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# Haplotypes of *BRCA1* Mutation Alleles in Japanese Ovarian and Breast-Ovarian Cancer Families: A Novel Method for Detecting *BRCA1* Associated Ovarian Cancer

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***Summary.*** **We examined 21 ovarian and 24 breast-ovarian cancer families with *BRCA1* mutations among a Japanese population. Twenty-four kinds of mutations were observed in these families. The DNAs of the mutation carriers (including the cancer patients) with these mutations were genotyped with microsatellite markers (D17S855, D17S1322, D17S1323 and D17S1325) located around the *BRCA1* gene. The microsatellite**

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**genotyping showed that the alleles with the same mutations had the same haplotypes. This suggests that patients with the same mutation may have a common ancestor. On the other hand, a loss of heterozygosity (LOH) in the 4 microsatellite markers was observed in 48 (96.0%) of 50 tumor tissues examined. A combination of microsatellite genotyping and LOH analysis revealed that DNAs of the tumors retained the alleles with haplotypes common to the patients with the same mutation. Based on these results, we speculated on which mutations would occur in ovarian cancer families, working inversely from the haplotypes determined by this combination of methods. Consequently, T307A mutation in 2 families and C2919T in 4 families could be easily found. Applying these methods, we were able to detect haplotypes of 22 mutation alleles common to persons with the same mutations. Moreover, we employed LOH analysis and discovered two patients with a germline mutation of *BRCA1* among 57 ovarian cancer patients without a family history of ovarian cancer. Determination of the retained haplotype by LOH analysis was proven to be an efficient tool to detect *BRCA1* mutations from both familial and non-familial ovarian cancer patients.**

**Key words**—Familial ovarian cancer, Sporadic ovarian cancer, *BRCA1*, LOH, Haplotype.

## INTRODUCTION

In 1994, Miki et al. isolated the *BRCA1* gene (MIM 113705), which harbors mutations with a high penetrance for breast and ovarian cancers[1]. This gene is thought to be one of the tumor suppressor genes, and its dysfunction is believed to play a leading role in the carcinogenesis of hereditary breast and/or ovarian cancers. Indeed, the lifelong risk of women with *BRCA1* mutations for ovarian cancers has been calculated as high as 79%[2].

Many *BRCA1* mutations have been reported thus far. In some ethnic groups, specific *BRCA1* mutations have been found and their haplotypes determined[3-7]. Persons who have the same mutations and the same haplotypes are thought to have descended from the same original ancestors, regardless of differences in appearance. One good example can be observed in Ashkenazi Jews. Approximately 1% of Ashkenazi Jews have the 185delAG mutation with almost the same haplotypes[3]. In the same way, mutations of 5382insC, 1675delA, and 943ins10 have been reported in Ashkenazi Jews, Norwegians, and African Americans, respectively[4-7]. Among Japanese as well, some identical mutations have been detected in families not related to each other at least within a range of third degree relatives[8-13]. One purpose of the present study was to determine haplotypes of the mutation alleles which were specific to Japanese, and to confirm the fact that the families with the same mutations did descend from common ancestors.

Some studies have reported that 2-8% of sporadic ovarian cancers are associated with germline mutations of *BRCA1*[14-16]. However, a greater effort is needed to determine the whole sequence of the *BRCA1* gene in each patient because of the large size of this gene. Some current studies have reported that a LOH was frequently observed in cancers associated with the *BRCA1* mutations[10,14,17,18], and the retained alleles in LOH were expected to harbor mutations according to the 'two hit' hypothesis[19]. A second purpose of this study was to devise an efficient method for finding patients with a *BRCA1* mutation among sporadic ovarian cancers based on haplotypes of the retained *BRCA1* alleles in the LOH analysis.

## MATERIALS AND METHODS

### Families

We selected ovarian and/or breast cancer patients whose first degree relatives included ovarian and/or breast cancer patients, as confirmed by the patient records of the authors' hospitals. If the cancer types of the relatives were solely ovarian or breast, the family was tagged as "a site-specific ovarian cancer family" or "a site-specific breast cancer family." If they included both ovarian and breast cancers, the family was tagged as "a breast-ovarian cancer family."

