Mutation status of RAD51C, PALB2 and BRIP1 in 100 Japanese familial breast cancer cases without BRCA1 and BRCA2 mutations

Katsutoshi Sato,1 Mio Koyasu,1 Sachio Nomura,1,2 Yuri Sato,1 Mizuho Kita,1 Yuumi Ashihara,1 Yasue Adachi,1 Shinji Ohno,1 Takuji Iwase,1 Dai Kitagawa,3 Eri Nakashima,3 Reiko Yoshida,1 Yoshio Miki2 and Masami Arai1

1Clinical Genetic Oncology, Cancer Institute Hospital, Japanese Foundation of Cancer Research, Tokyo; 2Translational Research Support, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Tokyo; 3Department of Surgery, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Tokyo; 4Division of Medical Genomics, Department of Molecular Genetics, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan

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Correspondence
Masami Arai, Clinical Genetic Oncology, Cancer Institute Hospital, Japanese Foundation of Cancer Research, 3-8-1, Koto-ku, Tokyo 135-8550, Japan.
Tel: +81-3-3520-0111; Fax: +81-3-3570-0343; E-mail: marai@fcr.or.jp

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In addition to BRCA1 and BRCA2, RAD51C, PALB2 and BRIP1 are known as breast cancer susceptibility genes. However, the mutation status of these genes in Japanese familial breast cancer cases has not yet been evaluated. To this end, we analyzed the exon sequence and genomic rearrangement of RAD51C, PALB2 and BRIP1 in 100 Japanese patients diagnosed with familial breast and ovarian cancer and without BRCA1 and BRCA2 mutations. We detected a large deletion from exons 6 to 9 in RAD51C, 4 novel BRIP1 missense variants containing 3 novel non-synonymous variants, c.89A>C, c.736A>G and c.2131A>G, and a splice donor site variant c.918+2T>C. No deleterious variant of PALB2 was detected. The results of pedigree analysis showed that the proband with a large deletion on RAD51C had a family history of both breast and ovarian cancer, and the families of probands with novel BRIP1 missense variants included a male patient with breast cancer or many patients with breast cancer within the second-degree relatives. We showed that the mutation frequency of RAD51C in Japanese familial breast cancer cases was similar to that in Western countries and that the prevalence of deleterious mutation of PALB2 was possibly lower. Furthermore, our results suggested that BRIP1 mutation frequency in Japan might differ from that in Western countries.

It is widely known that BRCA1 and BRCA2 are the most critical causative genes for hereditary breast cancer. The linkage analysis in 237 families with at least 4 breast cancer patients revealed that breast cancer was caused by abnormality of BRCA1 and BRCA2 in 52 and 32% of these families, respectively.1 In Japan, approximately 25% of patients are suspected to present with familial breast cancer have mutations in either BRCA1 or BRCA2.2 Although these studies indicate that a large part of familial breast cancer is caused by inheritance of abnormal BRCA1 and BRCA2 genes, other reports suggest that the mutation of other genes is likely to contribute to the remaining cases. Therefore, identification of these susceptible genes would be advantageous for precise diagnosis and for the prevention of breast cancer incidence.

