	43	BC	Stage 3a	Invasive ductal carcinoma
C	CII 1 #	—	BC	—	—
D	DI 2 #	—	BC	—	—
	DII 2	45	BC	Stage 1	Invasive ductal carcinoma
E	EIII 4	47	BC	Stage 2a	Invasive ductal carcinoma
F	FI 2 &	—	BC	—	—
	FII 1	50	BC	Stage 2a	Invasive ductal carcinoma

* Family: A—F; Generation: I—II; Age from oldest to youngest: 1—4. $ BC: breast cancer. # The patients were taken from external hospital, no information. & The patient was dead, no information.

Table 2. The nature of genetic variations at the BRCA1 and BRCA2 genes from 6 new breast cancer families in Taiwan

Sample ID*	Gene	Exon	Sequence variants	Amino acid change	The mutation site of family members
AII 2	BRCA2	11	A4806G	Thr1526	—
AII 2	BRCA2	11	A5885C	Gln1886Pro	—
BII 2	BRCA2	11	T4035C	Val1269	—
CII 1	BRCA1	13	T4427C	Ser1436	—
CII 1	BRCA1	16	A4956G	Ser1613Gly	Daughter CIII 1 is normal
DII 2	BRCA1	13	T4427C	Ser1436	—
DII 2	BRCA1	16	A4956G	Ser1613Gly	—
EIII 4	BRCA1	11	T2430C	Leu771	—
EIII 4	BRCA1	11	A3232G	Glu1038Gly	Sister EIII 5, EIII 7 have heterozygote mutation
EIII 4	BRCA1	13	T4427C	Ser1436	—
EIII 4	BRCA1	16	A4956G	Ser1613Gly	Sister EIII 1, EIII 2, EIII 3, EIII 5, EIII 6, EIII 7 have heterozygote mutation
#FII 1	BRCA1	11	C2731T	Pro871Leu	Son FIII2 has homozygote mutation; sister FII 5, 2 nieces FIII4, FIII10, and nephew FIII11 have heterozygote mutation
#FII 1	BRCA1	11	A3232G	Glu1038Gly	The same as above
#FII 1	BRCA1	11	A3667G	Lys1183Arg	The same as above

* Family: A—F; Generation: I—II; Age from oldest to youngest: 1—4. # These three BRCA1 mutations are on the same chromosome.

In the A family, the daughter had breast cancer diagnosed at age 40 years, and her mother had breast cancer diagnosed at age 63. Both patients had stage 1 invasive ductal carcinoma. One novel missense substitution of Gln1886Pro (A5885C), as well as one novel silent nucleotide change of A4806G at Thr1526, of the *BRCA2* gene was found to be heterozygous. In the B family, the younger sister had stage 3a invasive ductal carcinoma diagnosed at age 43, and her elder sister had stage 0 intraductal carcinoma diagnosed at age 53. Only one previously reported silent nucleotide change of T4035C at Val1269 of the *BRCA2* gene was detected. In the C family, the younger sister had breast cancer diagnosed at 51, and her elder sister also had breast cancer, but their pathological data were not available. In the D family, the daughter had stage 1 invasive ductal carcinoma diagnosed at age 45, and her mother also had breast cancer. In both C and D families, the previously reported missense substitution of Ser1613Gly (A4956G) and silent nucleotide change of T4427C at Ser1436 of the *BRCA1* gene were found. However, the variant alleles in the C family are heterozygous, while those in the D family are either homozygous or hemizygous (loss of normal allele). In the E family, the patient had stage 2a invasive ductal carcinoma diagnosed at age 47, and her cousin also had breast cancer. Two previously reported missense substitutions of Glu1038Gly (A3232G) and Ser1613Gly (A4956G), as well as two silent nucleotide changes of T2430C at Leu771 and T4427C at Ser1436, of the *BRCA1* gene were found to be heterozygous. Both normal and variant alleles were separately isolated by subcloning and sequenced to confirm these missense substitutions. In the F family, the daughter had stage 2a invasive ductal carcinoma diagnosed at age 50, and her mother died of breast cancer. Three previously reported missense substitutions of Pro871Leu (C2731T), Glu1038Gly (A3232G) and Lys1183Arg (A3667G) of the *BRCA1* gene were found to be heterozygous, and different alleles were subcloned using the 5' and 3' primers to amplify these three variants (Figure 1). It is of interest that all three normal amino acids (Pro-Glu-Lys) are on one haplotype, whereas all three variant amino acids (Leu-Gly-Arg) are on another haplotype. A younger sister and two nieces were found to be heterozygous for these three missense substitutions, but thus far they are free of breast cancer.

