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

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Key words

BRIP1, hereditary breast cancer, Japanese, *PALB2*, *RAD51C*

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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 nonsynonymous 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 who 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-telangiectagia 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] RPA[10] 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-irradiation[13] 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.

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Materials and Methods

Patients. From April 2000 to September 2016, 740 patients received genetic counseling. Of these patients, 440 probands affected with breast and 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).

Table 1. Subject information

Cancer type	Number of sample	Age median (minimum–maximum)
Breast	94	49.0 (28–82)
Breast and ovarian	6	53.5 (34–71)
Total	100	49.5 (28–82)

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 amplification, 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),[17,18] the variants were regarded as "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

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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
							ClinVar	HGMD	*P*-value	SIFT	Polyphen-2	Align-GVGD	
RAD51C	2	c.195A>G	Synonymous	—	rs44511291	5	B	—	1.00	—	—	—	B
	6–9	Deletion from exons 6 to 9	Deletion	—	—	1	—	—	—	—	—	—	Del
BRIP1	2	c.89A>C	Missense	p.Asp30Thr	—	1	—	—	—	T	Prob. D	C55	Prob. Del
	5	c.430G>A	Missense	p.Ala144Thr	rs116952709	2	B	—	0.27	T	Prob. D	C55	N
	5	c.736A>G	Missense	p.Ile246Val	rs376893571	1	—	—	—	T	Prob. D	C25	Prob. Del
	7	c.867A>C	Synonymous	—	rs147749458	1	LB	—	—	—	—	—	N
	7–8	c.918+2T>C	Splice site mutation	—	—	1	—	—	—	—	—	—	Del
	15	c.2131A>G	Missense	p.Thr711Ala	—	1	—	—	—	T	Prob. D	C55	Prob. Del
	17	c.2440C>T	Missense	p.Arg814Cys	rs201869624	1	B/U	DM?	0.22	Del	Prob. D	C65	N
	19	c.2637A>G	Synonymous	—	rs4986765	100	B	—	0.73		—	—	N
	19	c.2755T>C	Missense	p.Ser919Pro	rs4986764	96	B	DP	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	—	—	—	N
	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	rs749494645	1	LB/U	—	1.00	T	B	C35	N
	4	c.1492G>T	Missense	p.Asp498Tyr	rs75023630	4	B/LB/U	DM?	0.08	T	Poss. D	C65	N
	4	c.1676A>G	Missense	p.Gln559Arg	rs152451	41	B	DP	0.28	T	B	C35	N
	5	c.2228A>G	Missense	p.Tyr743Cys	rs141749524	1	LB/U	—	0.51	T	B	C65	N
	5	c.2509G>A	Missense	p.Glu837Lys	rs587778587	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.

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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_enst_submit.html), PolyPhen-2 (Polymorphism Phenotyping v2, http://genetics.bwh.harvard.edu/pph2/) and Align-GVGD (Align Grantham Variation Grantham Deviation, http://agvgd.hci.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 correspond 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 "C55," 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 *PALB*2 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.2131A>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