### Sporadic ovarian cancers

Epithelial ovarian cancers randomly chosen from the patients who underwent surgery in Niigata University Hospital between 1995 and 1999 were tagged as "sporadic ovarian cancers." Fifty-seven formalin-fixed, paraffin-embedded specimens were obtained from the ovarian cancer cases. All tissue sections were independently examined by two pathologists to determine the histologic type according to the classification of the World Health Organization. Surgical staging was defined according to the International Federation of Gynecology and Obstetrics (FIGO).

### DNA extraction

After obtaining informed consent, we extracted genomic DNAs using a standard phenol-chloroform method[20]. DNAs extracted from peripheral blood or

**Table 1.** Numbers of families tested for *BRCA1* mutation

| | Mutation of *BRCA1* | |
|---|---|---|
| | (+) | (−) |
| Breast-ovarian cancer families | 24 | 4 |
| Site-specific ovarian cancer families | 21 | 21 |
| Site-specific breast cancer families | 0 | 1 |

**Table 2.** Mutations of *BRCA1* observed in patients affected with ovarian and/or breast cancer in Japan

| Mutation | Aminoacid change | Exon | Codon | Predicted effect |
|---|---|---|---|---|
| T 121 G | Met to Arg | 2 | 1 | Disrupt start codon |
| 241 del A | Frameshift | 3 | 41 | Protein truncation |
| T 300 G | Cys to Gly | 5 | 61 | Lose zinc-binding motif |
| T 307 A | Leu to stop | 5 | 63 | Protein truncation |
| 437 del T | Frameshift | 7 | 106 | Protein truncation |
| C 624 T | Gln to stop | 8 | 169 | Protein truncation |
| 1096 del AA | Frameshift | 11 | 326 | Protein truncation |
| G 1173 T | Glu to stop | 11 | 352 | Protein truncation |
| 2080 del A | Frameshift | 11 | 654 | Protein truncation |
| 2194 del AT | Frameshift | 11 | 692 | Protein truncation |
| 2507 del AG | Frameshift | 11 | 796 | Protein truncation |
| 2730 del CC | Frameshift | 11 | 871 | Protein truncation |
| C 2919 T | Gln to stop | 11 | 934 | Protein truncation |
| 3226 del 6dp | Phe to stop | 11 | 1036 | Protein truncation |
| 3376 ins T | Frameshift | 11 | 1086 | Protein truncation |
| 3494 del TC | Frameshift | 11 | 1125 | Protein truncation |
| 3516 del TT | Frameshift | 11 | 1133 | Protein truncation |
| 3532 del G | Frameshift | 11 | 1138 | Protein truncation |
| G 3759 T | Glu to stop | 11 | 1214 | Protein truncation |
| T 3766 G | Leu to stop | 11 | 1216 | Protein truncation |
| 3834 del 3, ins C | Frameshift | 11 | 1239 | Protein truncation |
| 4046 del TACA | Frameshift | 11 | 1309 | Protein truncation |
| 4237 del AG | Frameshift | 12 | 1373 | Protein truncation |
| 5326 del T | Frameshift | 20 | 1736 | Protein truncation |

formalin-fixed, paraffin-embedded intact tissues were called "normal DNA." DNAs extracted from formalin-fixed, paraffin-embedded tumor tissues were called "tumor DNA."

### Mutation analysis of *BRCA1*

Direct sequencing of whole exons was performed for the extracted DNAs. Each exon was amplified by a polymerase chain reaction (PCR) method with specific primers[21]. The PCR products were sequenced by a dideoxy method using the Autocycle Sequencing Kit (Amersham Pharmacia Biotech, Buckinghamshire, England); these were electrophoresed on 6% denaturing polyacrylamide gels and analyzed with automatic sequencers (ALF Sequencer II, ALF Express).