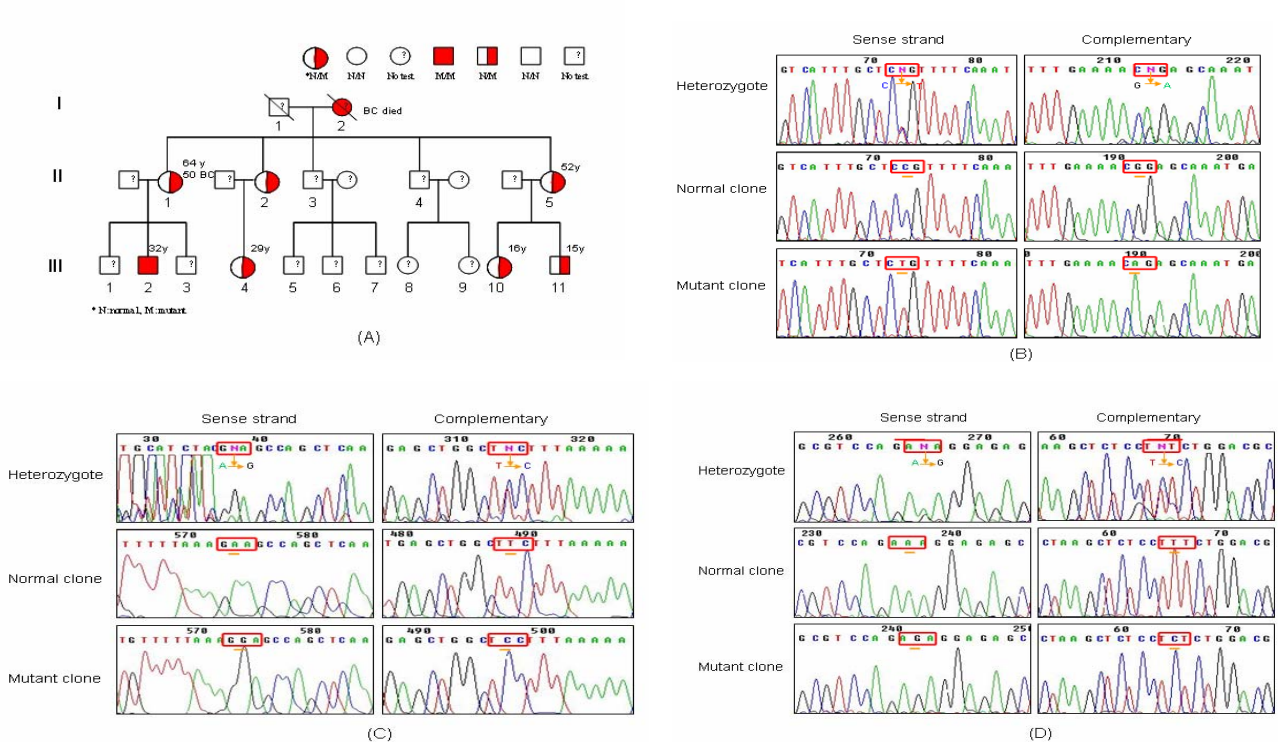

Figure 1. DNA sequence of exon 11 of the sense and complementary strand from a familial patient (designated as FII1) reveals three heterozygous mutation on the same allele. (A) The family F pedigree FII1 is a breast invasive ductal carcinoma patient and the detection of the (B) Pro871Leu (2731C→T), (C) Glu1038Gly (3232A→G) and (D) Lys1183Arg (3667A→G) missense mutation from exon 11 of BRCA1.

