Detection of BRCA1 and BRCA2 germline mutations in Japanese population using next-generation sequencing

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Keywords
BRCA1, BRCA2, diagnostic, familial, Japanese, next-generation sequencing

Abstract
Tumor suppressor genes BRCA1 and BRCA2 are the two main breast and ovarian cancer susceptibility genes, and their genetic testing has been used to evaluate the risk of hereditary breast and ovarian cancer (HBOC). While several studies have reported the prevalence of BRCA1 and BRCA2 mutations in Japanese populations, there is insufficient information about deleterious mutations compared with western countries. Moreover, because many rare variants are found in BRCA1 and BRCA2, both of which encode large proteins, it is difficult to sequence all coding regions using the Sanger method for mutation detection. In this study, therefore, we performed next-generation sequencing (NGS) analysis of the entire coding regions of BRCA1 and BRCA2 in 135 breast and/or ovarian cancer patients. Deleterious BRCA1 and BRCA2 mutations were detected in 10 patients (7.4%) by NGS analysis. Of these, one mutation in BRCA1 and two in BRCA2 had not been reported previously. Furthermore, a BRCA2 mutation found in a proband was also identified in two unaffected relatives. These data suggest the utility of screening BRCA1 and BRCA2 mutations by NGS in clinical diagnosis.

Introduction
Genetic testing for hereditary breast and ovarian cancer (HBOC) susceptibility has clinical importance for cancer prevention. Indeed, approximately 5–10% of breast and ovarian cancer patients are likely to be hereditary (Cancer Genome Atlas Research Network 2011; Cancer Genome Atlas Network 2012). Previous studies showed that BRCA1 (MIM #113705) and BRCA2 (MIM #600185) are the two major breast and ovarian cancer susceptibility genes (Easton et al. 1993; Narod et al. 1995; King et al. 2003), accounting for approximately 15% of inherited cases (Couch et al. 2014; Kanchi et al. 2014). Additionally, inherited mutations in TP53, PTEN, STK11, and CDH1 are associated with moderately high risks of breast cancer in the context of Li-Fraumeni syndrome, Cowden syndrome, Peutz–Jeghers syndrome, and hereditary diffuse gastric cancer syndrome, respectively (FitzGerald et al. 1998; Hearle et al. 2006; Schrader et al. 2008; Gonzalez et al. 2009; Walsh et al. 2010). Germline mutations in DNA repair genes such as PALB2, ATM, and CHEK2 also confer a risk for breast and ovarian cancer (Couch et al. 2014; Kanchi et al. 2014).

Genetic linkage studies localized BRCA1 and BRCA2 to chromosomes 17q and 13q, respectively (Hall et al. 1990; Wooster et al. 1994), and the genes were subsequently cloned (Miki et al. 1994; Tavtigian et al. 1996) and shown to play a role in DNA damage repair and the regulation of genomic stability. The functional importance of BRCA1 and BRCA2 in vivo is supported by mouse
models. For instance, Brca1 or Brca2 homozygous null mutants are embryonic lethal with growth retardation (Hakem et al. 1996; Connor et al. 1997; Suzuki et al. 1997), while Brca1 and Brca2 conditional knockout mice models showed that loss of Brca1 and Brca2 enhances tumorigenesis. This indicates that BRCA1 and BRCA2 are tumor suppressor genes (Xu et al. 1999; Jonkers et al. 2001).

BRCA1 (1863 amino acids) and BRCA2 (3418 amino acids) are large proteins with many repetitive elements (Welsh and King 2001). More than 1800 distinct mutations have been reported in BRCA1 and over 2,000 in BRCA2 in the Breast Cancer Information Core (BIC) database (Couch et al. 2014). A mutation was regarded to be deleterious if it led to premature truncation and loss of normal protein function. Because BRCA1 and BRCA2 loss of function mutations scattered throughout the genes, it is necessary to screen all coding regions of both genes in a genetic diagnosis of HBOC. However, this is an expensive and time-consuming process using the Sanger method. Furthermore, genetic testing of BRCA1 and BRCA2 has not been extensively performed in Japan compared with western countries, partly because genetic testing costs are currently no longer covered by health insurance. The present study therefore used next-generation sequencing (NGS) technology to determine the prevalence of breast and ovarian cancer patients carrying germline mutations in BRCA1 and BRCA2.