### Genotyping around *BRCA1*

Microsatellite markers flanking the *BRCA1* gene on 17q21 were used. These markers consisted of D17S855, D17S1322, and D17S1323 located within

*BRCA1*, and D17S1325 located upstream of *BRCA1* within an interval of less than 1cM[1,22]. Microsatellite markers of normal DNAs were amplified by PCR with primers specific to the markers, and the PCR products were mixed with 95% formamide, denatured, and separated on 6% denaturing polyacrylamide gels. DNA fragment analysis was performed by automated sequencers using Fragment Manager software (Amersham Pharmacia Biotech). Fragment lengths were calibrated using both internal length standards and PCR products of the standard DNA, CEPH 1347-02. When heterozygosity was detected in the genotyping of normal DNAs, the microsatellites were considered informative. The informative microsatellites of the tumor DNAs were also amplified and analyzed in the same way as those of the normal DNAs.

### LOH analysis

LOH in the present study was defined as follows: a positive loss occurred when the tumor sample had a reduction of 50% or more in the peak area of the DNA alleles compared with that of the normal sample.

In all samples with positive LOH at individual loci, the independent LOH analysis was repeated.

## RESULTS

### Mutations of *BRCA1*

We examined 42 site-specific ovarian, one site-specific breast, and 28 breast-ovarian cancer families. In 45 (63.4%) of the total of 71 families, *BRCA1* mutations were detected by a direct sequencing method. Twenty-four kinds of mutations were observed in 21 ovarian and 24 breast-ovarian cancer families (Tables 1 and 2). Twenty-two (91.7%) of those mutations were nonsense or frame shift mutations which caused truncation of the protein. The other two (8.3%) were missense mutations which caused disruption of the start codon or the zinc-binding motif.

Seventy-five ovarian tumors from the 45 ovarian cancer families consisted of 59 serous adenocarcinomas, 11 endometrioid adenocarcinomas, 4 undifferentiated carcinomas, and 1 unclassified carcinoma. There were no mucinous or clear cell adenocarcinomas.

### Haplotype analysis in the families carrying *BRCA1* mutations

Fig. 1 shows the positions of 4 microsatellite markers

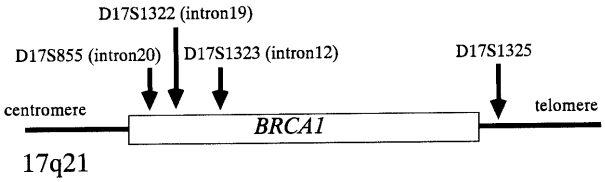


**Fig. 1.** Loci of four microsatellites around the *BRCA1* gene.

(D17S855, D17S1322, D17S1323, and D17S1325) in which genotypes were analyzed for all persons examined. Family trees with genotypes at those 4 microsatellite markers are shown in Figs. 2 and 3. In Fig. 2, the members with the T307A mutation, excluding 240 and 241 in family 015, had the 147-126-156-197 haplotype for the D17S855-D17S1322-D17S1323-D17S1325 microsatellite markers (bold-faced numbers in Fig. 2). Only in family 015 did the mutation alleles show the 195 genotype for D17S1325. Fig. 3 shows that the members with C2919T had haplotypes (157-132-150-199) completely identical with each other (bold-faced numbers in Fig. 3).

### LOH analysis in ovarian cancer with *BRCA1* mutation

Fifty ovarian cancer specimens were available for PCR in the families with *BRCA1* mutations. LOH analysis of the 4 microsatellite markers was performed in these specimens. Forty-eight (96.0%) of them showed LOH in at least one of the 4 microsatellite markers (data not shown).

In Figs. 2 and 3, the numbers in brackets are the lost genotype from the DNAs of tumors. When LOH was observed, the common alleles with identical haplotypes (bold-faced numbers in Figs. 2 and 3) were always retained.

