Frequency of *BRCA1* and *BRCA2* Germline Mutations Detected by Protein Truncation Test and Cumulative Risks of Breast and Ovarian Cancer among Mutation Carriers in Japanese Breast Cancer Families

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The purpose of this investigation is to study the frequency and penetrance of *BRCA1* and *BRCA2* germline mutations in Japanese familial breast cancer patients. Mutation analysis of *BRCA1* and *BRCA2* by protein truncation test was conducted on the 120 breast cancer patients (probands) with at least one breast cancer (site-specific breast cancer families, n=105) or one ovarian cancer (breast/ovarian cancer families, n=15) patient in their first-degree relatives. Eight *BRCA1* (7.6%) and ten *BRCA2* (9.5%) mutations were found in site-specific breast cancer families (n=105), and seven *BRCA1* (46.7%) but no *BRCA2* (0%) mutations were found in breast/ovarian cancer families (n=15). In site-specific breast cancer families, mutation frequency of *BRCA1* and *BRCA2* was high in families with more than three breast cancer patients (30%, 6/20), early onset (40≤ years old) breast cancer patients (41.1%, 14/34), or bilateral breast cancer patients (40%, 6/15). Cumulative incidence of breast cancer by age 70 was estimated to be 78% and 80% for *BRCA1* and *BRCA2* mutation carriers, respectively, and that of ovarian cancer was 40% and 0% for *BRCA1* and *BRCA2* mutation carriers, respectively. Family profiles are important determinants of risk for carrying a *BRCA1* or *BRCA2* mutation. Japanese women with *BRCA1* mutation have a high risk of breast and ovarian cancer and those with *BRCA2* mutation have a high risk of breast cancer but not ovarian cancer. **(Journal of Korean Breast Cancer Society 2002; 5:194-201)**

Key Words: Familial breast cancer, *BRCA1*, *BRCA2*, Germline mutation

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INTRODUCTION

BRCA1 and *BRCA2* are breast cancer susceptibility gene which were cloned through linkage analysis using large breast cancer families.(*1-4*) Recently, it has been reported that frequency of *BRCA1* mutations is 7.1∼26.5% and that of *BRCA2* is 13.0% for breast cancer patients with a modest to minimal family history in the Western countries.(*5-12*) It is well known that *BRCA1* and *BRCA2* mutation frequency can be different among ethnic groups.(*13-18*) Thus, it seems to be important to study the *BRCA1* and *BRCA2* mutation frequency as well as predictors of having such mutations s in Japanese breast cancer families. In addition, a low incidence of breast cancer in general population in Japan as compared with the Western countries seems to raise the possibility that risk for developing breast cancers among mutation carriers might be lower in Japanese women than Western women.

In the present paper, we have reviewed our recent study on the *BRCA1* and *BRCA2* mutation frequency by protein trun-

cation test (PTT) in Japanese breast cancer families as well as the relationship between family history profiles and the mutation frequency. Furthermore, risk for developing breast cancer or ovarian cancer has also been estimated using the families with deleterious mutations.

MATERIALS AND METHODS

1) Patients

Among the breast cancer patients treated in the seven key hospitals in Japan during the period from July 1995 to December 1998, those who had at least one breast cancer or one ovarian cancer patient in their first-degree relatives were consecutively entered in this study after the informed consent was obtained. Family history was taken from the probands and/or their relatives by trained nurses and further confirmed by the doctors. In total, 120 breast cancer families were recruited. Of these, three families were with a male breast cancer patient.

2) DNA and RNA isolation

Mononuclear cells were isolated by Ficoll-Paque from the EDTA-treated peripheral blood samples obtained from the probands, and were subjected to DNA and RNA extraction according to the phenol/chloroform method and AGPC method, respectively. In some cases, DNA and RNA were prepared from the fresh frozen tumor tissues using the same methods. When a mutation was found in tumor tissue-derived DNA, constitutional DNA was analyzed in order to confirm that the mutation was germline but not somatic.