Recently, various genes, other than BRCA1 and BRCA2, such as ataxia-telangiectasia mutated (ATM), checkpoint kinase 2 (CHEK2), tumor protein p53 (TP53), Cadherin 1 (CDH1), phosphatase and tensin homolog (PTEN), RAD51 paralog C (RAD51C), partner and localizer of BRCA2 (PALB2) and BRCA1 interacting protein 1 (BRIP1) have garnered attention as susceptibility genes of familial breast cancer.3 Of these genes, we focused on RAD51C, BRIP1 and PALB2 because these three genes have common features. Deleterious mutation of each gene commonly induces Fanconi anemia (FA), and the products of these three genes directly participate in homologous recombination (HR) repair interacting with BRCA1, BRCA2 and each other, whereas other factors, such as ATM, CHEK2 and TP53, are closely associated with functions of cell cycle checkpoint rather than HR repair. RAD51C promotes the strand exchange of DNA by coordinating with RAD51B and replication protein A1 (RPA) in the HR repair process.4,5 PALB2 interacts with many proteins, including BRCA1, BRCA2, RAD51, RAD51C, RPA and DNA polymerase η, and removes the collapsed replication fork by means of HR repair.6-8 BRIP1 is a BRCA1 interacting protein with a DEAH helicase domain. In addition to BRCA1, this protein interacts with TopBP1,9 RPA10 and MLH1.11,12 This interaction is required to repair the stalled replication fork in S phase. Dysfunction of these proteins significantly increased sensitivity to γ-irradiation13 and mitomycin C.7,8,14 Because DNA cross-linking reagent is removed by the HR repair process through the FA pathway, RAD51C, PALB2 and BRIP1 are essential for HR repair.

Deleterious mutations of these genes are likely to affect familial breast cancer incidence.15 However, the mutation spectrum of these genes in Japanese patients with familial breast cancer has not yet been revealed. In this study, we evaluated full exon sequence and genome rearrangement of these genes to assess their mutation spectrum in Japanese patients with familial breast cancer who were negative for BRCA1 and BRCA2 mutations.
Materials and Methods

Patients. From April 2000 to September 2016, 740 patients received genetic counseling. Of these patients, 440 probands affected with breast or ovarian cancer received genetic testing for BRCA1 or BRCA2 mutations. As a result, deleterious mutations on BRCA1 or BRCA2 were detected in 119 patients, while none were detected in the remaining 321 patients. Of the 321 probands without deleterious mutation in BRCA1 and BRCA2, 100 probands with breast cancer were enrolled into this study. A total of 99 patients satisfied the National Comprehensive Cancer Network (NCCN) criteria for BRCA1 and BRCA2 mutation testing.(16) Although a female proband did not meet the NCCN criteria for BRCA1 and BRCA2 testing, she was selected as a subject in our study because she, her mother and her maternal grandfather were affected with left breast cancer, metachronous bilateral breast cancer and pancreatic cancer, respectively. Of the 100 cases, 94 and 6 cases were probands with breast and both breast and ovarian cancers, respectively. The 94 breast cancer cases included 90 female and 4 male individuals (Table 1). No obvious deleterious mutation in BRCA1 and BRCA2 was detected by commercial genetic test for BRCA1/2 mutation (FALCO Biosystems, Kyoto, Japan). Although the variants of uncertain significance (VUS) in BRCA1 and BRCA2 were observed in 3 cases, we considered them eligible for this study because these variants could not explain the association with familial breast cancer occurrence and, therefore, suggested that they presented with other causative genetic mutations. For 1 individual, multiplex ligation-dependent probe amplification (MLPA) could not be performed to assess a large deletion of BRCA1 and BRCA2.

Written informed consent or broad consent was obtained from all participants. This study was approved by the ethical committee of the Cancer Institute Hospital, Japanese Foundation of Cancer Research (2014-1040).

Preparation of DNA samples. Genomic DNA was harvested from blood samples using a QIAamp DNA Blood Maxi Kit (Qiagen, Hilden, Germany) following the manufacturer’s protocol. NanoDrop (ND-1000, Thermo Fisher Scientific, Waltham, MA, USA) was used to determine DNA concentration.

PCR-direct sequencing. The genomic DNA was amplified by PCR with the Expand High Fidelity PCR System, dNTPack (Roche Diagnostics, Basel, Switzerland) following the manufacturer’s protocol. The forward and reverse primers for RAD51C, BRIP1 and PALB2 were designed for amplification of all the coding sequences for each gene (Table S1).

