Mutational Analysis of *BRCA1* and *BRCA2* and Clinicopathologic Analysis of Ovarian Cancer in 82 Ovarian Cancer Families: Two Common Founder Mutations of *BRCA1* in Japanese Population[1]

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ABSTRACT

We analyzed genetic alterations in *BRCA1* and *BRCA2* genes among 82 ovarian cancer families in Japan. The clinical characteristics of *BRCA*-associated ovarian cancer patients were compared with cases carrying no mutations as well as with population controls. Using a direct sequencing method, 45 of the 82 ovarian cancer families were found to carry *BRCA1* or *BRCA2* germ-line mutations (40 with *BRCA1* and 5 with *BRCA2*). In 24 independent mutations of *BRCA1*, 5 recurrent mutations were found and 2 of them, the L63X and Q934X mutations, were detected in seven and eight independent families, respectively. In addition, 16 mutations of *BRCA1* and 3 mutations of *BRCA2* have never been described previously. In consideration of clinicopathological features, there was a significantly higher proportion of tumors with serous adenocarcinoma and of cases of advanced stages in the *BRCA1* or *BRCA2* cases than in those of the controls. On the other hand, there were no differences of mean age at diagnosis between patients with *BRCA1* or *BRCA2* mutation and those of the controls. Our results indicate that the features of *BRCA*-associated ovarian can-

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cer in Japan appear to be similar to those in Western countries, and the L63X and Q934X mutations of *BRCA1* appear to be common founder mutations unique to the Japanese population.

INTRODUCTION

Ovarian cancer is the most lethal disease in gynecological malignancy. Approximately 5–10% of cases are thought to have a hereditary basis (1), and a positive family history of ovarian cancer is one of the strongest and most consistent of the risk factors for the development of the disease. It has been reported that first-degree relatives of ovarian cancer patients were found to be at a 2–4-fold increased risk for developing the disease (2, 3). Familial ovarian cancer occurs as part of two clinically distinct syndromes: site-specific ovarian cancer families and breast-ovarian cancer families (4). Predisposition to ovarian cancer also occurs as part of Lynch type II or hereditary nonpolyposis colorectal cancer syndrome (5). Inherited mutations of *BRCA1*, *BRCA2*, and the mismatch-repair genes are known to confer predisposition to ovarian cancer (6–8). Germ-line mutations of *BRCA1* are predicted to be responsible for ~45% of breast cancer families and 80% of breast-ovarian cancer families (8–10). Both male and female *BRCA2* carriers have a high risk of early onset breast cancer; however, ovarian cancer initially was thought to be a much less prominent feature of these families, but it is now thought that *BRCA2* may account for as much as 10–35% of familial ovarian cancers (8, 11).

The characteristics of familial ovarian cancer are not as well documented in larger studies. A high proportion of serous adenocarcinoma and of an advanced stage has been reported for *BRCA1*-associated tumors in several studies (12–14). However, the clinical features of *BRCA2*-associated ovarian cancers are still unknown. Recently, it was reported that germ-line *BRCA1* or *BRCA2* mutations were found in 43% of 112 ovarian cancer families in the United Kingdom (14, 15). We previously reported seven *BRCA1* mutations found in 19 ovarian cancer families (16). No other studies have reported on as many ovarian cancer families in Japan. It is very difficult to recruit a sufficient number of ovarian cancer families in Japan, because lifetime risk of ovarian cancer for women in Japan is 2 or 3 times lower than that in the United States (17), and a registry for ovarian cancer has not been established.

The mutation spectrum is similar in both *BRCA1* and *BRCA2* genes. Most germ-line mutations are predicted to result in protein truncation caused by frameshift, nonsense, or splice-site alterations, and the mutations are spread along the length of the coding region (The Breast Cancer Information Core). However, it was reported that genetic and epigenetic alterations in familial ovarian cancer in Japan may differ from those in Western countries (18). Founder mutations for *BRCA1* and *BRCA2* have been described in many racial and ethnic groups (19, 20). In Japan, few reports have been described about a common founder mutation for these genes.