### Determination of haplotypes at mutation alleles

Taking into account both the genotypes common to persons who had the same mutation and the retained genotypes in the LOH analysis, we determined the haplotypes of the 22 *BRCA1* mutation alleles (Table 1). There was a difference in the haplotypes of *BRCA1* between similar mutation alleles (2080delA) in the two families. The haplotypes of the 437delT and T3766G alleles could not be determined, as neither the tumors of the patients nor the peripheral blood of their relatives could be obtained.

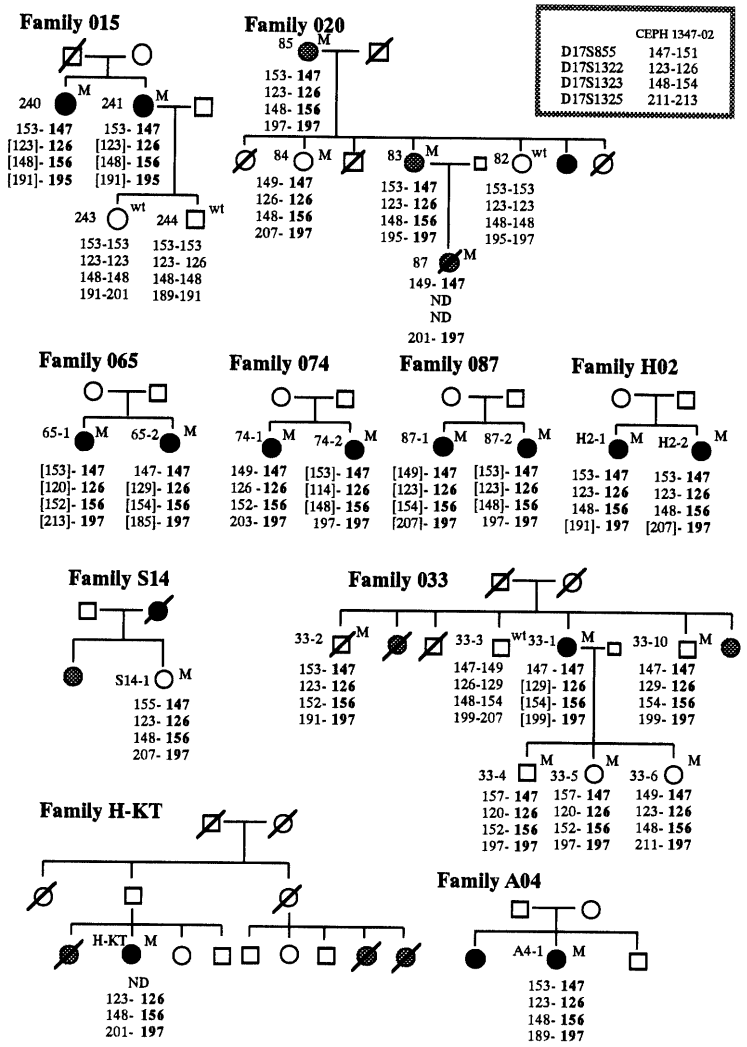


**Fig. 2.** Families with the T307A mutation of *BRCA1* and their genotypes. Circles represent females; boxes represent males; oblique lines through circle or square represent deceased persons. Black circles represent patients affected with ovarian cancer; Shaded circles represent patients affected with breast cancer. Numbers to the left of the symbol are individual names, and numbers under the symbols are genotypes of D17S855, D17S1322, D17S1323, and D17S1325, respectively. The letters 'ND' mean 'not done'. Genotypes in brackets are absent from DNA of tumors. Family members with the letter 'M' were the carrier of the T307A mutation in *BRCA1*, which was confirmed by direct sequence analysis. Numbers in bold indicate haplotypes of the allele with T307A mutation in *BRCA1*. Direct sequence analysis of DNA from the persons with the letters 'wt' did not allow detection of the same mutation.

### LOH analysis in sporadic ovarian cancers

We randomly chose 57 sporadic ovarian cancer patients who had no family members affected with ovarian cancer or breast cancer. In the DNAs extracted from their intact tissues and tumors, genotypes for the 4 microsatellite markers were examined. The retained haplotypes could be determined in 32 cases (56.1%) based on LOH analysis (Table 4). In 5 cases, the retained haplotypes corresponded to haplotypes of the mutation alleles which had been obtained from the familial cancer cases (Table 3). Two of them exhibited the speculated mutations, T307A and C2919T.

