PALB2 mutation in a woman with bilateral breast cancer: A case report

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Abstract. Partner and localizer of breast cancer 2 (*PALB2*) was identified as a moderate-risk gene of breast and pancreas cancer. The present authors previously reported that no *PALB2* germline mutations with a deleterious frameshift or stop codons were identified in 155 Japanese patients with breast and/or ovarian cancer who were estimated to be at risk of hereditary cancer, according to the National Comprehensive Cancer Network (NCCN) criteria. In the present study, one patient with a deleterious mutation of *PALB2* (c. 2834+2 T>C) has been identified from a study of an additional 128 cases. Therefore, the prevalence of *PALB2* among Japanese patients is now estimated to be 0.35% (1/283). The proband was a 63-year-old woman with bilateral breast cancer, although she had experienced no other cancers. The proband had two elder sisters, the eldest of whom died from pancreatic cancer at 60 years of age. The proband's 40-year-old daughter was affected, but did not show any malignancies. There are only a few reports concerning *PALB2* mutations in Japan. To the best of our knowledge, this is the first case study to reveal the significance of DNA-repair genes in the development of malignancies in Japanese patients with breast cancer.

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Introduction

The significance of the breast cancer 1 (*BRCA1*) and *BRCA2* mutations in familial breast and ovarian cancer has been well established (1,2). However, the mutations of these genes are estimated to cause, at most, 20-30% of cases of hereditary breast cancer (3). The present authors studied the *BRCA1/2* mutations in 191 patients in a previous study, but the prevalence was shown to be unexpectedly low (4,5). In fact, it was only 7% among the analyzed patients who had a family history of breast cancers.

Partner and localizer of BRCA2 (*PALB2*) was identified as a moderate-risk gene in breast and pancreas cancer (6). *PALB2* is located on chromosome 16p12.2 containing 13 exons and 12 introns, and is involved in *BRCA2*-associated pathways (6). Recently, Antoniou *et al* (7) reported that *PALB2* carriers have a high risk of developing breast cancer, and concluded that the cumulative risk of mutation carrier was 34% by the age of 70 in their prospective follow-up study on 154 families.

The prevalence of the *PALB2* mutation was reported to be 1.2-3.4% in European countries, whereas it is very rare in Asian countries (8-18). To the best of our knowledge, no study has been performed that has identified the *PALB2* deleterious mutation in Japanese patients with breast cancer. From our first cohort data, no deleterious *PALB2* mutations were identified in 155 patients with breast and/or ovarian cancer who were estimated to be at risk of hereditary cancer according to the National Comprehensive Cancer Network (NCCN) criteria (19). In the present case study, an additional 128 cases having breast and/or ovarian cancer were studied, and the case of a patient with bilateral breast cancer is presented who harbors the deleterious mutation in *PALB2*. Factoring in the first cohort of 155 cases, the frequency of the *PALB2* mutation is now estimated at 0.35 % (1/283) in the Japanese population.

Case report

A 63-year-old female was referred to our hospital (Department of Breast Surgery, Yamanashi Prefectural Central Hospital, Kofu, Japan) due to the presence of a lump in her left breast and