Materials and Methods

Patients and sample preparation

Peripheral blood samples were obtained from 135 breast and/or ovarian cancer patients and two unaffected individuals who attended Yamanashi Prefectural Central Hospital (Yamanashi, Japan) between 2013 and 2014. Lymphocytes were isolated following centrifugation of blood samples at 820g at 25°C for 10 min. Peripheral blood lymphocytes were stored at −80°C until required for DNA extraction. Total DNA was extracted from lymphocytes using the QIAamp® DNA Blood Mini kit (Qiagen, Tokyo, Japan) or QIAamp® DNA Blood Mini QIAcube Kit (QIAGEN) with the QIAcube (QIAGEN). The concentration of DNA was determined using the Nano Drop 2000 spectrophotometer (Thermo Fisher Scientific, Yokohama, Japan). Informed consent was obtained from all subjects, and this study was approved by the institutional review board at Yamanashi Prefectural Central Hospital.

Targeted next-generation sequencing

For targeted NGS analysis, the Ion AmpliSeq™ BRCA1 and BRCA2 Panel (Life Technologies, Tokyo, Japan) containing 167 primer pairs in three pools was used. Multiplex PCR was performed using 50–100 ng genomic DNA with a premixed primer pool and Ion AmpliSeq HiFi master mix (Ion AmpliSeq™ Library Kit 2.0) for 2 min at 99°C, followed by 19 cycles of 99°C for 15 sec and 60°C for 4 min, ending with a holding period at 10°C. The PCR amplicons were treated with 2 µL FuPa reagent to partially digest primer sequences and phosphorylate the amplicons at 50°C for 10 min, followed by 55°C for 10 min, then 60°C for 20 min. The amplicons were ligated to adapters with the diluted barcodes of the Ion Xpress™ Barcode Adapters kit (Life Technologies) for 30 min at 22°C then 72°C for 20 min. Adaptor ligated amplicon libraries were purified using Agencourt AMPure® XP reagents (Beckman Coulter, Tokyo, Japan). The library concentration was determined using an Ion Library Quantitation Kit (Life Technologies), then each library was diluted to 8–16 pmol/L and the same amount of libraries was pooled for one sequence reaction. Next, emulsion PCR was carried out using the Ion OneTouch™ System and Ion OneTouch™ 200 Template Kit v2 (Life Technologies) according to the manufacturer’s instructions. Template-positive Ion Sphere™ Particles were then enriched with Dynabeads® MyOne™ Streptavidin C1 Beads (Life Technologies) using an Ion OneTouch™ ES system (Life Technologies). Purified Ion Sphere particles were loaded on an Ion 314 or 318 Chip. Massively parallel sequencing was carried out on a Personal Genome Machine (PGM) sequencer (Ion Torrent™) using the Ion PGM Sequencing 200 Kit version 2 according to the manufacturer’s instructions. Sequencing was performed using 500 flow runs that generated approximately 200 bp reads.

Data analysis

The sequence data were processed using standard Ion Torrent Suite™ Software running on the Torrent Server (Life Technologies, Tokyo, Japan). Raw signal data were analyzed using Torrent Suite™ version 3.6.2 or 4.0.2. The pipeline included signaling processing, base calling, quality score assignment, adapter trimming, PCR duplicate removal, read alignment to human genome 19 reference (hg19), quality control of mapping quality, coverage analysis, and variant calling. Coverage analysis and variant calling used Torrent Variant Caller plugin software (version 3.6 or 4.0) in the Torrent Server. The variant caller parameter setting was germline PGM high stringency. Following data analysis, annotation of single-nucleotide variants, insertions, deletions, and splice site alterations was performed by the Ion Reporter™ Server System (Life Technologies), which identified nonsynonymous mutations. Splice site alteration were analyzed 2 bp upstream...
or downstream of exon–intron boundaries. Sequence data were visually confirmed with the Integrative Genomics Viewer (IGV) and any sequence, alignment, or variant call error artifacts were discarded. Nonsynonymous mutations were annotated using the BIC database (https://research.nhgri.nih.gov/projects/bic/index.shtml) and ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) (Landrum et al. 2014). Minor allele frequency was determined from the 1000 Genomes Project database (Abecasis et al. 2012), the 5000 Exome project (http://evs.gs.washington.edu/EVS/), and The Human Genetic Variation Database (HGVD) (http://www.genome.med.kyoto-u.ac.jp/SnpDB).