In this study, we have collected information on 82 ovarian cancer families and analyzed their genetic alterations and characteristics of ovarian cancer patients with *BRCA1* or *BRCA2* mutation in Japan.

Table 1 Results of *BRCA1* and *BRCA2* mutational analysis

	No. of families			
Family	*BRCA1*	*BRCA2*	No mutation	Total
Ovarian	22	2	31	55
Breast/ovarian	18	3	6	27
Total	40	5	37	82

PATIENTS AND METHODS

Families. We examined the clinical data from hospital records and pathological reports or asked physicians to answer questionnaires or hear from patients in nationwide major centers for gynecological cancer in Japan. Among ~7900 patients with epithelial ovarian cancer in approximately 80 centers for ~10 years, we recruited 82 probands and ovarian cancer families in which 198 patients were ascertained. The criterion for a site-specific ovarian cancer family is as follows: Two or more members with well-documented epithelial ovarian cancer in the second-degree relatives and no breast cancer cases in the third-degree relatives. When the family had at least one breast cancer case in a third-degree relative, it was classified as a breast-ovarian cancer family. All of the experiments were performed under informed consent. Population control patients with epithelial ovarian cancer were ascertained from the cancer registry of Niigata in Japan from 1983 to 1996 and a nationwide survey in Japan from 1980 to 1987 (21).

Mutational Analysis of *BRCA1* and *BRCA2*. Direct sequencing was performed with genomic DNA obtained from one individual with ovarian cancer from each family initially. When the individual was positive with a mutation and more than one material was available in the same family, we examined using direct sequencing as to whether other affected individuals were carrying the same mutation or not. Genomic DNA was prepared from lymphocytes and paraffin-embedded blocks using the standard phenol-chloroform methods. The entire exons, 23 exons in *BRCA1* and 26 exons in *BRCA2*, and the intronic boundary regions were sequenced in both forward and reverse directions for detecting germ-line mutations. The noncoding intronic regions that were analyzed did not extend more than 20-bp proximal to the 5′ end and 10-bp distal to the 3′ end of each exon. These regions were amplified by PCR respectively from 100 ng of genomic DNA (35 reactions for *BRCA1* and 47 reactions for *BRCA2*). The PCR products were sequenced by the dideoxy method using an Autocycle sequencing kit (Pharmacia Biotech, Tokyo, Japan) and an end-labeled primer by Cy5. PCR products were electrophoresed in 6% polyacrylamide gel and analyzed with an automatic sequencer, ALF express (Pharmacia Biotech).

Statistical Analysis. Clinical characteristics among ovarian cancer patients were tested by unpaired *t* test, χ^2 analysis, and Fisher's exact test.

RESULTS

Mutational Analysis of *BRCA1* and *BRCA2*. The existence of germ-line mutations of *BRCA1* or *BRCA2* was analyzed on 82 ovarian cancer families (Table 1), of which 55 were