DNA sequencing was performed by Eurofins DNA sequence service (Eurofins genomics, Tokyo, Japan). The DNA chromatogram was aligned to the National Center for Biotechnology Information (NCBI) reference sequence (RefSeq) for RAD51C (NM_058216.1), PALB2 (NM_024675.3) and BRIP1 (NM_032043.2) by SeqScape Software 3 (ver. 3.0, Applied Biosystems, Foster City, CA, USA). When variants were identified for each gene, the DNA sequences of the corresponding exon were analyzed again in our laboratory with a BigDye Terminator v1.1 Sequence Standard Kit (Applied Biosystems) following the manufacturer’s protocol. The detailed protocols are described in the supporting information (Doc. S1).

Multiplex ligation-dependent probe amplification. Genomic rearrangement was assessed by MLPA using commercial reagents for RAD51C (P260-A2 and P260-B1, MRC-Holland, Amsterdam, the Netherlands), PALB2 (P260-A2 and P260-B1, MRC-Holland) and BRIP1 (p240-A3, MRC-Holland) following the manufacturer’s instructions. During the hybridization step, 20 μL of Vapor-Lock (Qiagen) was used to prevent the evaporation of the reagents.

DNA from a patient with no mutation on the studied genes was used as the reference DNA. The detailed protocols are described in the supporting information (Doc. S2).

Statistical analysis. Statistical difference in allele counts was tested by Fisher’s exact test. The statistical values, including P-values and odds ratios, were calculated by using SAS software (University Edition, version 9.4, SAS Institute, Cary, NC, USA). P-values < 0.05 were considered statistically significant.

Interpretation of clinical significance. The interpretation of the clinical significance of the observed variants was based on the type of mutation and the statistical significance of the allele counts. When the observed variant resulted in a conformational change in the gene product due to a deletion, insertion and amputation, or was at the splice site, it was considered “deleterious.” When there was no significant difference in allele counts between our results and that registered in the Human Genome Variation Database (HGVD), the observed mutation was considered “Neutral.” When the statistical values could not be calculated due to no registration in HGVD, the family history, the information from other databases, including ClinVar and Human Genome Mutation Database (HGMD professional, version 2016.01), and the results of in silico analysis were interpreted as the variant being “probably deleterious” or not (Table 2).

Results

Mutations in RAD51, PALB2 and BRIP1. Two RAD51C variants, a synonymous variant and a large deletion from exons 6 to 9, were detected (Fig. S1). Six PALB2 missense variants were detected. A total of 12 BRIP1 variants, including 8 missense, 3 synonymous and 1 mutation at a typical splice donor site, were identified (Fig. S2, Table 2).

To evaluate whether the frequency of these variants was statistically significant, each allele count in our study was compared with cases registered in HGVD. Because HGVD registered the exome sequence data of more than 1200 Japanese individuals without any apparent disease, using the data in HGVD is suitable for estimation of statistical values and their significance in the Japanese population. The results of the statistical assessment indicated that the allele counts of already known RAD51C, PALB2 and BRIP1 missense variants were not significantly different from those registered in HGVD (Table S2). This means that these missense variants contributed less genetically to breast cancer occurrence. Thus, these variants were considered genetically “neutral” (Table 2).

In contrast, some variants, RAD51C deletion from exons 6 to 9 and BRIP1 missense variants c.89A>C, c.736A>G, c.867A>C, c.918+2T>C and c.2131A>G were not registered in HGVD, HGMD or ClinVar. The BRIP1 c.3508C>G variant was recorded as an uncertain significant variant in ClinVar, but not in HGVD and HGMD (Table 2). The large deletion in RAD51C would produce a truncated RAD51C protein, and the
Table 2. Summary of observed mutation on RAD51C, BRIP1 and PALB2 in Japanese breast and ovarian cancer patients