4. **Discussion**

Although the molecular nature of mutations in these two genes has been extensively analyzed among Caucasians, only some *BRCA1* and *BRCA2* mutations were reported among Asian populations (Table3), http://research.nhgri.nih.gov/bic/). In this investigation, the novel missense substitution of Gln1886Pro (A5885C) and silent nucleotide change of A4806G at Thr1526 of the *BRCA2* gene were found in one of these six Taiwanese breast cancer families. The four missense substitutions of Pro871Leu, Glu1038Gly, Lys1183Arg and Ser1613Gly of *BRCA1* gene which were detected in this investigation, as well as the substitutions of Pro346Ser of *BRCA1* gene and Asn289His, His372Asn, Asn991Asp and Ile3412Val of *BRCA2* gene, were previously reported among 18 Taiwanese breast cancer families [Li et al., 1999]. The pathological effect of these amino acid substitutions at *BRCA1* and *BRCA2* genes remain to be determined as either disease-associated mutations or benign polymorphisms (Table 4). Some of missense mutations at exon splicing enhancer sequences were shown to cause exon skipping, resulting in breast cancer [Fakenthal et al., 2002; Campos et al., 2003]. Among the 24 breast cancer families analyzed thus far in Taiwan, the same splicing mutation of *BRCA1* gene observed in two unrelated families and three different deletions of *BRCA2* gene are clearly associated with breast cancer [Li et al., 1999]. It may be noted that these breast cancer associated mutations deleted in Taiwan are different from those reported from Hong Kong [Khoo et al., 2000] and Tainjin (northern China) [Zhi et al., 2002].

Table 3. The *BRCA1* and *BRCA2* mutations were reported among Caucasians and Asian populations

Gene	Exon	Sequence variants	Amino acid change	Previously reported	[a] Alleles found in Taiwanese	[b] Alleles reported in Asia	[c] Alleles reported in the world
BRCA1	11	T2430C	Leu771	Yes	2	5	25
BRCA1	11	C2731T	Pro871Leu	Yes	2	5	26
BRCA1	11	A3232G	Glu1038Gly	Yes	2	7	36
BRCA1	11	A3667G	Lys1183Arg	Yes	2	5	32
BRCA1	13	T4427C	Ser1436	Yes	2	7	33
BRCA1	16	A4956G	Ser1613Gly	Yes	2	9	33
BRCA2	11	T4035C	Val1269	Yes	2	2	5
BRCA2	11	A4806G	Thr1526	No	1	1	1
BRCA2	11	A5885C	Gln1886Pro	No	1	1	1

[a] The mutations in Taiwanese from this study and Li, et al., (1999) [b] The mutations in Asia (including Taiwanese)
[a, b, c] Breast cancer information core (Bic) http://research.nhgri.nih.gov/bic/

Table 4. The BRCA1 and BRCA2 amino acid mutation effect

Gene	Exon	Sequence variants	Amino acid change	PI change	Mutation effect
BRCA1	11	T2430C	L771	5.98	Silent mutation
BRCA1	11	C2731T	P871L	6.48? 5.98	Small nonpolar residue ? Hydrophobic residue
BRCA1	11	A3232G	E1038G	3.22? 5.97	Acidic side chains ? Small nonpolar residue
BRCA1	11	A3667G	K1183R	9.74? 10.76	Basic side chains ? Basic side chains
BRCA1	13	T4427C	S1436	5.68	Silent mutation
BRCA1	16	A4956G	S1613G	5.68? 5.97	Small polar residue ? Small nonpolar residue
BRCA2	11	T4035C	V1269	5.97	Silent mutation
BRCA2	11	A4806G	T1526	5.87	Silent mutation
BRCA2	11	A5885C	Q1886P	5.65? 6.48	Uncharged polar side chains ? Small nonpolar residue

Acknowledgements

This investigation was supported by a grant from Kaohsiung Veterans General Hospital.

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