3) Protein truncation test (PTT)

Since 80～90% of *BRCA1* and *BRCA2* mutations so far reported are nonsense or frameshift mutations which lead to premature termination of the protein synthesis, we have adopted PTT as a screening method. PTT of *BRCA1* and *BRCA2* was carried out according to the method described by Hogervorst et al (*19*) and Krainer et al (*20*), respectively. In brief, the entire coding region of the *BRCA1* gene was divided into five

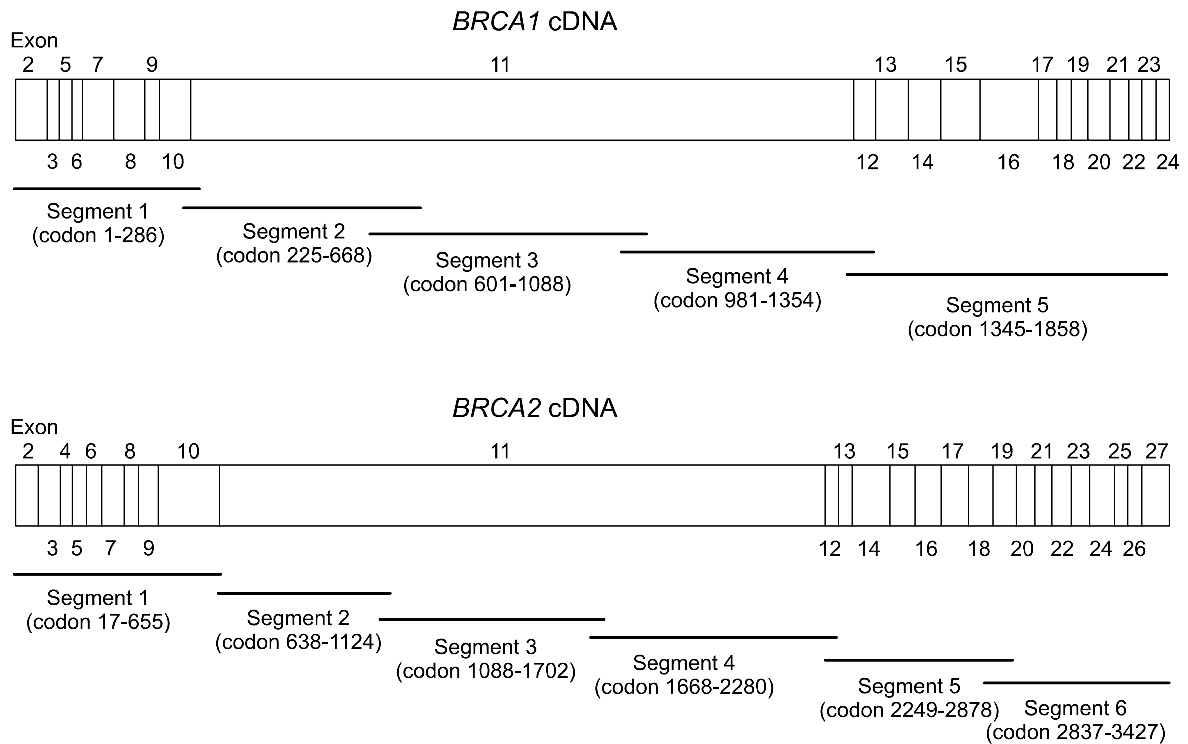

Fig. 1. Protein truncation test of *BRCA1* and *BRCA2* gene. The entire coding regions of the *BRCA1* and *BRCA2* were divided into five and six overlapping fragments, respectively, and amplified by polymerase chain reaction (PCR) using DNA for the fragments 2～4 of *BRCA1* and *BRCA2*, and by nested reverse-transcriptase PCR (RT-PCR) using RNA for the fragments 1 and 5 for *BRCA1* and fragments 1, 5, and 6 for *BRCA2*.