Sanger sequencing

PCR was performed using genomic DNA as a template and primer pairs flanking the deleterious variant sites. PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN) according to the manufacturer’s instructions. PCR products were purified and subsequently analyzed by the 3500 Genetic Analyzer (Applied Biosystems, Tokyo, Japan). GenBank sequences of human BRCA1 (accession number: NP_009225.1) and BRCA2 (accession number: NP_000050.2) were referred to at the NCBI Reference Sequence Database. Primer sequences are provided in the Table S1.

Validation NGS data

To assess the consistency of results between laboratories, we sent an aliquot of 25 genomic DNA samples to FALCO Biosystems Ltd. (Kyoto, Japan), and direct sequencing was performed. All variants detected by direct sequencing were interpreted according to the Myriad Genetics’ criteria.

Pathogenic variants in BRCA1 and BRCA2 genes

Of the 135 patients enrolled in our study, deleterious BRCA1 or BRCA2 truncation mutations were found in 10 (7.4%), including five BRCA1 mutations (3.7%) and five BRCA2 mutations (3.7%) (Table 1). Of the 10 mutations, five were nonsense, four were frameshift, and 1 Sanger sequencing using BigDye® Terminator v3.1 using M13 forward or reverse primers (Life Technologies). PCR products were purified and subsequently analyzed by the 3500 Genetic Analyzer (Applied Biosystems, Tokyo, Japan). GenBank sequences of human BRCA1 (accession number: NP_009225.1) and BRCA2 (accession number: NP_000050.2) were referred to at the NCBI Reference Sequence Database. Primer sequences are provided in the Table S1.

| Patient no. | Gene | Designation | Type | Coding | Var Freq (%) | Cov | Ref Cov | Var Cov | BIC | ClinVar |
|-------------|------|-------------|------|--------|--------------|-----|---------|---------|-----|---------|
| 1           | BRCA1| p.L63X      | SNV  | c.188T>A| 52           | 564 | 273     | 291     | CI  | Pathogenic |
| 2           | BRCA1| p.K85Qfs    | INS  | c.1952_1953insG | 45  | 448 | 245     | 203 | –     | –       |
| 3           | BRCA1| p.Q93X      | SNV  | c.2800C>T | 53  | 400 | 189     | 211 | CI  | Pathogenic |
| 4           | BRCA1| p.Q93X      | SNV  | c.2800C>T | 50  | 483 | 241     | 242 | CI  | Pathogenic |
| 5           | BRCA1| p.F125Qfs   | DEL  | c.3770_3771delAG | 48  | 446 | 231     | 215 | CI  | Pathogenic |
| 6           | BRCA2| p.Q850fs    | INS  | c.2547_2548insCC | 50  | 161 | 81      | 80  | –     | –       |
| 7           | BRCA2| p.S188X     | SNV  | c.5645C>A | 53  | 164 | 77      | 87  | CI  | Pathogenic |
| 8           | BRCA2| p.N213Qfs   | DEL  | c.6402_6406delTAACT | 42  | 296 | 173     | 123 | CI  | Pathogenic |
| 9           | BRCA2| p.R231Qfs   | SNV  | c.6952C>T | 32  | 91  | 62      | 29  | CI  | Pathogenic |
| 10          | BRCA2| p.I2675V    | SNV  | c.8023A>G | 56  | 173 | 76      | 97  | –    | Likely pathogenic |

Molecular Genetics & Genomic Medicine

Detection of BRCA1 and BRCA2 Germline Mutations

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**Figure 1.** Germline loss of function mutations identified in patients with breast and/or ovarian cancer. (A) Distribution of BRCA1 and BRCA2 mutations detected in 10 of 132 patients with breast and/or ovarian cancer. The small circles indicate the number of individuals with a mutation. The number under the square indicates the amino acid position. (B) Representative image of read alignments visualized with IGV. Arrow indicates the germline mutation (BRCA1 p.L63X, c.188T>A) detected by NGS analysis. (C) Representative validation of NGS data in (B) by Sanger sequencing. Germline mutation (BRCA1 p.L63X) was detected in the patient but not in the control subject. Arrow indicates position of the mutated nucleotide (c.188T>A).