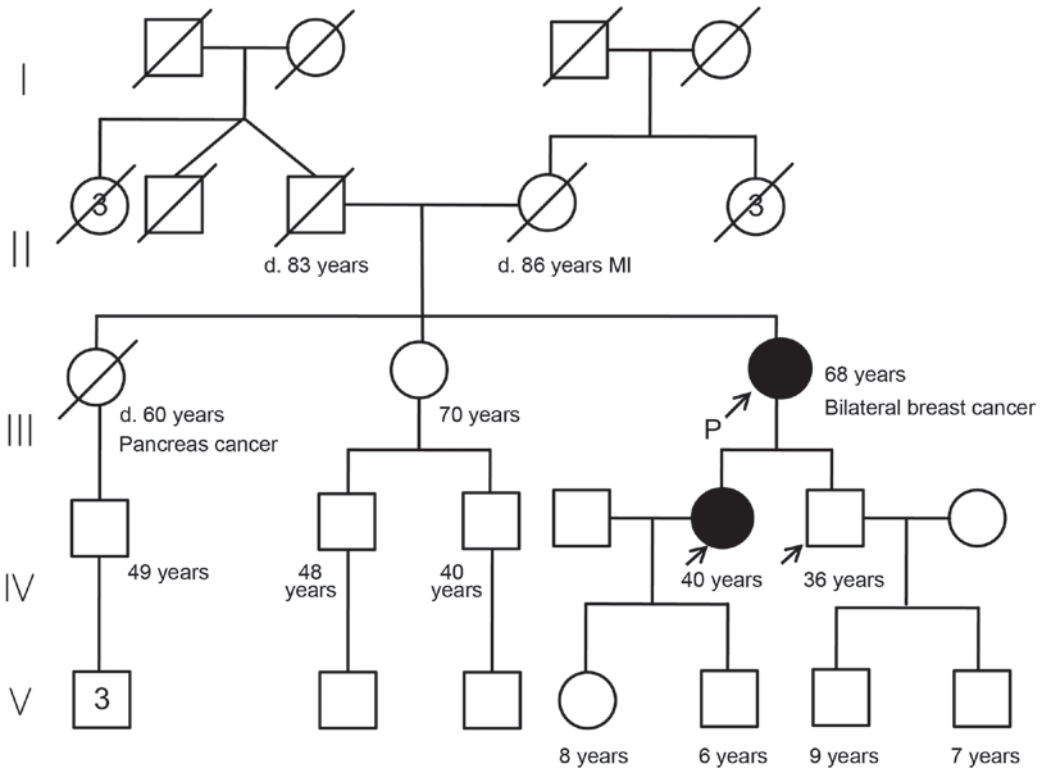

Figure 1. Chart showing the family pedigree of the case patient, showing that she had two elder sisters, one of whom (the eldest) died from pancreatic cancer at 60 years of age, while the other remained healthy at 70 years of age. The patient's parents died from causes unrelated to cancer. To the best of the patient's knowledge, no other family members (7 uncles or aunts, and 3 nephews and their descendants) have experienced cancer. The patient's 40-year-old daughter and 36-year-old son underwent genetic testing for the *PALB2* mutation, revealing the daughter is affected, whereas the son is not. The black circle indicates the affected individual, and the arrows indicate tested individuals. MI, myocardial infarction.

bloody discharge from the right-side nipple. The patient had no personal history of other cancers or diseases. Her family history is shown in the pedigree chart (Fig. 1). The patient had two gravidas and two parities.

The cytology of nipple discharge was performed by the clinic, revealing the presence of malignant cells. Mammography indicated segmental pleomorphic calcification in the right breast, and a spiculated polygonal tumor measuring 2 cm in diameter with pleomorphic calcification in the left breast. Furthermore, an irregularly shaped axillar lymph node was observed on the left side (Fig. 2).

Fine-needle aspiration cytology for the left-sided breast tumor also revealed the presence of malignant cells. The patient was diagnosed with bilateral breast cancer, and underwent a right-sided mastectomy and breast reconstruction, and left-sided breast-conserving therapy. Pathological findings revealed that the right-sided breast cancer was ductal carcinoma *in situ* (DCIS), with no lymph node metastasis, grade 2, estrogen receptor (ER) (7+) and progesterone receptor (PR) (3+) according to the Allred Score (20), and human epidermal growth factor 2 (HER2) (1+) according to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (*CAP*) criteria (21). The left-sided breast cancer was invasive ductal carcinoma (non-specific type) with lymph node metastases (2/12), grade 2, ER (8+), PR (6+), and HER2 (1+). Epirubicin-cyclophosphamide (EC) adjuvant chemotherapy (epirubicin, 90 mg/m^2, and cyclophosphamide, 600 mg/m^2, 3 times a week for 4 cycles, followed by docetaxel, 75 mg/m^2, 3 times a week for 4 cycles) was administered, and subsequently, radiation therapy (50 Gray) for the left-side breast was performed. The patient received oral hormone therapy with toremifen (40 mg/day) for 5 years.

The benefits and disadvantages of knowing the results of genetic testing were explained to the patient. Added to the explanation was the possibility that there could be uncertain results that would need to be clarified in future investigations. The patient and her family (40-year-old daughter and 36-year-old son) were referred to genetic counseling (S.N. and T.K.). Written informed consent was obtained from the patient and from her daughter and son.