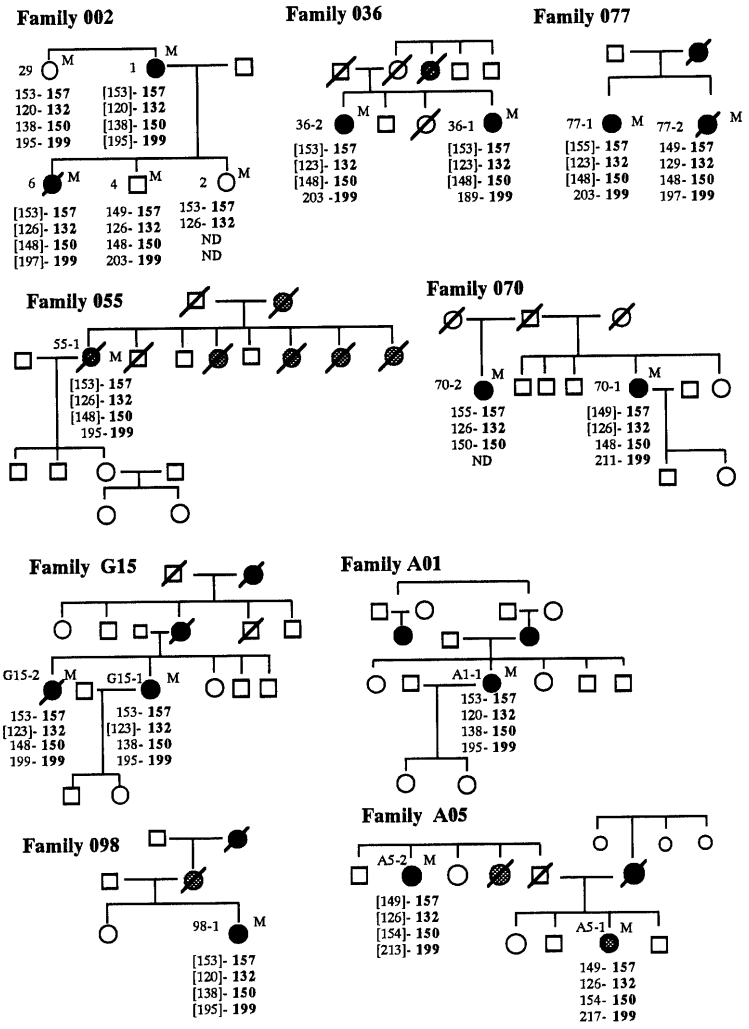


**Fig. 3.** Families with the C2919T mutation of *BRCA1* and their genotypes. Shaded circles with thick borders represent patients affected with both ovarian cancer and breast cancer. The persons with letter 'M' were the carrier of the C2919T mutation in *BRCA1*, which was confirmed by direct sequence analysis. The other symbols and marks were drawn in the same way as in Fig. 2.

## DISCUSSION

We investigated ovarian cancer families with *BRCA1* mutations and analyzed them genetically to clarify the Japanese ancestors of those mutations. Among 43 families with *BRCA1* mutations, 24 different kinds of mutations were found. Microsatellite genotyping showed that alleles with the same mutations had the same haplotypes. These results suggest that patients with the same mutations may have descended from the same ancestors, and that the haplotypes of *BRCA1* have been maintained well throughout many generations. Similar results have been reported for other ethnic groups such as Ashkenazi Jews[3].