one was a missense mutation (Table 1). No splice site mutations in the exon–intron boundaries were identified in this study. The BRCA2 missense mutation p.12675V (c.8023A>G) was predicted to be causative because it generates a donor site within exon 18 that causes an in-frame deletion and splicing defect (Bonnet et al. 2008). BRCA1 nonsense mutations p.L63X (c.188T>A) and p.Q934X (c.2800C>T) have previously been recognized as Japanese founder mutations (Sekine et al. 2001; Nakamura et al. 2013). In our study, BRCA1 L63X and Q934X were found in one and two patients, respectively (Table 1), indicating that they occur frequently in the Japanese population. Of the other deleterious mutations identified in this study, one BRCA1 mutation (p.K652fs; c.1952_1953insG) and two BRCA2 mutations (p.Q850fs; c.2547_2548insCC and p.12675V; c.8023A>G) were not registered in the BIC database (Table 1), suggesting that they are novel. Furthermore, the founder mutations BRCA1 c.185delAG, BRCA1 c.5382insC, and BRCA2 c.6174delT, previously identified in an Ashkenazi Jewish population, were not observed in the current cohort, indicating that BRCA mutations vary among different populations as described before (Kim and Choi 2013).

**Missense variants in BRCA1 and BRCA2**

NGS analysis detected a total of 23 missense variants of uncertain significance (VUS) including 10 BRCA1 variants and 13 BRCA2 variants (Tables 2, 3). Of these, six BRCA1 VUS and eight BRCA2 VUS are rare variants according to 1000 Genome Project data (<1% population minor allele frequency) (Table 2). By contrast, four BRCA1 and five BRCA2 variants were present at high frequencies in the study subjects and in ≥1% of the population according to 1000 Genome Project data, indicating that they are common polymorphisms (Table 3). We next used HGVD, which contains Japanese exome sequencing data, to examine whether variants identified in this analysis were Japanese genetic variations. One BRCA1 variant (p.M1628T: 2%) and three BRCA2 variants (p.K322Q: 1%, p.M784V: 9.7% and p.K2729N: 1.7%) were found in ≥1% of Japanese population (Table 2), indicating these variants were comparatively unique to Japanese.

**Cross-validation of NGS data**

To further analysis the accuracy of NGS data, we out-sourced a set of 25 samples for genetic analysis and compared the results at the two independent laboratories (our laboratory and FALCO Biosystems Ltd.). The result performed with two institutions showed concordance was 100% for all 25 samples (Table 4), therefore, we confirmed the reproducibility of our NGS data. Functional significance of five nonsense mutation, four indels and sixteen missense variants were interpreted by FALCO Biosystems (the Myriad Genetics criteria). According to this, all nonsense mutation and indels were interpreted as deleterious and one missense variant is suspected deleterious (Table 4). In contrast, five missense variants were uncertain one was favor polymorphism and nine were polymorphism (Table 4). Interestingly, five missense variants (BRCA1: p.L52F, p.N1018S, p.N1236S, p.V1653L, BRCA2: p.D1990A) interpreted as an uncertain were rare variants according to HGVD database (Table 2). Although it remains unclear whether these five missense VUS have influence on BRCA1 or BRCA2 functions, it is possible that some of variants are associated with HBOC.
### Table 2. Rare variants of uncertain significance found in patients with breast and/or ovarian cancer (n = 135).

| Gene | Designation | Case Freq (n = 135) | BIC | Clin Var | dbSNP | 1000 genome MAF (%) | 5000 exomes | HGVD exome (%) |
|------|-------------|---------------------|-----|----------|-------|----------------------|-------------|---------------|
| BRCA1 | p.L52F      | 1 (0.73%)           | Unknown | Uncertain significance | rs80357084 | – | – | 0.4 |
| BRCA1 | p.V271M     | 1 (0.73%)           | Unknown | Uncertain significance | rs80357244 | <0.1 | – | 0.5 |
| BRCA1 | p.N1018S    | 1 (0.73%)           | – | – | – | – | – | – |
| BRCA1 | p.N1236S    | 1 (0.73%)           | – | – | – | – | – | – |
| BRCA1 | p.M1628T    | 6 (4.41%)           | Unknown | Conflicting data | rs4986854 | 0.4 | AMAF = 0.02% | EMAF = 0.03% | GMAF = 0.03% |
| BRCA1 | p.V1653L    | 2 (1.47%)           | – | – | rs80357261 | – | – | – |
| BRCA2 | p.K322Q     | 4 (2.94%)           | Unknown | Conflicting data | rs11571640 | <0.1 | – | 1 |
| BRCA2 | p.M784V     | 21 (15.4%)          | Unknown | Uncertain significance | rs11571653 | 0.7 | – | 9.7 |
| BRCA2 | p.I1929V    | 1 (0.73%)           | NCS | Benign | rs79538375 | 0.1 | AMAF = 0% | EMAF= 0.01% | GMAF = 0.01% |
| BRCA2 | p.D1990A    | 1 (0.73%)           | – | Uncertain significance | rs148618542 | <0.1 | – | – |
| BRCA2 | p.G2044V    | 5 (4%)              | Unknown | Conflicting data | rs56191579 | 0.1 | AMAF = 0.02% | – | – |
| BRCA2 | p.V2109I    | 2 (1.47%)           | Unknown | Uncertain significance | rs79456940 | <0.1 | – | 0.7 |
| BRCA2 | p.V2503I    | 1 (0.73%)           | – | – | – | – | – | 0.1 |
| BRCA2 | p.K2729N    | 6 (4.41%)           | Unknown | Uncertain significance | rs80359065 | 0.3 | – | 1.7 |