overlapping fragments (fragments 1～5) (Fig. 1) and amplified by polymerase chain reaction (PCR) using DNA for the fragments 2～4 and by nested reverse-transcriptase PCR (RT-PCR) using RNA for the fragments 1 and 5. Primers used for PCR and RT-PCR amplification are shown in Table 1. The PCR condition for genomic DNA consisted of 30 cycles (94°C for 1 minute, 58°C for 1 minute, and 70°C for 4 minutes). For RT-PCR, RNA was subjected to reverse transcription using random hexamers before nested PCR amplification. The first PCR condition consisted of 32 (94°C for 1 minute, 58°C for 1 minute, and 70°C for 4 minutes) and the second PCR condition consisted of 30 cycles (94°C for 0.5 minute, 58°C for 1 minute, and 70°C for 3.5 minutes).Each sense primer for genomic DNA PCR and each inner sense primer for nested RT-PCR contained the 5' extension, GCTAATACGACTCA-CTATAGGAACAGACCACCATGG, which encoded a T7 RNA polymerase recognition site, a start codon, and Kozak translation-initiation consensus sequence, to allow efficient in vitro transcription and translation. An aliquot of the PCR products was incubated with T7 polymerase, rabbit reticulocyte lysate, and $[^{35}S]$methionine in a coupled transcription-translation reaction, followed by electrophoresis with 10～20% gradient polyacrylamide sodium docecyl sulfate gel. The PCR products that gave rise to truncated peptides were analyzed by automated nucleotide sequencing of both strands with dye-labeled dideoxy terminators. PTT of *BRCA2* was carried out according to the method described above for *BRCA1* using the primers shown in Fig. 1 and Table 1.

4) Statistics

The cumulative incidence of breast and ovarian cancer in the first-degree relatives (mothers and sisters) of the probands was calculated according to the Kaplan-Meier method. There were 52 and 24 first-degree relatives in the *BRCA1*- and *BRCA2*-associated breast cancer families, respectively. The *BRCA1*-associated breast cancer families had 17 breast and six ovarian cancer patients, and the *BRCA2*-associated breast cancer families had ten breast and no ovarian cancer patients.

RESULTS

1) *BRCA1* and *BRCA2* mutations

Representative results of PTT for *BRCA1* (segment 4) are shown in Fig. 2, where the sample 3 showed a truncated protein band. Direct sequencing revealed one base insertion at 3581 (codon 1155). In total, 15 mutations including eight nonsense

Table 1. Primers used for protein truncation test of *BRCA1* and *BRCA2*

*BRCA1**		Primer sequences (5'-3')	*BRCA2*†		Primer sequences (5'-3')
Segment 1	Outer F‡	GTGGGGTTTCTCAGATAACTGG	Segment 1	Outer F	CATTGGAGGAATATCGTAGGT
	Outer R§	ATGAGTTGTAGGTTTCTGCTGTG		Outer R	AAAGAGCTAGTTAAGGACAAAGT
	Inner-F‖	GTTCATTGGAACAGAAAGAAATGG		Inner F‖	CACGCTGCAACAAAGCA
	Inner-R	TTCTCATGCTGTAATGAGCTGG		Inner-R	GGTTCTTCAGAATCATTCTGTG
Segment 2	F‖	CTTGTGAATTTTCTGAGACGG	Segment 2	F‖	TTATTGCATTCTTCTGTGAAAAGA
	R	ATGAGTTGTAGGTTTCTGCTGTG		R	CTGACTTCCTGATTCTTCTAAT
Segment 3	F‖	ACAATTCAAAAGCACCTAAAAAG	Segment 3	F‖	CTCAGATGTTATTTTCCAAGCA
	R	AACCCCTAATCTAAGCATAGCATTC		R	GTTGACCATCAAATATTCCTTC
Segment 4	F‖	CACCACTTTTTCCCATCAAGTC	Segment 4	F‖	GCCTTAGCTTTTTACACAAGTTG
	R	TTATTTTCTTCCAAGCCCGTTCC		R	CACTAAGATAAGGGGCTCT
Segment 5	Outer F	TCACAGTGCAGTGAATTGGAAG	Segment 5	Outer F	GCAGTAGAAATTGCTAAAGCT
	Outer R	GTAGCCAGGACAGTAGAAGGA		Outer R	GTTAGTGCACGTGATGGTA
	Inner-F‖	AAGAAAGAGGAACGGGCTTGG		Inner-F‖	GCCACACATTCTCTTTTTACATGT
	Inner-R	GATCTGGGGTATCAGGTAGGTG		Inner-R	CTTCATGTTCTTCAAATTCCTC
			Segment 6	Outer F	AAGAGCATACCCTATACAGT
				Outer R	CTGGAAAGGTTAAGCGTCAAT
				Inner-F‖	GATTATACATATTTCGCAATGAAAG
				Inner-R	TTATTGTCGCCTTTGCAAATG