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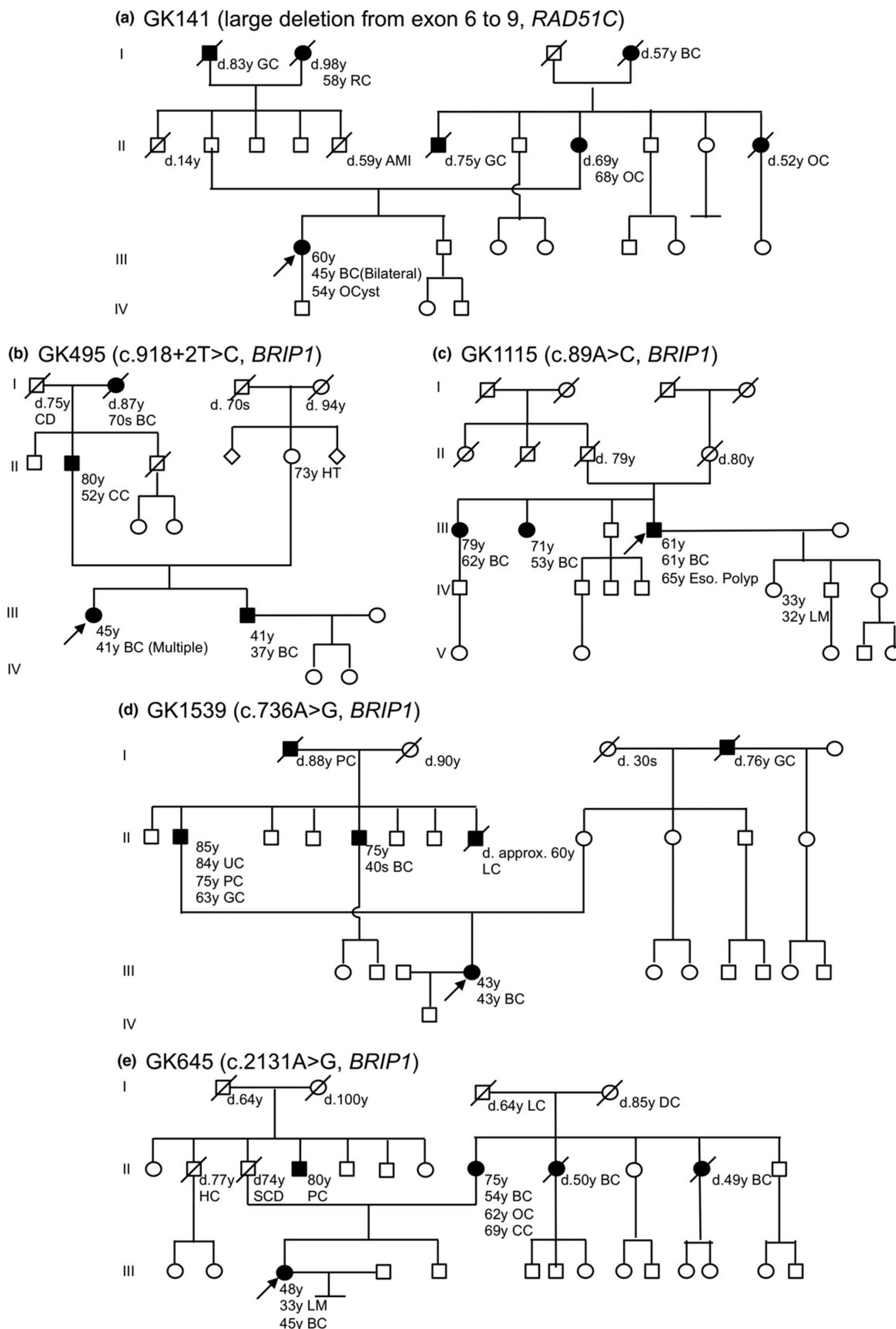

Fig. 1. Pedigree of patients with breast cancer and deleterious or probably damaging variants of *RAD51C* and *BRIP1*. The GK number is an anonymous identifier for each patient in our laboratory. AMI, acute myocardial infraction; BC, breast cancer; CC, colon cancer; CD, cardiac disease; d., dead at; DC, duodenum cancer; eso. polyp, esophageal polyp; GC, gastric cancer; HC, hepatic cancer; HT, Hashimoto's disease; LC, lung cancer; LCir, liver cirrhosis; LM, leliomyoma; OC, ovarian cancer; OCyst, ovarian cyst; PC, prostate cancer; RC, rectal cancer.

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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 breast cancer predisposition.[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 are reported by Hirotsu[37] and Nakagomi *et al.*[38,39] They also detected 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.[9,42–44] 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, other novel and functionally deleterious variants 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 statuses 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[19] are required to effectively explain the association between these novel variants and familial breast cancer occurrence.

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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.

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