Table 2 Germline mutations in *BRCA1* and *BRCA2*

No.	Family	Designation	Exon	Nucleotide	Codon	AA change	Predicted effect
BRCA1							
1	Ov[a]	M1R[b]	2	121	1	Met to Arg	Disrupt start codon
2	Ov	241delA[b]	3	241	41	Frameshift	PT
3	Br/Ov	C61G	5	300	61	Cys to Gly	Lose zinc-binding motif
4	Ov	L63X[c]	5	307	63	Leu to stop	PT
5	Br/Ov	L63X[c]	5	307	63	Leu to stop	PT
6	Ov	L63X[c]	5	307	63	Leu to stop	PT
7	Ov	L63X[c]	5	307	63	Leu to stop	PT
8	Ov	L63X[c]	5	307	63	Leu to stop	PT
9	Br/Ov	L63X[c]	5	307	63	Leu to stop	PT
10	Br/Ov	L63X[c]	5	307	63	Leu to stop	PT
11	Br/Ov	N169X[c]	8	624	169	Gln to stop	PT
12	Ov	E352X[b]	11	1173	352	Glu to stop	PT
13	Ov	2080delA	11	2080	654	Frameshift	PT
14	Ov	2080delA	11	2080	654	Frameshift	PT
15	Br/Ov	2194–2195delAT[b]	11	2194–2195	692	Frameshift	PT
16	Br/Ov	2507–2508delAG[b]	11	2507–2508	796	Frameshift	PT
17	Br/Ov	2507–2508delAG[b]	11	2507–2508	796	Frameshift	PT
18	Br/Ov	2730–2731delCC[b]	11	2730–2731	871	Frameshift	PT
19	Ov	Q934X[c]	11	2919	934	Gln to stop	PT
20	Ov	Q934X[c]	11	2919	934	Gln to stop	PT
21	Br/Ov	Q934X[c]	11	2919	934	Gln to stop	PT
22	Ov	Q934X[c]	11	2919	934	Gln to stop	PT
23	Br/Ov	Q934X[c]	11	2919	934	Gln to stop	PT
24	Ov	Q934X[c]	11	2919	934	Gln to stop	PT
25	Ov	Q934X[c]	11	2919	934	Gln to stop	PT
26	Br/Ov	Q934X[c]	11	2919	934	Gln to stop	PT
27	Br/Ov	3226–3231delTTAAAG[b]	11	3226–3231	1036	Frameshift	PT
28	Ov	3376–3377insT[b]	11	3376–3377	1086	Frameshift	PT
29	Br/Ov	3493–3494delCT[c]	11	3493–3494	1125	Frameshift	PT
30	Ov	3516–3517delTT[b]	11	3516–3517	1133	Frameshift	PT
31	Ov	3532delG[b]	11	3532	1138	Frameshift	PT
32	Br/Ov	E1214X	11	3759	1214	Glu to stop	PT
34	Br/Ov	L1216X[c]	11	3766	1216	Leu to stop	PT
33	Ov	3834–3836del3,insC[b]	11	3834–3836	1239	Frameshift	PT
35	Br/Ov	4046–4049delTACA[b]	11	4046–4049	1309	Frameshift	PT
36	Br/Ov	4237–4238delAG[b]	12	4237–4238	1373	Frameshift	PT
37	Ov	4237–4238delAG[b]	12	4237–4238	1373	Frameshift	PT
38	Ov	IVS14-2A>G[b]	15	—	IVS	—	Splice aberration
39	Ov	5326delT[b]	20	5326	1736	Frameshift	PT
40	Ov	D1778Y[b]	21	5451	1778	Asp to Tyr	Splice aberration
BRCA2							
1	Ov	4567delG[b]	11	4567	1447	Frameshift	PT
2	Ov	5804–5807delTTAA	11	5804–5807	1859	Frameshift	PT
3	Br/Ov	7384–7385insT[b]	14	7384–7385	2386	Frameshift	PT
4	Br/Ov	8941–8944delTATG[b]	21	8941–8944	2905	Frameshift	PT
5	Br/Ov	Q3026X[c]	23	9304	3026	Gln to stop	PT

[a] Ov, ovarian; Br, breast; PT, protein truncation; IVS, a noncoding intervening sequence.
[b] Novel and unique mutation in Japan.
[c] Unique mutation in Japan.

site-specific ovarian cancer families and 27 were breast-ovarian cancer families. In the 55 site-specific ovarian cancer families, 22 families were carrying germ-line mutations in *BRCA1* (22/55, 40.0%); however, in 27 breast-ovarian cancer families, 18 families were positive with the mutation (18/27, 66.7%). A very small proportion of the families were carrying germ-line mutations in *BRCA2*, 2 families among the 55 site specific ovarian cancer families (2/55, 3.6%) and 3 families among the 27 breast ovarian cancer families (3/27, 11.1%). Thirty-seven families were negative for germ-line mutations of *BRCA1* or *BRCA2* (37/82, 45.1%). *BRCA1* mutations were 8 times more common than *BRCA2* mutations (40 *versus* 5).