*,† Segments 1 and 5 *for B*RCA1 and segments 1, 5, and 6 *for B*RCA2 were amplified by nested PCR; ‡ forward primer; §reverse primer; ‖ These primers contained the 5' extension, GCTAATACGACTCACTATAGGAACAGACCACCATGG.

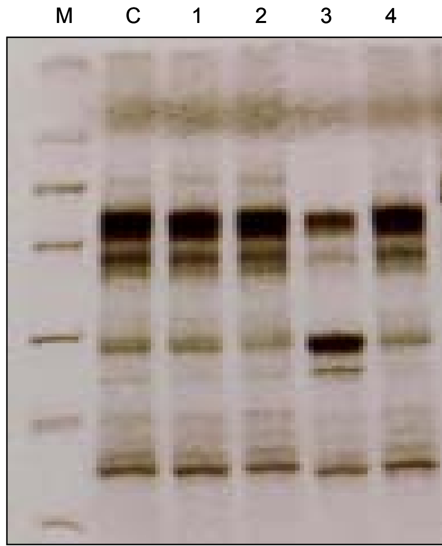

Fig. 2. Representative results of PTT for *BRCA1*. Four samples (lanes 1～4) were analyzed by PTT for *BRCA1* (fragment 4). A mutant band is seen in sample 3. M, molecular marker; C, healthy control.

and seven frameshift mutations were identified for *BRCA1*, and ten mutations including three nonsense and seven frameshift mutations were identified for *BRCA2* (Table 2). The same nonsense mutation (codon 934) of *BRCA1* was observed in three unrelated patients and the same frameshift mutation (4 bp deletion (5802-5805)) was observed in two unrelated patients.

2) Frequency of *BRCA1* and *BRCA2* mutations according to family history profiles

Eight *BRCA1* (7.6%) and ten *BRCA2* (9.5%) mutations were found in site-specific breast cancer families (n=105), and seven *BRCA1* mutations (46.7%) but no *BRCA2* (0%) mutations were found in breast/ovarian cancer families (n=15) (Table 3). In site-specific breast cancer families, mutation frequency of *BRCA1* and *BRCA2* was higher in families with more than three breast cancer patients (30%) as compared to families with two breast cancer patients (14.5%). Families with patients diagnosed at younger than 40 years old showed a high frequency of *BRCA1* and *BRCA2* mutations (41.1%), and the frequency decreased as the age at diagnosis became older. Families with bilateral breast cancer patients showed a higher frequency of *BRCA1* and *BRCA2* mutations (40%) than those with only unilateral breast cancer patients (13.3%) (Table 4). No mutation in*BRCA1* and *BRCA2* was found in three families with a male breast cancer patient.

