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Identification and evaluation of 55 genetic variations in the *BRCA1* and the *BRCA2* genes of patients from 50 Japanese breast cancer families

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Abstract We sequenced approximately 23 kb genomic regions containing all the coding exons and their flanking introns of two breast cancer susceptibility genes, *BRCA1* and *BRCA2*, of 55 individuals from 50 unrelated Japanese breast cancer families. We identified 55 single-nucleotide polymorphisms (SNPs) (21 in *BRCA1* and 34 in *BRCA2*) containing nine pathogenic protein-truncating mutations (four in *BRCA1* and five in *BRCA2* from ten patients). Among the remaining 46 SNPs, allele frequencies of 40 were examined in both the breast cancer patients and 28 healthy volunteers with no breast cancer family history by PCR-RFLP or by direct DNA sequencing. Twenty-eight SNPs were common and were also found in the healthy volunteers and/or a SNP database. The remaining 18 were rare (allele frequency <0.05) and were not found in the healthy volunteers and/or the database. The pathogenic significance of these coding SNPs (cSNPs) remains to be clarified. The SNP in-

formation from this study will be useful in the future genetic testing of both *BRCA1* and *BRCA2* genes in the Japanese population.

Keywords Single-nucleotide polymorphism (SNP) · Breast cancer susceptibility gene · *BRCA1* · *BRCA2* · Japanese population · Direct DNA sequencing

Introduction

Mutations in the *BRCA1* and the *BRCA2* genes have been linked with the susceptibility to breast and ovarian cancer (Miki et al. 1994; Wooster et al. 1995; Tavtigian et al. 1996). Mutation carriers of these genes are at high risk of breast and ovarian cancer (Narod et al. 1995; Ford et al. 1998; Thorlacius et al. 1998; Neuhausen 1999; Rebbeck 1999; Struwing et al. 1997; Anglian Breast Cancer Study Group 2000). The two genes have large coding sequences consisting of 48 exons in total, and a large number of mutations and SNPs are reported in the Breast Cancer Information Core (BIC) database (<http://research.nhgri.nih.gov/bic/>) and the dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP/index.html>). The majority of mutations described are protein-truncating mutations containing frame-shift mutations and non-sense mutations. In the BIC database, 55.9% of genetic variants are reported as pathogenic mutations containing mainly protein-truncating mutations and a small fraction of pathogenic missense mutations, and 39.3% of variants are categorized as “unclassified variants.” These variants contain coding SNPs (cSNPs) that result in amino-acid substitutions or SNPs located at exon–intron boundaries. We have also reported five protein-truncating mutations and 12 “unclassified variants” that have been found only once in 24 breast cancer families (Sakayori et al. 2003). To elucidate whether detected SNPs are pathogenic mutations or not, it is important to accumulate SNP information (both the type and allele frequency) in patients and the general population.

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Table 1 Sequence variations detected in the *BRCA1* and the *BRCA2* genes