Germ-line mutations in *BRCA1* or *BRCA2* are listed in Table 2. Twenty-four independent *BRCA1* mutations were identified in 40 of the 82 families (40/82, 48.8%). Thirty-six of the 40 mutations are either frameshift or nonsense mutations (36/40, 90.0%) that would be predicted to result in premature truncation of the *BRCA1* protein. Three mutations were missense mutations that were presumably predicted to disrupt the start codon of exon 2, to lose a zinc-binding motif of exon 5, or to induce splice aberration by occurring at the end of exon 21. These mutations were not found in healthy women in this family or in a substantial number of healthy volunteers who had no family history of ovarian and/or breast cancer, indicating that these

Table 3 Genetic variants and polymorphisms in *BRCA1* and *BRCA2*

Exon	Designation	Nucleotide	Codon	In BIC[a] site
BRCA1				
Genetic variants				
16	M1628T	5002	1628	Yes
24	A1843P	5646	1843	Yes
Polymorphisms				
3	233G>A	233	38	Yes
11	2201C>T	2201	694	Yes
11	2430T>C	2430	771	Yes
11	P871L	2731	871	Yes
11	E1038G	3232	1038	Yes
11	K1183R	3667	1183	Yes
13	4427T>C	4427	1436	Yes
16	S1613G	4956	1613	Yes
BRCA2				
Genetic variants				
7	L184P	779	184	Yes
11	A737S	2437	737	Yes
11	V2109I	6553	2109	Yes
Polymorphisms				
2	203G>A	203	IVS	Yes
10	H372N	1342	372	Yes
10	1593A>G	1593	455	Yes
11	M784V	2578	784	Yes
11	3624A>G	3624	1132	Yes
11	4035T>C	4035	1269	Yes
14	7470A>G	7470	2414	Yes
27	I3412V	10462	3412	Yes

[a] BIC, The Breast Cancer Information Core; IVS, a noncoding intervening sequence.

genetic variants could be diagnosed as pathogenic mutations but not polymorphisms (data not shown). One mutation, IVS14–2A>G, consisting of a nucleotide substitution in a noncoding intervening sequence was expected to prevent mRNA processing. We identified five recurrent mutations and, among these recurrent mutations, a substitution of T to A at nucleotide 307 (L63X) was detected in 7 independent families (7/40, 17.5%), and a substitution of C to T at nucleotide 2919 (Q934X) was detected in 8 families (8/40, 20.0%). Five independent *BRCA2* mutations were identified (5/82, 6.1%), and all of the mutations are predicted to result in premature truncation of the *BRCA2* protein. Sixteen of 24 *BRCA1* and three of five *BRCA2*-independent mutations detected in this study were not found in any other reports. In 38 of 45 families carrying mutation in *BRCA1* or *BRCA2*, multiple affected members including patients with breast cancer shared a same mutation (7 families with >3 members and 31 families with 2 members). In 7 other families, no available DNA was obtained from any affected individual except the proband.

Genetic variants of uncertain significance and common polymorphisms in *BRCA1* or *BRCA2* are listed in Table 3. Two genetic variants and eight common polymorphisms were observed in *BRCA1*, and three variants and eight polymorphisms were observed in *BRCA2*.

Clinicopathological Analysis. The clinical and pathological characteristics of familial and sporadic patients with epithelial ovarian cancer are summarized in Table 4. There are the characteristics of 110 patients with *BRCA1* mutation, 10 patients with *BRCA2* mutation, 78 patients with no mutation in *BRCA1* or *BRCA2*, 1299 patients with epithelial ovarian cancer from the cancer registry of Niigata in Japan as control 1 and 1185 patients with epithelial ovarian cancer from a nationwide survey in Japan as control 2 (21). The mean age at diagnosis of tumors with no mutation (49.7 years of age) was significantly younger than control cases, 54.2 years of age ($P = 0.0076$). However, there were no significant differences of mean age at diagnosis between patients with *BRCA1* or *BRCA2* mutation, 52.1 and 58.4 years of age, respectively, and those of control cases. The major histological type for *BRCA1*- or *BRCA2*-associated tumors was serous adenocarcinoma in 79.8% and 88.9% of tumors, respectively. There were significant differences in the proportion of tumors with serous adenocarcinoma between these two groups and the control groups; however, no difference was found between the nonmutation groups and the control groups. No tumor with mucinous or clear cell adenocarcinoma occurred in the *BRCA1*-related cases. In regard to the clinical stage at diagnosis, there was a significantly higher proportion of stage III or IV tumors in the *BRCA1* or *BRCA2* cases than in the control cases. On the other hand, no difference was seen in the stage distribution between the tumors with no mutation and those of the control 1 ($P = 0.25$).