Germline mutations for *BRCA1/2* and *PALB2* were analyzed using targeted sequencing, as previously reported (4,19,22). Briefly, the Ion AmpliSeq™ BRCA1 and BRCA2 and the Ion AmpliSeq™ BRCA Reflex Hereditary Cancer Research panels (Thermo Fisher Scientific, Inc., Waltham, MA, USA) were used, targeting the whole exons of the *BRCA1/2* genes and an additional 25 hereditary cancer-associated genes (22,23). Buffy coat DNA was used as a template, and the sequencing library was generated using an AmpliSeq Library kit 2.0 (Thermo Fisher Scientific, Inc.) (24-31). Next-generation sequencing analysis was subsequently performed on an Ion PGM or Ion Proton platform (Thermo Fisher Scientific, Inc.) (24-31).

A deleterious mutation of *PALB2* (chr16: 23635328, c. 2834+2 T>C) was identified (Fig. 3A), which is the first case in 283 analyzed patients in our hospital during the period between 2013 and 2016, i.e., 0.35% or 1/283 of Japanese patients were revealed to have the *PALB2* deleterious mutation. Furthermore

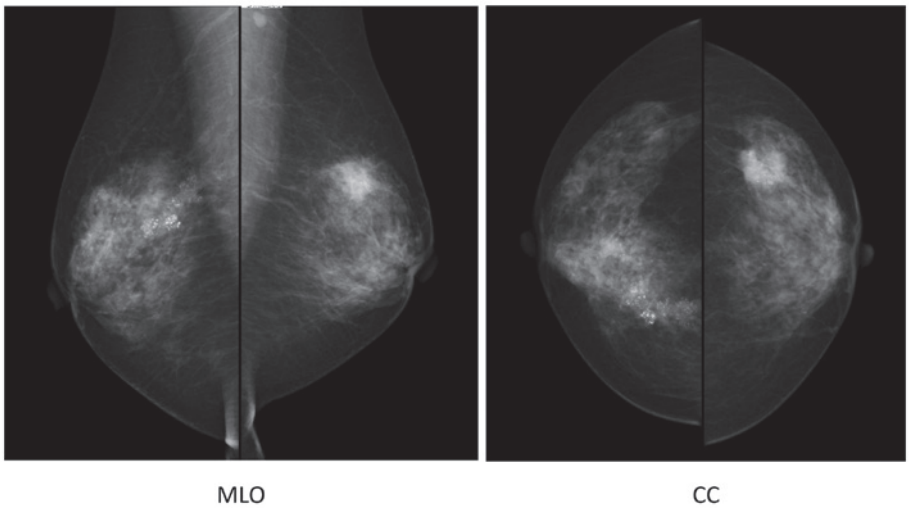

Figure 2. Mammography, showing segmental pleomorphic calcification in the right breast and a spiculated polygonal tumor measuring 2 cm in diameter with pleomorphic calcification in the left breast. Furthermore, an irregularly shaped axillar lymph node was observed on the left side. MLO, mediolateral-oblique (view); CC, cranial-cadual (view).

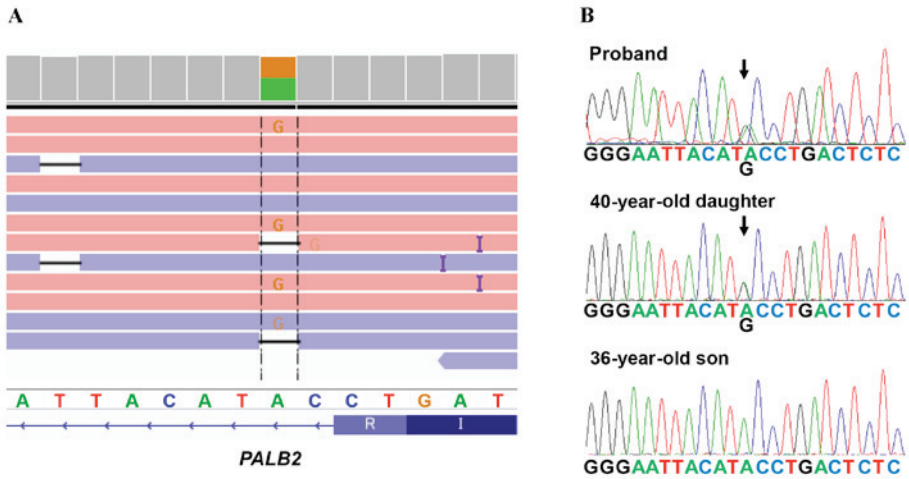

Figure 3. Genetic analysis of *PALB2* mutations. (A) Next-generation sequencing analysis for *PALB2* mutations. The patient had a deleterious mutation of *PALB2* (c. 2834+2 T>C). (B) Sanger sequencing revealed that the proband's 40-year-old daughter harbored the *PALB2* mutation, whereas the 36-year-old son did not.

the splice-site mutation in *PALB2* was not identified in the Exome Aggregation Consortium (ExAC), the Human Genetic Variation Database (HGVD), the Integrative Japanese Genome Variation (iJGVD) or the Catalogue Of Somatic Mutations In Cancer (COSMIC) databases. To the best of our knowledge, this variant has therefore not been reported previously, suggesting that our identified variant is novel one.