SNP ID ^a	Location	Variation ^b	Flanking sequence (5' to 3')	dbSNP ID ^c	BIC ^d	Volunteers ^e
<i>BRCA1</i> gene						
BRCA1-1	Intron 1	IVS1-115C > T	tggtttgtat C/T attctaaaac	rs3765640		+
BRCA1-2	Exon 3	K38 (silent) (114G > A)	tctccacaaa G/A tgtgaccaca	rs8176099	+	+
BRCA1-3	Intron 8	IVS8-58delT	tacatttttt T/- aacctttta		+	+
BRCA1-4 [#]	Exon 11	G275D (824G > A)	gagccatgtg G/A cacaaatact			
BRCA1-5	Exon 11	S694 (silent) (2082C > T)	gacatgacag C/T gatactttcc	rs1799949	+	+
BRCA1-6	Exon 11	L771 (silent) (2311T > C)	cagtatttca T/C tggtagctgg	rs16940	+	+
BRCA1-7 [#]	Exon 11	2389-2390delGA*	ggcaaaaaa GA/- accaaataaa			
BRCA1-8	Exon 11	P871L (2612C > T)	tcatttgctc C/T gttttcaaat	rs799917	+	+
BRCA1-9	Exon 11	E1038G (3113A > G)	gttttttaag A/G agccagctca	rs16941	+	+
BRCA1-10	Exon 11	K1183R (3548A > G)	agcgtccaga A/G aggagagctt	rs16942	+	+
BRCA1-11 [#]	Exon 12	C1372X (4116T > A)*	catctgggtg T/A gagagtga			
BRCA1-12	Exon 13	S1436 (silent) (4308T > C)	taagtgtact T/C tctgcccttg	rs1060915	+	+
BRCA1-13 [#]	Intron 14	IVS14 + 14A > G	agaaacatca A/G tgtaaagatg		+	
BRCA1-14	Exon 16	S1613G (4837A > G)	atctgccag A/G gtccagctgc	rs1799966	+	+
BRCA1-15 [#]	Exon 16	M1628T (4883T > C)	tataatgcaa T/C ggaagaaagt	rs4986854	+	
BRCA1-16	Intron 18	IVS18 + 66G > A	tacacctaac G/A tttaaacct	rs3092994	+	+
BRCA1-17 [#]	Intron 22	IVS22 + 33A > T	gagagggagg A/T cacaatatc			
BRCA1-18 [#]	Intron 23	IVS23 + 8G > T	atggttaagt G/T cctgcatgta		+	
BRCA1-19	Exon 5	L63X (188T > A)*	cagtgtcctt T/A atgtaagaat		+	
BRCA1-20	Exon 3	H41R (122A > G)	aagtgtgacc A/G catattttgcaaa			
BRCA1-21	Exon 8	470-471delCT*	tccaactct CT/- aaccttgga		+	
<i>BRCA2</i> gene						
BRCA2-1	Exon 2	5'UTR-26G > A	tatttaccaa G/A cattggagga	rs1799943	+	+
BRCA2-2 [#]	Intron 2	IVS2-16T > A	taaggtggga T/A tttttttta			
BRCA2-3 [#]	Intron 2	IVS2-9T > G	ggattttttt T/G ttaatatagat			
BRCA2-4	Intron 4	IVS4 + 67A > C	tggtctataa A/C gatgaatctg			+
BRCA2-5	Intron 4	IVS4-89T > C	acaatttata T/C gaatgagaat		+	+
BRCA2-6	Intron 7	IVS7 + 183T > A	caaatacatt T/A agtggtagtc			+
BRCA2-7	Intron 8	IVS8 + 56C > T	ttggaaatgc C/T ttgttaaat		+	+
BRCA2-8 [#]	Exon 10	F266 (silent) (798T > C)	gtcatggatt T/C ggaaaaacat			
BRCA2-9	Exon 10	N289H (865A > C)	gtcaatgcc A/C atgtctaga	rs766173	+	+
BRCA2-10	Exon 10	H372 N (1114C > A)	aaatgtagca C/A atcagaagcc	rs144848	+	+
BRCA2-11	Exon 10	S455 (silent) (1365A > G)	tacaaaaac A/G gagaagccat	rs1801439	+	+
BRCA2-12 [#]	Exon 10	T582P (1744A > C)	tttaatatcc A/C ctttgaaaaa		+	
BRCA2-13	Exon 11	H743 (silent) (2229T > C)	cagtacaaca T/C tcaaaagtgg		+	+
BRCA2-14	Exon 11	M784V (2350A > G)	aaacctagtc A/G tgatttctag		+	+
BRCA2-15	Exon 11	N991D (2971A > G)	tgattacatg A/G acaaatgggc	rs1799944	+	+
BRCA2-16	Exon 11	K1132 (silent) (3396A > G)	agtttagaaa A/G ccaagctaca	rs1801406	+	+
BRCA2-17 [#]	Exon 11	S1140 (silent) (3420T > C)	tgcaagaag T/C acatttgaag			
BRCA2-18 [#]	Exon 11	3830delA*	aagatagaaa A/- tcaaatgat			
BRCA2-19 [#]	Exon 11	L1522F (4566G > T)	ctactctgtt G/T gggtttcata			
BRCA2-20 [#]	Exon 11	G2044V (6131G > T)	tcccaaaaag G/T cttttcatat		+	+
BRCA2-21	Exon 14	S2414 (silent) (7242A > G)	aaactaaac A/G cattttcaca	Rs1799955	+	+
BRCA2-22 [#]	Exon 11	6491-6495delAGTTG*	gacaaacaac AGTTG/- gtattaggaa			
BRCA2-23	Intron 16	IVS16 + 47A > G	gtattccctc A/G tccctcttc			
BRCA2-24	Intron 16	IVS16-14T > C	aatattctac T/C tttatttgtt		+	+
BRCA2-25	Exon 11	V2109I (6325G > A)	acttctcgt G/A ttgataagag		+	
BRCA2-26 [#]	Exon 20	S2835X (8504C > A)*	gagaagacat C/A atctggatta			
BRCA2-27	Intron 22	IVS22-147A > G	cagataaagt A/G taaagttagt			
BRCA2-28	Exon 25	R3128X (9382C > T)*	AacctccagtggC/Tgaccagaatcc		+	
BRCA2-29	Exon 10	1278delA*	ttcagaaaa A/- gacctattag			
BRCA2-30	Exon 10	K322Q (964A > C)	aaatctacaa A/C aagtaagaactagc		+	
BRCA2-31	Exon 10	E425 (silent) (1275A > G)	aaatatttcaga A/G aaagacct			
BRCA2-32	Exon 11	V1269 (silent) (3807T > C)	catgattctgt T/C gtttcaatgt		+	
BRCA2-33	Exon 11	E1455 (silent) (4365A > G)	cagaaccaga A/G gaattgcata			
BRCA2-34	Exon 18	K2729N (8187G > T)	tatgctgttaa G/T gccagtttagatcct		+	