Table 2. *BRCA1* and *BRCA2* germline mutations

Family	Codon	Nucleotide change	Effect
BRCA 1			
1	290	1 bp (988) deletion	Frameshift
2	374	1 bp (1239) deletion	Frameshift
3	445	2 bp (1453-4) deletion	Frameshift
4	503	AAG to TAG	Nonsense
5	934	CAG to TAG	Nonsense
6	934	CAG to TAG	Nonsense
7	934	CAG to TAG	Nonsense
8	1041	TCA to TGA	Nonsense
9	1085	AGA to TGA	Nonsense
10	1112	5 bp (3453) insertion	Frameshift
11	1125	2 bp (3493-4) deletion	Frameshift
12	1140	1 bp (3538) deletion	Frameshift
13	1155	1 bp (3581) insertion	Frameshift
14	1214	GAG to TAG	Nonsense
15	1618	1 bp (4971) deletion	Frameshift
BRCA 2			
1	1827	5 bp (5707-11) deletion	Frameshift
2	1858	4 bp (5802-5) deletion	Frameshift
3	1858	4 bp (5802-5) deletion	Frameshift
4	1883	TCA to TAA	Nonsense
5	1924	4 bp (5999-6002) deletion	Frameshift
6	2004	4bp (6240-3) deletion	Frameshift
7	2052	TCA to TGA	Nonsense
8	2172	1 bp (6744) deletion	Frameshift
9	3026	CAG to TAG	Nonsense
10	3026	CAG to TAG	Nonsense

Table 3. Frequency of *BRCA1* and *BRCA2* mutations in site-specific breast or breast/ovarian cancer families

	No. of families	Germline mutation		
		BRCA 1	*BRCA 2*	Total
Breast cancer family	105	8 (7.6%)	10 (9.5%)	18 (17.1%)
Breast/ovarian cancer family	15	7 (46.7%)	0 (0%)	7 (46.7%)

3) Cumulative incidence of breast and ovarian cancers among *BRCA1* or *BRCA2* mutation carriers

Cumulative incidence of breast cancer among the first-degree relatives of the *BRCA1* and *BRCA2*-associated families were 39% and 40%, respectively, by the age of 70, and that of

ovarian cancer among the first-degree relatives of the *BRCA1*- and *BRCA2*-associated families was 20% and 0%, respectively, by the age of 70 (Fig. 3). Among the first-degree relatives of probands with *BRCA1* or *BRCA2* mutation, approximately 50% would be expected to be mutation carriers under an autosomal dominant genetic model, whereas the remaining 50% would be expected to be normal homozygotes. The observed Kaplan-Meier risk estimates for breast cancer and ovarian cancer seen for the first-degree relatives of probands represent an average between the mutation carriers and non-carriers. Therefore, cumulative risks of breast cancer and ovarian cancer among mutation carriers may be estimated twice the cumulative risk among the first-degree relatives. According to this estimation, cumulative incidence of breast cancer among *BRCA1* and *BRCA2* mutation carriers can be calculated to be 78% and 80%, respectively, by the age of 70, and that of ovarian cancer among *BRCA1* and *BRCA2* mutation carriers was 40% and 0%, respectively, by the age of 70.

Table 4. Frequency of *BRCA1* and *BRCA2* mutations according to family profiles

	No. of families	Germline mutation		
		BRCA1	*BRCA2*	Total
No. of breast cancer patients in family				
2	85	5 (6.0%)	7 (8.4%)	12 (14.5%)
3	20	3 (15%)	3 (15%)	6 (30%)
Age at diagnosis*				
~40	34	6 (17.6%)	8 (23.5%)	14 (41.1%)
41~51	43	1 (2.3%)	2 (4.7%)	3 (7.0%)
51~60	20	1 (5.0%)	0 (0%)	1 (5%)
60~	8	0 (0%)	0 (0%)	0 (0%)
Bilaterality				
Unilateral	90	5 (5.6%)	7 (7.8%)	12 (13.3%)
Bilateral	15	3 (20%)	3 (20%)	6 (40%)

*The youngest age at diagnosis in a family.