Table 5 represents the characteristics of the cases with L63X or Q934X and other mutations in *BRCA1*. The mean age at diagnosis for cases with either mutation did not significantly differ from that for the others. In addition, no difference was seen in the histological subtypes between each mutation status. On the other hand, there was a significantly lower proportion of stage III or IV tumors in the cases with L63X than in the others ($P = 0.016$).

The geographic distribution of ovarian cancer families with these two mutations is shown in Fig. 1 and Table 6. We divided the Japanese Islands into east and west based on the Fossa Magna. The Fossa Magna, named by German geologist, means a great chasm in the earth in Latin. All of the families with L63X were in the eastern part of Japan, and the proportion of families with L63X in the east was significantly higher than that in the west ($P = 0.035$).

DISCUSSION

The clinical and genetic characteristics of familial ovarian cancer are not as well documented as those of familial breast cancer. Recently, Gayther *et al.* (15) reported that germ-line *BRCA1* or *BRCA2* mutations were found in 43% of 112 ovarian cancer families in the United Kingdom, and *BRCA1* mutations were ~4 times more common than *BRCA2* mutations. In this study, we identified germ-line mutations of *BRCA1* or *BRCA2* in slightly more than one-half (55%) of 82 families and found that *BRCA1* mutations were 8 times more common than *BRCA2* mutations in Japanese ovarian cancer families. In our study, most of the *BRCA1* mutations were predicted to result in protein truncation similar to observations in many other reports (14, 15), suggesting that this is one of the unique characteristics of the *BRCA1* gene. Contrary to our findings, in Japanese breast-ovarian cancer families, a much lower frequency of the mutation of *BRCA1* in which missense mutations tended to predominate was observed (18). This contradictory result could be related to

Table 4 Clinical and pathological characteristics of familial and sporadic ovarian cancer

	No. of cases (%)				
	BRCA1	*BRCA2*	No mutation	Population controls 1	Population controls 2
Total	110	10	78	1299	1185
Age (yr)					
Mean ± SD	52.1 ± 9.7	58.4 ± 13.6	49.7 ± 9.6[a]	54.2 ± 13.5	ND[b]
Range	28–79	41–74	25–68	12–94	
Histology					
Serous	79 (79.8)[c]	8 (88.9)[d]	40 (54.8)[e]	524 (44.3)	575 (50.1)
Endometrioid	14 (14.1)	0	8 (11.0)	157 (13.3)	134 (11.7)
Mucinous	0	0	10 (13.7)	273 (23.1)	250 (21.8)
Clear cell	0	1 (11.1)	11 (15.1)	164 (13.9)	143 (12.5)
Others	6 (6.1)	0	4 (5.5)	66 (5.6)	46 (4.0)
Unknown	11	1	5	115	37
Stage					
I	13 (13.8)[f]	1 (11.1)[g]	25 (45.5)[h]	553 (43.1)	390 (32.9)
II	7 (7.4)[f]	0[g]	9 (16.4)[h]	167 (13.0)	167 (14.1)
III	61 (64.9)[f]	7 (77.8)[g]	13 (23.6)[h]	434 (33.9)	480 (40.5)
IV	13 (13.8)[f]	1 (11.1)[g]	8 (14.5)[h]	128 (10.0)	148 (12.5)
Unknown	16	1	23	17	0