^aThe SNP ID that have been found in this study. [#]Published previously (Sakayori et al. 2003)

^bThe nucleotide number in the coding region indicates the position downstream of the first nucleotide of ATG (initiation codon) in the *BRCA1* gene or the *BRCA2* gene. *: Nonsense or frame-shift mutation

^cNational Center for Biotechnology Information (NCBI) dbSNP database

^dBreast Cancer Information Core (BIC) database, +: listed in BIC

^e28 Japanese healthy volunteers, +: found at least one individual

Table 2 Allele frequencies of SNPs in the *BRCA1* and the *BRCA2* genes

SNP ID ^a	Allele frequency		
	Breast cancer patients ^b	Volunteers ^c	dbSNP ^d
<i>BRCA1</i> gene			
BRCA1-1	C=0.43, T=0.57	C=0.52, T=0.48	C=0.343, T=0.657
BRCA1-2	G=0.93, A=0.07	G=0.98, A=0.02	G=0.994, A=0.006
BRCA1-3	T7=0.65, T6=0.35*	T7=0.50, T6=0.50	
BRCA1-4	G=0.98, A=0.02	G=1.00, A=0.00	
BRCA1-5	C=0.62, T=0.38	C=0.56, T=0.44	C=0.657, T=0.343
BRCA1-6	T=0.62, C=0.38	T=0.54, C=0.46	T=0.678, C=0.322
BRCA1-8	C=0.62, T=0.38	C=0.54, T=0.46	C=0.619, T=0.381
BRCA1-9	A=0.62, G=0.38	A=0.54, G=0.46	A=0.725, G=0.275
BRCA1-10	A=0.62, G=0.38	A=0.54, G=0.46	A=0.703, G=0.297
BRCA1-12	T=0.62, C=0.38	T=0.54, C=0.46	T=0.747, C=0.253
BRCA1-13	A=0.98, G=0.02*	A=1.00, G=0.00	
BRCA1-14	A=0.62, G=0.38	A=0.54, G=0.46	A=0.696, G=0.304
BRCA1-15	T=0.98, C=0.02	T=1.00, C=0.00	T=0.995, C=0.005
BRCA1-16	G=0.63, A=0.37	G=0.54, A=0.46	G=0.693, A=0.307
BRCA1-17	A=0.98, G=0.02*	A=1.00, G=0.00	
BRCA1-18	G=0.99, T=0.01	G=1.00, T=0.00	
BRCA1-20	A=0.99, G=0.01	ND	
<i>BRCA2</i> gene			
BRCA2-1	G=0.55, A=0.45	G=0.49, A=0.51	G=0.762, A=0.238
BRCA2-2	T=0.97, C=0.03*	T=1.00, C=0.00	
BRCA2-3	T=0.98, G=0.02*	T=1.00, C=0.00	
BRCA2-4	A=0.84, C=0.16*	A=0.84, C=0.16	
BRCA2-5	T=0.84, C=0.16*	T=0.84, C=0.16	
BRCA2-6	T=0.32, A=0.68*	T=0.58, A=0.42	
BRCA2-7	C=0.96, T=0.04*	C=0.96, T=0.04	
BRCA2-8	T=0.98, C=0.02	T=1.00, C=0.00	
BRCA2-9	A=0.86, C=0.14	A=0.84, C=0.16	A=0.838, C=0.024, G=0.009, T=0.129
BRCA2-10	A=0.80, C=0.20	A=0.82, C=0.18	A=0.607, C=0.281, G=0.026, T=0.085
BRCA2-11	A=0.86, G=0.14	A=0.84, G=0.16	A=0.875, G=0.125
BRCA2-12	A=0.99, C=0.01	A=1.00, C=0.00	
BRCA2-13	T=0.86, C=0.14	T=0.84, C=0.16	
BRCA2-14	A=0.93, G=0.07	A=0.95, G=0.05	
BRCA2-15	A=0.85, G=0.15	A=0.84, G=0.16	A=0.970, G=0.030
BRCA2-16	A=0.47, G=0.53	A=0.50, G=0.50	A=0.705, G=0.295
BRCA2-17	T=0.99, C=0.01	T=1.00, C=0.00	
BRCA2-19	G=0.98, T=0.02	G=1.00, T=0.00	
BRCA2-20	G=0.97, T=0.03	G=0.98, T=0.02	