In family 015, the mutation allele of T307A indicated the 195 genotype for D17S1325. This might have been caused by a slippage after the occurrence of the mutation. In families 087 and H-KT, we examined the microsatellite genotyping of patients before direct sequencing. By observing genotypes common to affected family members and genotypes retained in the LOH analysis, we found that they had a similar haplotype (147-126-156-197) characteristic of those patients with mutation T307A. As the T307A is known to be located in exon 5, we were able to easily

**Table 3.** Haplotypes of *BRCA1* mutant alleles

| | D17S855 | D17S1322 | D17S1323 | D17S1325 |
|---|---|---|---|---|
| T 121 G | 149 | 123 | 148 | 189 |
| 241 del A | 149 | 126 | 152 | 217 |
| T 300 G | 149 | 123 | 148 | 211 |
| T 307 A | 147 | 126 | 156 | 197/195 |
| C 624 T | 155 | 123 | 148 | 207 |
| 1096 del AA | 149 | 126 | 154 | 213 |
| G 1173 T | 153 | 123 | 148 | 195 |
| 2080 del A | 153/149 | 123 | 148 | 191/195 |
| 2194 del AT | 153 | 123 | 148 | 191 |
| 2507 del AG | 147 | 129 | 152 | 197 |
| 2730 del CC | 147 | 126 | 156 | 195 |
| C 2919 T | 157 | 132 | 150 | 199 |
| 3226 del 6bp | 153 | 129 | 154 | 199 |
| 3376 ins T | 155 | 126 | 148 | 197 |
| 3494 del TC | 149 | 126 | 156 | 203 |
| 3516 del TT | 149 | 123 | 154 | 217 |
| 3532 del G | 153 | 123 | 148 | 191 |
| G 3759 T | 153 | 114 | 148 | 197 |
| 3834 del 3, ins C | 149 | 123 | 148 | 195 |
| 4046 del TACA | 149 | 129 | 152 | 201 |
| 4237 del AG | 147 | 126 | 158 | 195 |
| 5326 del T | 149 | 126 | 156 | 197 |
| Genotype of CEPH 1347-02 | 147/151 | 123/126 | 148/154 | 211/213 |

detect this mutation by the direct sequencing of exon 5 alone, thereby avoiding the sequencing of whole genes. In the same way, we could ascertain mutation C2919T in families 098 and A01 with a common haplotype (157-132-150-199) by sequencing a part of exon 11 alone. Here, we have proven that this haplotype analysis, that is, observing the genotypes common to affected family members and the genotypes retained in the LOH analysis, is an easy method for firding mutations of *BRCA1* among familial ovarian cancers.

Although various mutations of *BRCA1* cause hereditary breast and/or ovarian cancers, greater effort is needed to perform a direct sequencing of its whole gene in order to identify the mutations since *BRCA1* is a very large gene. To lessen this effort, several methods, such as the protein truncation test (PTT)[23], the stop codon assay[24] and the single-strand conformation polymorphism (SSCP), have been developed. During the course of the present study, we revealed that each mutation of *BRCA1* specific to the Japanese was derived from a common ancestor with the same haplotype, and based on this fact, determined a novel method for efficiently detecting mutation alleles of *BRCA1*. The crux of this method is to speculate which mutation occurs by observing the haplotypes of alleles which are common to patients from the same family at the time of microsatellite genotyping, and/or those which are retained in the tumors at the time of the LOH analysis. The establishment of this method is a by-product of the present study.

The latter stage of this method (LOH analysis) can be applied to sporadic ovarian cancers. Using this method, we detected 2 cases with *BRCA1* mutations among 57 sporadic ovarian cancers (3.5%) (Tables 3 and 4). Some authors have reported that 2-8% of sporadic ovarian cancers are associated with germline mutations of *BRCA1*[14,15,16]. The incidence in the present study was consistent with those of the previous reports.