[a] $P = 0.0076$ (*vs.* controls 1).
[b] ND, not done.
[c] $P = 1.0 \times 10^{-10}$ (*vs.* controls 1), $P = 4.1 \times 10^{-9}$ (*vs.* controls 2).
[d] $P = 0.0083$ (*vs.* controls 1), $P = 0.020$ (*vs.* controls 2).
[e] $P = 0.051$ (*vs.* controls 1), $P = 0.26$ (*vs.* controls 2).
[f] $P = 1.0 \times 10^{-10}$ (*vs.* controls 1), $P = 5.4 \times 10^{-7}$ (*vs.* controls 2).
[g] $P = 0.0077$ (*vs.* controls 1), $P = 0.030$ (*vs.* controls 2).
[h] $P = 0.25$ (*vs.* controls 1), $P = 0.022$ (*vs.* controls 2).

Table 5 Clinical and pathological characteristics of *BRCA1*-associated ovarian cancer by mutation status

	No. of cases (%)		
	L63X	Q934X	Others
Total	14	17	79
Age (yr)			
Mean ± SD	52.2 ± 6.7	53.1 ± 11.5	51.9 ± 9.6
Range	39–65	28–72	37–79
Histology			
Serous	9 (69.2)	11 (64.7)	59 (85.5)
Endometrioid	3 (23.1)	3 (17.6)	8 (11.6)
Mucinous	0	0	0
Clear cell	0	0	0
Others	1 (7.7)	3 (17.6)	2 (2.9)
Unknown	1	0	10
Stage			
I + II	6 (46.2)[a]	5 (29.4)	9 (14.1)[a]
III + IV	7 (53.8)[a]	12 (70.6)	55 (85.9)[a]
Unknown	1	0	15

[a] $P = 0.016$.

technical problems of mutation analysis, *e.g.* a direct sequencing on the whole coding region *versus* a screening by single strand conformation polymorphism analysis.

Currently, various *BRCA1* mutations have been identified, though a few have been detected recurrently. One such mutation, 185delAG, is observed in ~1% of Ashkenazi Jews (22). These studies demonstrate that some mutations of *BRCA1* are segregated geographically and racially. On the basis of our findings, the L63X and the Q934X were seen in seven and eight independent families, respectively. These two mutations were reported in breast cancer families in Japan (eight families with L63X and three families with Q934X, respectively) but not in other countries (23–26). Haplotyping analysis showed that the cases with these two mutations were likely derived from common ancestors (data not shown). These results indicate that these kinds of mutations are common founder mutations in the Japanese population. The geographic distribution of ovarian cancer patients with these two mutations seems to be skewed. Japanese are a relatively uniform population ethnically, so this difference of geographic prevalence based on mutation status may be caused by migration and marriage patterns in the population structure in Japan. The Japanese islands are divided into east and west based on the Fossa Magna according to culture. In fact, people in the eastern part of Japan traditionally eat square rice cakes during New Year's ceremony. On the other hand, people in the west eat round ones.

The risk of ovarian cancer is not believed to be the same for all of the *BRCA1* mutations and varies according to the position of the mutation along the gene (27). In particular, several studies suggested that the risk of ovarian cancer in females with 185delAG mutation was higher than that with 5382 insC mutation (28, 29). Furthermore, Hedenfalk *et al.* (30) suggested that a heritable mutation influenced the gene expression profile of the cancer. Therefore, we examined the differences of the clinical features between cases with L63X and with Q934X. As a result, there was a significantly lower proportion of advanced tumors in the cases with L63X than in other mutations. These results suggest that the position of the germ-line mutation along the *BRCA1* gene may influence the process of carcinogenesis and progression of the disease. The question of whether differ-