| Gene | Exon | Location | Nucleotide exchange | Protein exchange | SNP ID | Carrier counts | Clinical significance | In silico analysis | Our interpretation |
|------|------|----------|---------------------|------------------|-------|----------------|----------------------|-------------------|-------------------|
|      |      |          |                     |                  |       |                |                      |                   |                   |
| RAD51C | 2   | c.195A>G | Synonymous          |                  | rs44511291 | 5            | B                    | 1.00              | Del, B            |
|       | 6-9 | Deletion from exons 6 to 9 | Deletion |                  |       | 1            |                      |                   | Del, B            |
| BRIP1  | 2   | c.89A>C  | Missense            | p.Asp30Thr       |        | 1            |                      |                   | T, Prob. D, C55   |
|       | 5   | c.430G>A | Missense            | p.Ala144Thr      | rs116952709 | 2          | B                    | 0.27              | T, Prob. D, C55   |
|       | 5   | c.736A>G | Missense            | p.Ile246Val      | rs376893571 | 1          |                      |                   | T, Prob. D, C25   |
|       | 7   | c.867A>C | Synonymous          |                  | rs147749458 | 1          | LB                   |                   | —                 |
|       | 7-8 | c.918+2T>C | Splice site mutation |                |        | 1            |                      |                   | Del, B            |
|       | 15  | c.2131A>G | Missense          | p.Thr711Ala      |        | 1            |                      |                   | T, Prob. D, C55   |
|       | 17  | c.2440C>T | Missense          | p.Arg814Cys      | rs201869624 | 1          | B/U                  | 0.22              | Del, Prob. D, C65 |
|       | 19  | c.2637A>G | Synonymous          |                  | rs4986765 | 100         | B                    | 0.73              | —                 |
|       | 29  | c.2775T>C | Missense          | p.Ser919Pro      | rs4986764 | 96         | B                    | 0.05              | T, B, C0, N       |
|       | 19  | c.2830C>G | Missense          | p.Gln944Glu      | rs140233356 | 3          | U                    | 0.11              | T, B, C25, N      |
|       | 20  | c.3411T>C | Synonymous          |                  | rs4986763 | 95         | B                    | 0.54              | —                 |
|       | 20  | c.3508C>G | Missense          | p.Leu1170Val     | rs587782552 | 1          | U                    |                   | T, B, C25, N      |
| PALB2  | 4   | c.925A>G | Missense            | p.Ile309Val      | rs3809683 | 1          | B/LB                  | 0.58              | T, B, C25, N      |
|       | 4   | c.1379A>G | Missense          | p.Gln460Glu      | rs749434645 | 1          | LB/LB                 | 1.00              | T, B, C35, N      |
|       | 4   | c.1492G>T | Missense          | p.Asp498Tyr      | rs75023630 | 4          | B/LB/U               | 0.08              | T, Poss. D, C65   |
|       | 5   | c.1676A>G | Missense          | p.Gln559Arg      | rs152451  | 41         | B                    | 0.28              | T, B, C35, N      |
|       | 5   | c.2228A>G | Missense          | p.Tyr743Cys      | rs141749524 | 1          | LB/LB                | 0.51              | T, B, C65, N      |
|       | 5   | c.2509G>A | Missense          | p.Glu837Cys      | rs587785874 | 2          | U                    | 0.40              | T, B, C55, N      |

B, benign; Del, Deleterious; DM?, possibly disease causing mutation; DP, Disease associated polymorphism; LB, likely benign; N, neutral; Poss. D, possibly damaging; Prob. D, probably damaging; Prob. Del, probably deleterious; T, Tolerated; U, uncertain significance. In ClinVar column, the abbreviations separated by a splash indicate conflicting interpretation of the clinical significance. Dash means no registered or not calculated.


**BRIP1** c.918+2T>C mutation was located at a typical splice donor site. Therefore, these variants were considered “deleterious” (Table 2).