MAF, minor allele frequency; AMAF, African American minor allele frequency; EMAF, European American minor allele frequency; GMAF, global minor allele frequency; HGVD, the human genetic variation database.

1Allele frequency data from Yokohama City University.

### Table 3. Common variants found in patients with breast and/or ovarian cancer (n = 135).

| Gene | Designation | Case Freq (n = 135) | BIC | ClinVar | dbSNP | 1000 genome MAF (%) | 5000 exomes | HGVD exome (%) |
|------|-------------|---------------------|-----|---------|-------|----------------------|-------------|---------------|
| BRCA1 | p.P871L     | 78 (57%)            | NCS | Benign | rs799917 | 48.3 | AMAF = 20% | EMAF = 33.59% | 33.4 |
| BRCA1 | p.E1038G    | 78 (57%)            | NCS | Benign | rs16941 | 30.3 | AMAF = 18.84% | EMAF = 32.55% | 33.2 |
| BRCA1 | p.K1183R    | 78 (57%)            | NCS | Benign | rs16942 | 32.4 | AMAF = 23.83% | EMAF = 32.44% | 33.3 |
| BRCA1 | p.S1613G    | 78 (57%)            | NCS | Benign | rs1799966 | 32.7 | AMAF = 24.26% | EMAF = 32.66% | 33.4 |
| BRCA2 | p.N289H     | 31 (23%)            | NCS | Benign | rs766173 | 5.8 | AMAF = 2.02% | EMAF = 3.68% | 13.7 |
| BRCA2 | p.N372H     | 46 (34%)            | NCS | Conflicting data | rs144848 | 24.0 | AMAF = 12.89% | EMAF = 28.59% | 22.3 |
| BRCA2 | p.N991D     | 30 (22%)            | Unknown | Conflicting data | rs1799944 | 6.2 | AMAF = 3.86% | EMAF = 3.66% | 13.5 |
| BRCA2 | p.V2466A    | 135 (100%)          | Unknown | Uncertain significance | rs169547 | 2.2 | AMAF = 6.47% | EMAF = 0.06% | 99.9 |
| BRCA2 | p.I3412V    | 3 (2.2%)            | Unknown | Conflicting data | rs1801426 | 4.3 | AMAF = 10.64% | EMAF = 0.19% | 2 |

NCS, not clinically significant; MAF, minor allele frequency; AMAF, African American minor allele frequency; EMAF, European American minor allele frequency; GMAF, global minor allele frequency; HGVD, the human genetic variation database.
To determine whether unaffected relatives carry the same deleterious mutations as the probands, we enrolled two unaffected relatives of an ovarian patient harboring BRCA2 p.S1882X (c.5645C>A). Sanger sequencing analysis showed that both relatives also carried the BRCA2 mutation (Fig. 2). Taken together, these results suggested the screening of BRCA1/2 mutation carrier using NGS has a role in aiding cancer prevention and reducing the cancer risk in unaffected individuals.

**Discussion**

In this study, we determined the sequence of BRCA1 and BRCA2 in 135 breast and/or ovarian cancer patients by NGS, which was then validated using Sanger sequencing. We identified deleterious BRCA1 or BRCA2 truncation mutations in 10 patients, including one BRCA1 (p.K652 fs; c.1952_1953insG) and two BRCA2 (p.Q850 fs; c.2547_2548insCC and p.I2675V; c.8023A>G) mutations, which, to the best of our knowledge, are novel. An aliquot of 25 samples were outsourced for genetic analysis and there was concordance in results obtained by two independent laboratories. Furthermore, two unaffected