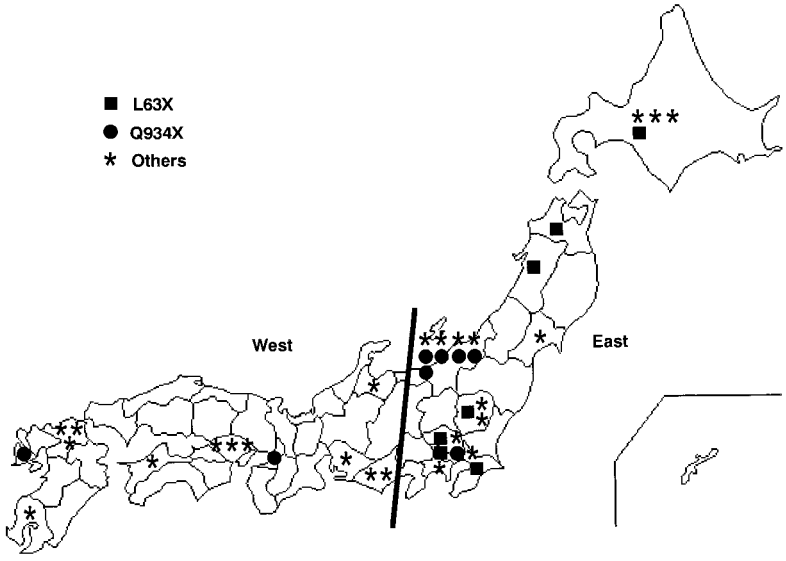

Fig. 1 Geographic distributions of ovarian cancer families with common founder mutations of *BRCA1* in Japanese populations. ■, ●, and ∗ denote a family with L63X, Q934X, and other mutations of *BRCA1*, respectively. There were seven and eight families with L63X and Q934X mutations, respectively. The proportion of families with L63X in the eastern part of Japan was higher than that in the western part of Japan.

Table 6 Geographic distributions of ovarian cancer families with germ-line mutation in *BRCA1*

	BRCA1 (No. of families)			
	L63X	Q934X	Others	Total[a]
East	7[b]	6[c]	13	44
West	0[b]	2[c]	12	38

[a] Families in which the genetic alterations of *BRCA1* and *BRCA2* were analyzed.
[b] $P = 0.035$.
[c] $P = 0.41$.

ent mutations result in a different prognosis in this population will require additional study.

The results of our clinical analysis revealed that there was a significantly higher proportion of tumors of an advanced stage in the *BRCA1* or *BRCA2* cases than in the control cases; however, we previously reported that 13 patients treated with stage III disease with mutations of *BRCA1* showed more favorable outcomes compared with sporadic cases (31). In regard to the mean age at diagnosis, no difference was seen between patients with *BRCA1* or *BRCA2* mutation and those of the control cases. Boyd *et al.* (32) reported that ovarian cancers with *BRCA1* mutation in the United States were diagnosed more than 8 years earlier compared with those of the sporadic cases. The later onset of *BRCA1*-associated ovarian cancer in Japan might be attributed to the sensitiveness of subjects to environmental differences (*e.g.*, lower parity, lower use of talc, lower population of obese women, lower serum level of gonadotropin, and lower intakes of fat or milk in Japan) as well as to the pathogenesis of sporadic ovarian cancer, because the tumor cell was caused by both initial germ cell mutation and proceeding somatic cell mutation.

We cannot exclude the possibility that our screening results presented were likely to be an underestimate of the contribution of these two genes to familial ovarian cancer, because, for example, any mutation altering a primer site would have been missed. Nevertheless, about one-half of the ovarian cancer families were not associated with mutation of *BRCA1* or *BRCA2*. The clinical features in relation to the younger mean age at diagnosis of tumors with no mutation other than that of sporadic cases suggested that these patients were influenced with the hereditary genetic instability succeeded from the ancestral patients. Consistent with the previous reports (14), these findings raise the possibility that additional novel susceptibility genes for familial ovarian cancer might exist (33). Therefore, it seems urgent to perform genome-wide linkage analysis and an association study in ovarian cancer families for additional investigation to identify novel susceptibility genes for familial ovarian cancer.

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Clinical Cancer Research

Mutational Analysis of *BRCA1* and *BRCA2* and Clinicopathologic Analysis of Ovarian Cancer in 82 Ovarian Cancer Families: Two Common Founder Mutations of BRCA1 in Japanese Population

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