Among these Japanese patients, many of the reported mutations overlapped[8-13]. The mutations observed in 26 (57.8%) of 45 families with mutations of *BRCA1* in this study were also included in other

**Table 4.** Haplotype analysis of *BRCA1* in sporadic ovarian cancer

| Case | Age at onset | Histology | Stage | LOHa) at BRCA1 | D17S855 | D17S1322 | D17S1323 | D17S1325 | Haplotype | Direct sequence |
|---|---|---|---|---|---|---|---|---|---|---|
| 148 | 44 | Serous | Ia | + | 153 or 157 | 123 | 152 | 191 or 195 | | |
| K09 | 64 | Serous | Ia | RERb) | RER | RER | RER | RER | | |
| 120 | 50 | Serous | Ic | + | 147 | 126 | 156 | 197 | =T307A | T307A |
| 142 | 57 | Serous | Ic | + | 157 | 132 | 150 | 199 | =C2919T | C2919T |
| 147 | 45 | Serous | Ic | − | | | | | | |
| K02 | 51 | Serous | Ic | − | | | | | | |
| K03 | 68 | Serous | Ic | − | | | | | | |
| K04 | 69 | Serous | Ic | + | 153 | 120 | | 195 | | |
| K21 | 41 | Serous | Ic | − | | | | | | |
| 130 | 57 | Serous | IIc | + | 153 | 120 | 148 | 213 | | |
| 133 | 59 | Serous | IIc | + | 151 | 126 | 154 | 189 | | |
| 134 | 45 | Serous | IIc | + | 149 | 129 | 154 | 193 | | |
| 138 | 67 | Serous | IIc | + | 149 | 126 | 156 | 193 | | |
| 139 | 40 | Serous | IIc | − | | | | | | |
| 146 | 38 | Serous | IIc | − | | | | | | |
| 149 | 59 | Serous | IIc | − | | | | | | |
| 104 | 45 | Serous | IIIc | + | 155 | 123 | 148 | 203 | | |
| 109 | 76 | Serous | IIIc | + | 153 | 123 | 148 | 199 | =3226 del 6bp | no mutation |
| 121 | 57 | Serous | IIIc | + | 149 | 126 | 148 | 207 or 211 | | |
| 128 | 80 | Serous | IIIc | + | 149 | 126 | 148 | 207 or 211 | | |
| 131 | 49 | Serous | IIIc | − | | | | | | |
| 132 | 49 | Serous | IIIc | − | | | | | | |
| 137 | 57 | Serous | IIIc | + | 147 | 126 | 154 | 213 | | |
| 144 | 53 | Serous | IIIc | − | | | | | | |
| K01 | 36 | Serous | IIIc | − | | | | | | |
| K05 | 70 | Serous | IIIc | + | 147 | 126 | 148 | 195 | | |
| K06 | 61 | Serous | IIIc | + | 153 or 157 | 111 | 138 | 191 | | |
| K07 | 70 | Serous | IIIc | + | 149 | 123 | 150 or 152 | 211 | | |
| K08 | 53 | Serous | IIIc | + | 147 | 126 | 156 | 195 or 197 | | |
| 135 | 49 | Serous | IV | + | 149 or 153 | 123 | 148 | 203 | | |
| 401 | 46 | Endometrioid | Ib | + | 149 | 123 | 148 or 150 | 203 | | |
| 102 | 37 | Endometrioid | Ic | + | 153 | 123 | 148 | 195 | =G1173T | no mutation |
| 402 | 38 | Endometrioid | Ic | + | 147 | 120 or 126 | 152 or 158 | 191 | | |
| K22 | 48 | Endometrioid | Ic | RER | | | | | | |
| K23 | 37 | Endometrioid | Ic | − | | | | | | |
| K19 | 45 | Endometrioid | IIIa | + | 153 or 155 | 129 | 154 | 193 or 195 | | |
| 106 | 52 | Endometrioid | IIIc | + | 153 | 123 | 148 | 195 or 197 | | |
| 405 | 44 | Endometrioid | IIIc | + | 149 | 123 or 126 | 152 | 201 or 203 | | |
| 400 | 46 | Endometrioid | IV | + | 149 | 132 | 154 | 203 | | |
| 151 | 67 | Mucinous | Ia | − | | | | | | |
| 156 | 55 | Mucinous | Ic | − | | | | | | |
| 162 | 74 | Mucinous | Ic | + | 149 | 123 or 126 | 148 | 205 or 211 | >T300G | no mutation |
| 164 | 75 | Mucinous | Ic | − | | | | | | |
| K14 | 59 | Mucinous | IIIb | + | 157 | 132 | 150 | 199 | | |
| 163 | 57 | Mucinous | IIIc | + | 147 | 129 | 138 or 154 | 189 | | |
| K11 | 63 | Mucinous | IIIc | − | | | | | | |
| K12 | 48 | Mucinous | IIIc | RER | 153 | 123 | 148 | RER? | | |
| 152 | 61 | Mucinous | IV | − | | | | | | |
| K15 | 43 | Mucinous | IV | + | 149 | 120 or 123 | 148 or 150 | 195 | | |
| 180 | 44 | Clear cell | Ia | − | | | | | | |
| 181 | 61 | Clear cell | Ic | + | 149 or 147 | 123 | 146 or 154 | 189 or 195 | | |
| 182 | 45 | Clear cell | Ic | + | 149 | 126 | 148 or 195 | 191 or 195 | | |
| K17 | 52 | Clear cell | Ic | − | | | | | | |
| K18 | 51 | Clear cell | Ic | + | 153 | 123 or 126 | 138 | 197 | | |
| 183 | 66 | Clear cell | IIc | − | | | | | | |
| 192 | 52 | Clear cell | IIc | − | | | | | | |
| 184 | 45 | Clear cell | IIa | − | | | | | | |
| Genotype of CEPH 1347-02 | | | | | 147/151 | 123/126 | 148/154 | 211/213 | | |