Because the statistical significance was hardly evaluated for four novel **BRIP1** variants as there was no record in HGVD, the influence of the amino acid substitution corresponding to the missense mutation on **BRIP1** protein function was assessed using the multiple sequence alignment software SIFT (Sorting Intolerant From Tolerant, http://sift.jcvi.org/www/SIFT_e
xst_submit.html), PolyPhen-2 (Polymorphism Phenotyping v2, http://genetics.bwh.harvard.edu/pph2/), and Align-GVGD (Align Grantham Variation Grantham Deviation, http://agvgd.
hc.utah.edu). SIFT predicts amino acid substitution in conserved site as “Deleterious.” PolyPhen-2 predicts “possibly damaging” or “probably damaging” when the amino acid substitution is in a functionally important site. Align-GVGD calculates the chemical features of the substituted amino acid and scores its impact on protein function. In silico analysis using PolyPhen-2 showed that **BRIP1** p.Asp30Thr, p.Ile246Val and p.Thr711Ala mutations, which corresponded to c.89A>C, c.736A>G and c.2131A>G, respectively, were predicted as “probably damaging,” although these variants were calculated as “tolerated” by SIFT. The **BRIP1** p.Leu1170Val, which corresponds to c.3508C>G, was “benign” and “tolerated” by PolyPhen-2 and SIFT, respectively. Align-GVGD predicted that **BRIP1** p.Asp30Thr and p.Thr711Ala mutations were “C25,” which means that they likely affect the protein functions. **BRIP1** p.Ile246Val and p.Leu1170Val mutations were classified as “C25,” indicating little interference with the protein function. The p.Asp30Thr and p.Ile246Val mutations are located on the ATP binding domain and p.Thr711Ala is located in the C-terminal domain of helicase (accession number Q9BX63. UniprotKB, http://www.uniprot.org); thus, the three novel **BRIP1** variants, c.89A>C, c.736A>G and c.2131A>G, probably affect protein function. In contrast, the influence of p.Leu1170Val, which corresponds to c.3508C>G, on **BRIP1** function might be weak because SIFT, Polyphen-2 and Align-GVGD predicted this mutant as “tolerated,” “benign” and “C25,” respectively. These scores suggest that **BRIP1** p.Leu1170Val mutation was not located in a homologically conserved sequence or in a functional domain and, therefore, had little influence on the protein structure.

Taken together, our data indicate that a large deletion from exons 6 to 9 in **RAD51C**, and a **BRIP1** variant on a typical splice donor site, c.918+2T>C, were clearly deleterious. In addition, three **BRIP1** missense variants, c.89A>C, c.736A>G and c.2131A>G, were suspected to be functionally deleterious. However, no deleterious or probably deleterious **PALB2** variants were identified in this study.

**Pedigree of the patients with truncating and novel missense mutations in **RAD51C** and **BRIP1**.** The results of direct sequencing and MLPA identified a large deletion in **RAD51C**, a **BRIP1** variant on a typical splice donor site, and three **BRIP1** missense variants, which were predicted as functionally damaging. We next evaluated the family history of each proband.

The deletion of exons 6 to 9 on **RAD51C** was identified in a proband who presented with simultaneous bilateral breast cancer at 45 years of age (Fig. 1a). Her mother and maternal aunt developed ovarian cancer at 68 and 52 years of age, respectively. Moreover, her maternal grandmother was diagnosed with breast cancer at 57 years of age. Although one of her aunts presented with no cancer when the proband was genetically counseled first, ovarian cancer occurred later, at 75 years of age.