The pedigree chart of the patient is shown in Fig. 1. The patient had two elder sisters, the eldest of whom succumbed to pancreatic cancer at 60 years of age, whereas the other sister is alive and well at 70 years of age. The patients' parents died from causes unrelated to cancer. To the best of the patient's knowledge, no other family members (7 uncles or aunts, and 3 nephews and their descendants) have experienced cancer. The patient's 40-year-old daughter and 36-year-old son underwent gene informed consent to have genetic testing for the *PALB2* mutation. It was revealed that the daughter was affected, whereas the son was not (Fig. 3B). The mutation was also confirmed using Sanger sequencing (Fig. 3B). The 40-year-old daughter is now receiving regular check-ups for malignancies, including those of the breast and pancreas.

Discussion

PALB2 serves a crucial role in the localization and stabilization of *BRCA2* in nuclear chromatin, which is essential for *BRCA2* to function in double-strand-break DNA repair by homologous recombination. *PALB2* mono-allelic mutations result in cancer development, and bi-allelic mutations lead to a type of Fanconi anemia (6).

Recently, Antoniou *et al* (7) reported that *PALB2* carriers have a high risk of developing breast cancer, and determined that the cumulative risk of mutation carrier was 34% by the age of 70 in their prospective follow-up study on 154 families. In the USA, Canada, and Europe, the frequency of *PALB2* deleterious mutations was revealed to vary from 1.1

to 3.4% (8-15). A total of 4 previous studies have arisen from Asia. One study by Cao *et al* (16) from China revealed 3 cases out of 360 (0.8%) with the deleterious mutations, although there were none from Korea (300 cases) or from Malaysia (122 cases) (17,18). The previous study by the present authors on Japanese patients (n=155) revealed that none of them had the deleterious mutation (19).

The *PALB2* mutation has been reported to be associated with the development of pancreatic cancer. The prevalence of the *PALB2* mutation among familial pancreatic cancer was reported to be ~3-4% in the USA and European countries (32,33). In Japan, Takai *et al* (34) recently reported that two deleterious *PALB2* mutations were detected in 54 familial pancreas cancer families, as well as three *BRCA2* and two *ATM* deleterious mutations. However, the association between *PALB2* mutations and the risk of pancreatic cancer has yet to be fully elucidated among the Japanese population.

In the present case study, a 60-year-old elder sister was known to have had pancreatic cancer. However, it was impossible to examine the *PALB2* germline mutations, since a DNA sample was not available from the sister. To reveal whether the identified *PALB2* splice-site mutation has affected tumor development, it will be better to perform segregation analysis in this family. As a minimum at the present time, the proband's daughter, who has the *PALB2* mutation, should continue to have regular check-ups assessing the risk of developing pancreatic cancer, as well as breast cancer.

Compared with the USA and European countries, analysis of *BRCA1/2* for the detection of hereditary breast and/or ovarian cancer has not been widely accepted in Japan. Reports originating from Japan remain few in number (5,35,36). Further investigations are required to reveal the genetic features of Japanese patients with breast and/or other cancers (ovary, pancreas, prostate, and so forth).

It is important to understand the association between carcinogenesis and the dysfunction of DNA-repair genes in Japanese patients due to the up-and-coming therapeutic strategies that employ poly(ADP-ribose) polymerase (PARP) inhibitors, such as Orapalib (37,38). Recently, multi-gene assays for hereditary cancer have been developed (23,39), and other genes associated with double-strand DNA repair, such as *PALB2*, *ATM*, *BARD1*, and *RAD51*, will be analyzed for patients with hereditary cancer. These analyses are expected to reveal the association between DNA-repair genes and carcinogenesis with various types of cancer.

In conclusion, to the best of our knowledge, this is the first identified case of *PALB2* mutations in a Japanese patient with breast cancer. The present study therefore suggests that the *PALB2* mutation is associated with the development of breast and pancreas cancer, even in Japanese patients. At present, the frequency of the germline mutation in *PALB2* is 0.35% (1/283 cases).

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