DISCUSSION

Eight *BRCA1* (7.6%) and ten *BRCA2* (9.5%) mutations were found in site-specific breast cancer families (n=105). The risk factors for carrying a *BRCA1* or *BRCA2* mutation in the site-specific breast cancer families have been found to be the presence of(*1*) three and more breast cancer patients (30%),(*2*) early onset breast cancer patient (41.1%), or(*3*) with bilateral breast cancer patient (40%) in a family. These risk factors as well as mutation frequencies seem to be similar to those reported on the Western women.(*8,9*) Our results that seven *BRCA1* (46.7%), but no *BRCA2* (0%), mutations were found in breast/ovarian cancer families (n=15) seem to suggest that family history of ovarian cancer is associated with a high risk for carrying a mutation in *BRCA1* but not *BRCA2*. These results are consistent with the previous reports that frequency of *BRCA1* mutation is higher than that of *BRCA2* mutation in breast/ovarian cancer families.(*3,4*) Although Gayther et al reported an association of ovarian cancer family history with a specific region of *BRCA2* (ovarian cancer cluster region (OCCR), codon 936-2092),(*21*) we have failed to show such

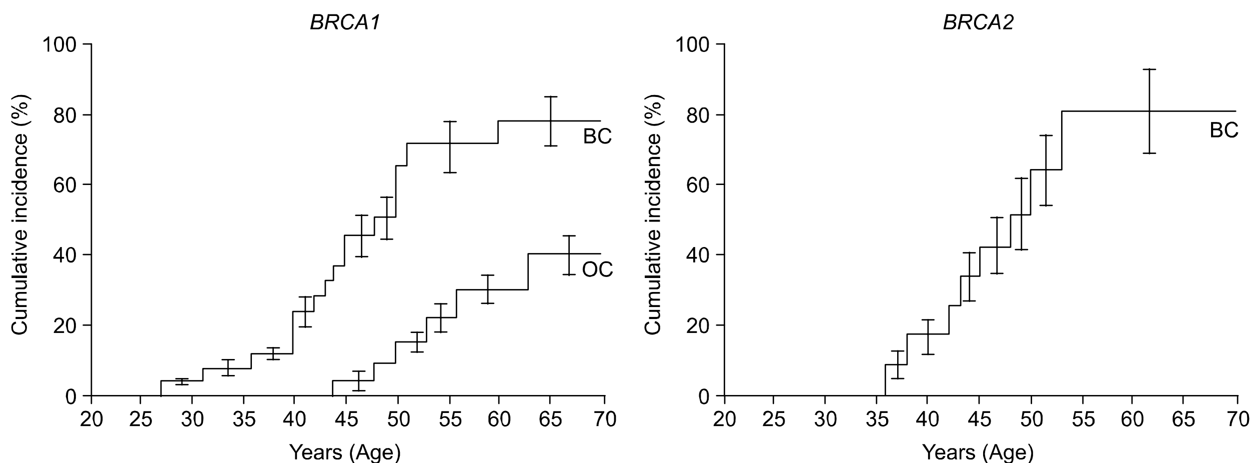

Fig. 3. Cumulative incidence of breast cancer and ovarian cancer according to age among the first-degree relatives of the probands carrying a *BRCA1* or *BRCA2* mutation. BC = breast cancer; OC = ovarian cancer; Bars = standard errors.

an association.

A low incidence of breast cancer in general population in Japan as compared with western countries suggests a possibility that risks of breast cancer among *BRCA1* or *BRCA2* mutation carriers might be lower in Japanese women than Western women. In the present study, however, we could demonstrate high risks of breast cancer among *BRCA1* or*BRCA2* mutation carriers (78% and 80%, respectively, by the age of 70), which are comparable to the risks observed in breast cancer families in the Western countries.(*22-25*) As pointed out by Easton,(*26*) breast cancer risks can be overestimated when they are calculated on the women selected for family history. Recently, lower risks of breast cancer among *BRCA1* and *BRCA2* mutation carriers, 36～37%,(*27,28*) have been reported when they are calculated on women not selected for family history (population-based study). These results suggest that actual risks of breast cancer among these mutation carriers are lower than initially expected from high-risk breast cancer families. However, these population-based studies are vulnerable to the criticism that they were conducted on a few specific mutations (185delAG for *BRCA1* and 6174delT and 999del5 for *BRCA2*) in specific populations (Ashkenazi Jewish and Icelandic people) and, so, the risks obtained from these studies might not be generalized to other mutations in other populations. Further studies need to be done before the accurate risks of breast cancer among *BRCA1* and *BRCA2* mutation carriers are established.