The **BRIP1** splice site variant c.918+2T>C was identified in a female proband. She was affected by multiple breast cancer at 41 years of age. Her younger brother and grandfather were diagnosed with breast cancer at 37 and 70 years of age, respectively. In addition, her father was diagnosed with colon cancer at 52 years of age (Fig. 1b). The **BRIP1** missense variant c.89A>C was detected in a 61-year-old male proband with breast cancer. His sister and brother also developed breast cancer at 62 and 53 years of age, respectively (Fig. 1c). The **BRIP1** missense variant c.736A>G was detected in a 43-year-old female proband with breast cancer. Her father was affected with various cancers, including gastric, prostate and urethral cancer at 63, 74 and 84 years of age, respectively. In addition, the proband had two uncles who had cancer. Of these uncles, one was diagnosed with breast cancer at the age of 40, and the other was diagnosed with lung cancer at 60 years of age. Her maternal grandfather was diagnosed with gastric cancer at 76 years of age (Fig. 1d). The **BRIP1** missense variant c.736A>G was detected in a 48-year-old female proband. She was diagnosed with leiomyoma and breast cancer at 33 and 45 years of age, respectively. Her mother was diagnosed not only with breast cancer at 54 years of age, but also with ovarian and colon cancer at 62 and 69 years of age, respectively. Her two aunts were both diagnosed with breast cancer at 50 and 49 years old, respectively (Fig. 1e). Finally, we confirmed the family history of the proband with the **BRIP1** missense variant c.3508C>G. This variant was predicted as being less pathogenic based on ClinVar and in silico analysis, but this missense variant was novel in Japanese population. Although this variant was observed in a female proband who was diagnosed with breast cancer at 36 years of age, there was no family history of breast or ovarian cancer (Fig. S3). Thus, we concluded that the **BRIP1** c.3508C>G variant was not linked to family history.

Altogether, the family of the proband with a large deletion in **RAD51C** included both patients with breast and ovarian cancer. The families of the probands with the novel **BRIP1** variants, c.918+2T>C, c.89A>C and c.736A>G, included at least 1 male patient with breast cancer. The family of the proband with **BRIP1** c.2131A>G included many patients with breast cancer within the second-degree relatives. These results suggest that three novel **BRIP1** missense variants, c.89A>C, c.736A>G and c.2131A>G, in addition to a large deletion from exons 6 to 9 in **RAD51C** and c.918+2T>C on **BRIP1**, might be closely associated with the susceptibility to familial breast cancer in Japan.

**Discussion**

Clinical characteristics of **RAD51C** mutations have been well investigated by Meindl et al.(19) By means of clinical DNA sequencing and in vitro functional analysis, they revealed that 6 of 1100 German patients with familial breast and ovarian cancers have functionally deleterious mutations on **RAD51C**, including insertion, deletion and splicing mutation. They demonstrated that these mutations are detected in 1.3% of probands with family history of both breast and ovarian cancer, but not in probands with family history of only breast cancer. (19) Similarly, most deleterious mutations were identified in families of patients with both breast and ovarian cancer, (20,22) although a few cases were identified in a family including only patients with breast cancer.(23–25) In addition, Osorio et al. reported that the prevalence of **RAD51C** mutation in a family of patients with ovarian cancer was 1%, while the value in a
Fig. 1. Pedigree of patients with breast cancer and deleterious or probably damaging variants of RAD51C and BRIP1. The GK number is an anonymous identifier for each patient in our laboratory. AMI, acute myocardial infarction; BC, breast cancer; CC, colon cancer; CD, cardiac disease; d., dead at; DC, duodenum cancer; eso. polyp, esophageal polyp; GC, gastric cancer; HC, hepatic cancer; HT, Hashimoto’s disease; LC, lung cancer; LCir, liver cirrhosis; LM, leiomyoma; OC, ovarian cancer; OCyst, ovarian cyst; PC, prostate cancer; RC, rectal cancer.
family including only patients with breast cancer was 0.2%.26

The results of these studies suggest that deleterious mutations in RAD51C contribute to not only to the risk of breast cancer, but also to the risk of ovarian cancer. Likewise, we determined that the large deletion of exons 6 to 9 in RAD51C was detected in a proband with family history of both breast and ovarian cancer (Table 2, Fig. 1a). The prevalence of this mutation was approximately 1%, consistent with another report.19

Although a study using a larger sample size is necessary to evaluate the prevalence of this deleterious mutation in Japanese patients with familial breast cancer, the RAD51C deleterious mutation likely contributes to the risk of breast and ovarian cancer in Japan, as observed in Western countries.