a) Loss of heterozygosity; b) Replication error.

reports. Therefore, it is supposed that the variety of *BRCA1* mutations is limited among the Japanese population. This may be supported by the fact that the Japanese people comprise a limited number of ethnic groups due to geographic isolation and the low incidence of marriage to other ethnic groups.

The sensitivity of this method to detect mutations will become improve with the addition of more data of alleles with *BRCA1* mutations. Since the existence of LOH is essential for this method, the mutations are unable to be detected in the tumors without LOH at the *BRCA1* region. Accordingly, some false negative cases may have been included in our negative cases. However, 64-100% of the ovarian cancer cases associated with germline mutations of *BRCA1* have been reported to have LOH at the *BRCA1* locus[10,14,16,17]. In fact, we observed LOH at that locus in 96.0% (48 of 50) of familial ovarian cancers with the mutations of *BRCA1*, suggesting that the number of false negative cases may be very small.

In this study we refer to the relationship between the mutation and the histological type of the ovarian cancers. The incidences of serous adenocarcinomas and endometrioid adenocarcinomas were 78.7% (59/75) and 14.7% (11/75), respectively, in the ovarian cancers with *BRCA1* mutations. On the other hand, 57 serous adenocarcinomas were included in the total 118 ovarian cancers treated at Niigata University Hospital from 1983 to 1996. The ratios of the serous adenocarcinomas differed significantly between the cases with *BRCA1* mutations and the total number of ovarian cancer cases ($P<0.05$, Chi-square test). This result was coincident with the other reports[25,26]. It should be noted that neither mucinous adenocarcinoma nor clear cell adenocarcinoma was included in the cases with *BRCA1* mutations. Both serous and endometrioid adenocarcinomas were observed even in the tumors with a mutation of completely the same sequence. There was no relationship between the histological finding and the pattern of the mutation.

Because average family size is decreasing in Japan, it is difficult to clarify further details of cancer-associated relatives of patients by examining patient history. It is thus supposed that many "latent" breast and/or ovarian cancer families associated with *BRCA1* mutations may be involved in the sporadic cases. Accordingly, members of such "latent" breast and/or ovarian cancer families are not aware of their predisposition to cancer until advanced cases appear in the next generation. For this reason, it is important to identify those families at risk among the sporadic ovarian and/or breast cancer groups and to advise them of the necessity of short term checkups in order to detect and treat these cancers in earlier stages. We believe that the present report will prove helpful for further determination of the families at risk for ovarian and breast cancers.

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