Incidence of ovarian cancer in general population is much lower in Japan as compared with western countries. Thus, it is also possible that risk of ovarian cancer among the mutation carriers might be low in Japanese women. But risk of ovarian cancer among *BRCA1* mutation carriers (40%) was found to be comparable to that reported on Western women.(*22-25*) Recently, Takana et al have reported a high life-time risk (80%) of ovarian cancers among Japanese *BRCA1* mutation carriers, (*29*) but their data are based on rare and large ovarian cancer families which were not experienced in the present study. In analogy with breast cancer risks, ovarian cancer risks among *BRCA1* (185delAG and 5382InsC) or *BRCA2* (6174delT) mutation carriers obtained from the population-based study are reported to be low (16% by the age of 70 (*24*)).

Several studies have shown that *BRCA2* mutation carriers are also at high risk for developing ovarian cancer(*9,20,21*) but no ovarian cancer patients was identified in any family members of *BRCA2* mutation carriers in the present study. This may be simply due to a small number of *BRCA2*-associated families but it might be also possible that a difference in susceptibility of *BRCA2*-mutation carriers to ovarian cancer exists between Japanese and Western women. Future studies of larger series may clarify this issue.

Katagiri et al. reported a high proportion of missense mutations, eight of 15 *BRCA1* mutations and seven of 14 *BRCA2* mutations, in Japanese familial breast cancer patients.(*18*) However, biological significance of these missense mutations has not been proven yet. In addition, other studies on Japanese familial breast cancer patients have shown that most mutations are nonsense or frameshift mutations.(*15-17*) Although PTT can not detect missense mutations, *BRCA1* and *BRCA2* mutation frequency in the present study is very similar to those reported by Shattuck-Eidens et al(*6*) and Thomas et al(*9*) who studied the mutation frequency by whole sequencing. These results seem to support the previous findings that almost all *BRCA1* mutations are those leading to premature termination of the protein synthesis that can be detected by PTT. Since PTT is a more simple and rapid test than whole sequencing, PTT is considered to be attractive and useful as the first screening method for the detection of *BRCA1* and *BRCA2* mutations.

Most *BRCA1* and *BRCA2* mutations were seen in the exon 11, of which mutations were analyzed using DNA. This result implies the possibility that nonsense or frameshift mutations decrease mRNA stability and might decrease the detection of the mutated allele derived mRNA. However, PTT is a sensitive test that can detect a mutant mRNA to a level of 10～20% of the normal mRNA. Moreover, we could actually detect nonsense and frameshift mutations of *BRCA1* and *BRCA2,* respectively, *outside* the exon 11, and successful detection of carriers using RNA has been also reported for DMD and FAP.(*30,31*) Therefore, it is unlikely that PTT was unable to detect mutations outside the exon 11.

In conclusion, we have shown that families at high risk for carrying a *BRCA1* or *BRCA2* mutation can be identified by family history profiles being characterized by the first degree relatives with ovarian cancer or breast cancer along with young age at diagnosis, bilateral occurrence or increased number of the affected relatives. Genetic testing is likely to be of clinical value for families having one of these risk factors. Although incidence of breast and ovarian cancer in general population is much lower in Japanese women than in Western women, the cumulative incidence of breast and ovarian cancer among *BRCA1* or *BRCA2* mutation carriers are similar except for that of ovarian cancer among *BRCA2* mutation carriers.

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