As are CHEK2 and ATM, PALB2 is an important gene for determining breast cancer susceptibility because truncating and deletion mutations significantly increase breast cancer susceptibility. An extensive study in the USA and European countries revealed that the relative risk of breast cancer was estimated to range from 2.3 to 13.4, with 95% confidence interval overlapping.27–34 Moreover, truncating mutations in PALB2 were also detected in Chinese familial breast cancer, although the relative risk has not been estimated.35,36 These studies simultaneously identified many non-synonymous missense variants in PALB2, but there was no evidence that these variants were associated with familial breast cancer.30–34 In our study, no deleterious truncating variant was identified, and the allele counts of the observed missense variants were not statistically different from that in HGVD (Tables 2 and S2). Similar results were reported by Hirotsu37 and Nakagomi et al.38,39

They also reported no obvious deleterious truncating variants in Japanese familial breast cancer cases. Although further study is necessary to estimate the relative risk of PALB2 mutations for familial breast cancer incidence by investigating how many patients with breast cancer present with any truncating and deletion mutations in PALB2, our results suggest that PALB2 deleterious mutation was likely to be significantly rare in Japanese cases compared to that in Western countries.

The relationship between BRIP1 mutation and familial breast cancer susceptibility has been evaluated in other countries, such as the UK and the USA.40,41 Seal et al. detected BRIP1 truncating mutations in 9 of 1212 patients without BRCA1 and BRCA2 mutations. They found that the prevalence of BRIP1 truncating mutations was approximately 0.7% in patients with familial breast cancer, and the relative risk of developing breast cancer for the BRIP1 truncating mutant was 2.0.40

In contrast, a statistically significant difference in the carrier frequency of missense mutation between their cases and controls was not detected.40 Other studies also reported that carrier frequency of possibly and most likely deleterious missense variants ranged from 0.6 to 3.0% in patients with familial breast cancer depending on the sample size.42–44

Similar to the report from Seal et al., no statistically significant difference was detected when compared with that in their control case.42–44 Furthermore, Easton et al. recently evaluated whether BRIP1 truncating and functionally deleterious missense variants increased the risk of breast cancer in patients of European origin by comparing more than 48 000 breast cancer cases and 43 000 healthy controls.41 Their results showed that the carrier frequencies of the BRIP1 truncating variant, p.Arg798Ter, were 0.05 and 0.04% in cases and healthy controls, respectively. In addition, the frequency of BRIP1 deleterious missense variants ranged from 0.09 to 1.4% for every variant. Because no statistical difference was detected in these frequencies, they concluded that both BRIP1 truncating and missense variants did not significantly increase breast cancer risk in a European population.41 Other groups also showed no association between large deletion in BRIP1 and familial breast cancer.45,46 Based on these reports, BRIP1 mutations marginally affect familial breast cancer incidence in Western countries. However, we identified four novel BRIP1 variants, including a splice site mutation, c.918+2T>C, and three functionally affected mutations, c.89A>C, c.736A>G and c.2131A>G. The carrier frequencies were approximately 1% and 3% for a splice site and functionally affected mutations, respectively, and, therefore, the values were similar to those in other studies.40–44 However, these variants were not identified by Easten et al.41 even though the sample size of their study was significantly larger than in ours and any other study. It is noteworthy that the proband with BRIP1 c.89A>C was a male patient with breast cancer, and the families of the probands with BRIP1 c.736A>G and c.918+2T>C included male patients with breast cancer within 2nd-degree relatives. The mother and 2 maternal aunts of the proband with BRIP1 c.2131A>G had breast cancer. Therefore, the results of prediction analyses and family history suggested that these four novel BRIP1 variants were potentially deleterious. These results suggested that BRIP1 c.89A>C, c.736A>G and c.2131A>G might be pathogenic mutations (Table 2).