**BRCA2 germline mutations in unaffected individuals**

To determine whether unaffected relatives carry the same deleterious mutations as the probands, we enrolled two unaffected relatives of an ovarian patient harboring BRCA2 p.S1882X (c.5645C>A). Sanger sequencing analysis showed that both relatives also carried the BRCA2 mutation (Fig. 2). Taken together, these results suggested the screening of BRCA1/2 mutation carrier using NGS has a role in aiding cancer prevention and reducing the cancer risk in unaffected individuals.
relatives were shown to carry the same deleterious BRCA2 mutation observed in the proband. This study demonstrated the utility of NGS for performing the molecular diagnosis of HBOC based on BRCA1 and BRCA2 genetic alterations. We propose that the technique is suitable to detect mutations in tumor suppressor genes such as BRCA1 and BRCA2 where causative mutations are distributed throughout the genes (Costa et al. 2013). It is also likely to be informative in the analysis of the many other susceptible genes related to breast and ovarian cancer that have been discovered (Couch et al. 2014). Indeed, several studies have developed a system for the simultaneous detection of multiple target genes using NGS (Walsh et al. 2010, 2011; Castéra et al. 2014). The identification of mutations in genes other than BRCA1 and BRCA2 will also help understand the genetic heterogeneity and penetrance of HBOC (Walsh et al. 2010; Castéra et al. 2014).

Identifying founder mutations would enable us to examine specific loci in the screening of high-risk subpopulations for inherited breast and ovarian cancer without performing a full sequence analysis of BRCA1 and BRCA2. Founder mutations have previously been described in an Ashkenazi Jewish population in which 3% of individuals carried BRCA1 c.185delAG, BRCA1 c.5382insC, or BRCA2 c.6174delT mutations (Ferla et al. 2007). The KOHBRA study carried out large population in Korea showed BRCA2 p.R2494X, BRCA1 p.Y130X, and BRCA1 p.V1833fs were candidate founder mutations (Han et al. 2011). In the Japanese population, BRCA1 L63X and Q934X were reported as founder mutations (Ikeda et al. 2001; Sekine et al. 2001; Sugano et al. 2008; Nakamura et al. 2013), and were observed in three patients of the present study. Although another study showed that BRCA2 5802delTTAA mutation was considered as common in Japanese breast cancer patients (Ikeda et al. 2001), we did not detect this type of mutation. These observations implies that the BRCA1 and BRCA2 founder mutation status in the Japanese population differs from that of other countries. Because BRCA1 and BRCA2 testing is less commonly undertaken in Japan compared with western countries, a large-scale cohort study is required to obtain more precise information about founder mutations in Japan.

This study demonstrated, among 135 breast and/or ovarian cancer patients, 10 patients (7.4%) were deleterious carriers. Previous study with Japanese subject showed BRCA1 and BRCA2 germline mutation in 36 subjects out of 135 (26.8%) (Sugano et al. 2008). In our study, the proportion of mutation carriers was lower than that of previous study, because we enrolled the subjects at diagnosis and not selected for family history.

Large genomic alterations in BRCA1 and BRCA2 are pathogenic, but a limitation of our study was that we did not examine these gene rearrangements. Therefore, additional analysis such as multiplex ligation-dependent probe amplification is required. In western countries, large genomic rearrangements were frequently found in BRCA1 gene locus (Gad et al. 2002; Montagna et al. 2003). Contrary to these findings, large genomic deletion were thought to be rare in Japanese population (Sugano et al. 2008). We expect that the combinatorial use of the NGS system with large genomic analysis will be desirable for screening HBOC.

In conclusion, we showed that multiplex PCR followed by NGS is useful for screening BRCA1 and BRCA2 germline mutations of probands and could be applicable to cancer prevention in unaffected relatives carrying the same mutation. This method is both cost- and time-effective for the screening of genetic variants, and will be beneficial in clinical and diagnostic use.

Acknowledgments

We thank Takeo Kubota and Satoko Nakagomi for their advice, and Takuro Uchida and Yumi Kubota for their help. This study was supported by a Grant-in-Aid for Genome Research Project from Yamanashi Prefecture (Y. H. and M. O.).

Conflict of Interest

None declared.

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Deleterious mutation identified by NGS data. Image of read alignments visualized with IGV. Arrows indicate the position of the mutations in the patients.

**Figure S2.** DNA sequencing analysis of deleterious BRCA1 and BRCA2 mutations. PCR followed by Sanger sequencing validated the nonsense mutations and frameshift insertions/deletions using genomic DNA from control subjects and each patient. Arrows indicate the position of the nonsense mutation in the patient and the corresponding position in the control subject.

**Table S1.** Primer sequences used for Sanger sequencing.

**Table S2.** Coverage analysis data.