BRCA2-21	A=0.58, G=0.42	A=0.50, G=0.50	A=0.758, G=0.242
BRCA2-23	A=0.96, G=0.04*	A=1.00, G=0.00	
BRCA2-24	T=0.36, C=0.64	T=0.27, C=0.73	
BRCA2-25 [#]	G=0.99, A=0.01	G=1.00, A=0.00	
BRCA2-27 [#]	A=0.98, G=0.02*	A=1.00, G=0.00	
BRCA2-30	A=0.97, C=0.03	ND	
BRCA2-31	A=0.97, C=0.03	ND	
BRCA2-32	T=0.86, C=0.14	ND	
BRCA2-33	A=0.98, G=0.02*	ND	
BRCA2-34	G=0.98, T=0.02	ND	

^aIdentical to the SNP ID listed in Table 1, [#]: found in a relative but not in the proband

^b110 alleles from 55 patients, *: data derived from 68 alleles (34 patients)

^c56 alleles from 28 healthy volunteers, ND: not determined

^dNational Center for Biotechnology Information (NCBI) dbSNP database

In this study, we extended our study on the *BRCA1* and *BRCA2* sequencing project to 55 breast cancer patients from 50 Japanese breast cancer families. We evaluated the detected SNPs by comparing the allele frequency of the SNPs in healthy volunteers.

Subjects and methods

Fifty-five enrolled patients (including 50 probands and five relatives) with a history of breast cancer from 50 unrelated high-risk breast cancer families were selected according to the criteria defined by the Tohoku Familial Cancer Society (Sakayori et al. 2003). An additional 28

Japanese volunteers with no breast cancer family history were also enrolled to analyze the specific *BRCA1* and *BRCA2* variations detected in the breast cancer patients. We obtained informed consent from all patients and volunteers, and the independent studies were approved by the Familial Cancer Society and the Ethical Committee of Tohoku University Graduate School of Medicine. To identify the sequence variations in the *BRCA1* and *BRCA2* genes of the familial breast cancer patients, we sequenced approximately 23 kb genomic regions of the *BRCA1* (8.4 kb) and the *BRCA2* (14.6 kb) containing all coding exons and their flanking introns using a method described previously (Sakayori et al. 2003). The identified SNPs found in the familial breast cancer

patients were also examined in the genomic DNA from the healthy volunteers by PCR-RFLP analysis using PCR primers, cycle conditions and restriction enzymes for PCR-RFLP analysis (<http://www.idac.tohoku.ac.jp/dep/co/data/saka/brca02.htm>) or by DNA sequence analysis. We used Genbank (U14680 and U61268 for *BRCA1*, U43746 and X95152-77 for *BRCA2*) and the BIC database as the reference sequences of *BRCA1* and *BRCA2* genes.