Interestingly, only one novel deleterious variant of BRIP1, which have not been reported in studies, including patients from Western countries, were also identified in Korean and Chinese populations.47,48 Therefore, BRIP1 mutation status in not only Japanese, but also Asian familial breast cancer cases, might be different from that in Western countries. Similar to BRIP1 mutation, we found that carrier frequency of PALB2 deleterious mutation also differed from that in Western populations. While difficult to explain, this difference might be based on the uniqueness of the Japanese genome. In fact, Nagasaki et al.49 found that the number of rare variants with minor allele frequency less than 0.1% in the Japanese genome was larger than that in other populations registered in the 1000 Genome Project. Their results suggest that the BRIP1 and PALB2 mutation status observed in our study reflect the differences in genomic structure between Japanese and Western populations.

Our study presents some experimental limitations. To elucidate whether these potentially deleterious mutations in BRIP1 were closely associated with familial breast cancer incidence and were inherited in family of the probands, functional in vitro analysis and segregation analysis should be performed. However, these analyses could not be performed in our study because informed consent for further analysis could not be obtained from the probands and their family members. As the clinical significance of these novel variants was still unknown, the need for additional studies was difficult to explain to the patient and further study was not approved by the committee of the ethical guidelines for human genome/gene analysis research at the Cancer Institute Hospital, Japanese Foundation of Cancer Research. Moreover, the sample size in our study was too small to accurately evaluate the allele frequency and the relative risk of the variants, which are registered in HGMD. Given these notions and the uniqueness of the Japanese genome, integration of genotyping data obtained from multiple institutions and validation of functional affected variants by in vitro functional assay such as measuring the chemosensitivity and the binding capacity of each variant are required to effectively explain the association between these novel variants and familial breast cancer occurrence.
In conclusion, we identified a large deletion from exons 6 to 9 in RAD51C, 4 novel BRIP1 missense variants, including three novel non-synonymous BRIP1 missense variants and one novel BRIP1 variant at a typical splice donor site in 100 Japanese patients with hereditary breast and/or ovarian cancer. No deleterious PALB2 mutation was detected in the present study. The large deletion from exons 6 to 9 in RAD51C and the BRIP1 splice site variant, c.918+2T>C, are strongly suspected to be pathogenic, and the three novel BRIP1 missense variants, c.89A>C, c.736A>G and c.2131A>G, probably affect its helicase function. We showed that RAD51C mutation status in Japanese familial breast cancer cases was similar to that in other countries, and the prevalence of PALB2 deleterious mutation in Japan might be lower than that in other countries. Furthermore, our results suggest that BRIP1 mutation status in Japanese familial breast cancer cases might be different from that in Western countries.

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Disclosure Statement
The authors have no conflict of interest to declare.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Doc. S1. Polymerase chain reaction-direct sequencing.

Doc. S2. Multiplex ligation-dependent probe amplification.

Table S1. Primers for PCR and DNA sequence.

Table S2. Comparison between minor allele counts in our results and that registered in Human Genome Variation Database.

Fig. S1. Large deletion from exons 6 to 9 in RAD51C. The copy number of RAD51C exons 6, 7, 8 and 9 was half of that of the other exons.

Fig. S2. DNA chromatograph of BRIP1 splice donor site. The sequence with c.918+2T>C (GK495) was compared with the reference sequence (NG_007409.2). The arrowhead indicates the mutated position. (a) and (b) show the wide and magnified view of the mutation containing sequence, respectively.

Fig. S3. Pedigree of the patients with breast cancer presenting with BRIP1 c.3508C>G. The GK number is an anonymous identifier for each patient in our laboratory. AdT, adrenal tumor; ALD, aldosteronism, CC, colon cancer; d., dead at; HT, Hashimoto’s disease; SC, skin cancer.