Results and discussion

By DNA sequencing analysis of the *BRCA1* and *BRCA2* genes for 55 patients from 50 unrelated breast cancer families; we detected 55 SNPs (21 SNPs in *BRCA1* and 34 SNPs in *BRCA2*). Among these SNPs, we found nine protein-truncating mutations (four in *BRCA1* and five in *BRCA2*) in ten patients containing six novel mutations that were not found in the BIC database (<http://research.nhgri.nih.gov/bic/>) (Table 1). The existence of mutations were also confirmed by the stop codon assay in yeast (Ishioka et al. 1997; Sakayori et al. 2003). The percentage of protein-truncating mutations in the examined families was 20% (ten of 50), comparable with several reports describing the frequency of protein-truncating mutations of the two genes in Japanese breast cancer families (Inoue et al. 1995; Takano et al. 1997; Inoue et al. 1997; Ikeda et al. 2001). Although the frequency was also similar to results from Western populations, the number of patients studied in our and other Japanese populations was smaller than that in Western countries. Therefore, it is necessary to study a larger number of patients to clarify the mutation frequency in Japanese familial breast cancer.

Among the remaining 46 SNPs (17 SNPs in *BRCA1* and 29 SNPs in *BRCA2*), 18 and 31 have been reported in the dbSNP database and in the BIC database, respectively, (Table 1). To evaluate whether the SNPs are also found in Japanese healthy volunteers, all but six SNPs were further examined by PCR-RFLP analysis or by DNA sequence analysis. Twenty-six SNPs were found in the volunteers at least once with different allele frequencies (Table 2). In these 40 SNPs, we predicted that 27 SNPs were common polymorphisms and probably played no direct role in the tumorigenesis of breast cancer. The remaining 13 SNPs were quite low in allele frequency (<0.05) and were not found in the healthy volunteers or the dbSNP database. In addition, there were six SNPs that failed to examine in the volunteer group. In these six SNPs, one was predicted to be common and five to be rare from the allele frequencies in the patients. Overall, we predicted the 18 rare SNPs are candidates of pathogenic mutations and that the remaining 28 were common polymorphisms and probably have no direct role in tumorigenesis of breast cancer. In the 18 rare SNPs, five (*BRCA1*-13, *BRCA1*-17, *BRCA1*-18, *BRCA2*-2, *BRCA2*-3) were located at the exon-intron boundaries and two (*BRCA2*-23, *BRCA2*-

27) were located in introns far from exon-intron boundaries. Although SNPs at the exon-intron boundaries may affect normal RNA splicing, our RT-PCR analysis showed negative data for splicing abnormalities. In the remaining 11 rare cSNPs, seven (*BRCA1*-4, *BRCA1*-20, *BRCA2*-12, *BRCA2*-19, *BRCA2*-25, *BRCA2*-30 and *BRCA2*-34) were nonsynonymous substitution resulting in the amino-acid substitutions, and four (*BRCA2*-8, *BRCA2*-17, *BRCA2*-31 and *BRCA2*-33) were synonymous (silent) changes. These SNPs may directly affect the functions of *BRCA1* and *BRCA2* proteins or affect normal splicing by acting as possible cryptic splicing sites. Unfortunately, we have failed to clarify this issue mainly because there are no reliable functional assays of either *BRCA1* or *BRCA2* protein for many nonsynonymous changes.

To confirm the pathogenic effect of rare SNPs, both the development of functional assays for these gene products and more intensive SNP analysis including an investigation into whether these SNPs cosegregate with breast cancer onset in families are necessary.

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