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ORIGINAL RESEARCH

PALB2 germline pathogenic variants: frequency, clinical features, and functional analysis of c.3350+5G>A variant in 3987 Korean cancer patients

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Background: Germline *PALB2* variants increase the risks of various cancers. However, these have not been comprehensively investigated in Korean patients with cancer. Our study aimed to evaluate the prevalence and clinical characteristics of *PALB2* germline variants in Korean patients with cancer and compare these findings with existing data.

Patients and methods: We analyzed the clinicopathological and germline next-generation sequencing data of 3987 patients with cancer from the National Cancer Center in Korea. Additionally, we carried out functional analysis of the *PALB2* splicing variant, c.3350+5G>A.

Results: A total of 104 patients presented *PALB2* germline variants with eight pathogenic variants (PVs), 14 likely pathogenic variants (LPVs), and 82 variants of uncertain significance (VUS). *PALB2* PV/LPVs were detected at an overall frequency of 0.6% (22/3987) across all patients. Among patients with PV/LPVs, 95.5% were women, and 19 and 3 carriers were diagnosed with breast and ovarian cancer, respectively. Further, we reclassified c.3350+5G>A as a PV rather than VUS, according to the American College of Medical Genetics and Genomics guidelines. Patients with *PALB2* PV/LPVs had a younger age at first cancer diagnosis (44.6 ± 10.1 years versus 50.2 ± 12.0 years, $P = 0.019$) and were more likely to have multiple primary organ cancer diagnoses (22.7% versus 8.3%, $P = 0.032$) compared with those without these variants.

Conclusion: Age at first cancer diagnosis and the presence of multiple primary organ cancers are key risk factors for suspected germline *PALB2* PV. Hence, strategies are required to improve adherence to the National Comprehensive Cancer Network guidelines for cancer screening and family genetic testing among Korean patients with cancer.

Key words: genetic counseling, breast cancer, ovarian cancer, *PALB2*

INTRODUCTION

Genetic susceptibilities significantly influence the emergence of various types of cancer.[1] Since their identification in the 1990s, *BRCA1* and *BRCA2* have become well established as significant predisposition factors for breast, ovarian, prostate, and pancreatic cancer.[2-4] Moreover, the advent of multigene panel testing has led to the discovery of additional cancer susceptibility genes, including *PALB2* (partner and localizer of BRCA2), which play a significant role in hereditary cancer risk.[5]

PALB2 (NM_024675.4) acts as a tumor suppressor gene by playing a vital role in DNA double-strand breaks (DSB) repair via the homologous recombination (HR) pathway.[6] Initially identified as a BRCA2-interacting protein, subsequent research revealed its interaction with BRCA1 as well.[7,8] Homologous recombination and non-homologous end joining are cellular mechanisms for repairing DNA DSB. Unlike non-homologous end joining, which joins broken ends directly, homologous recombination repairs DSB more accurately using a homologous DNA sequence as a template, utilizing the undamaged sister chromatid.[9,10] During homologous recombination, the PALB2 protein interacts with BRCA1 and BRCA2 through its N-terminal coiled-coil and C-terminal WD40 domains, respectively,

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forming a BRCA complex.[8,11] This complex is essential for initiating homologous recombination in response to DNA damage, specifically facilitating the recruitment of RAD51, a key component in the repair process, to the site of damage.[12]

Carriers of *PALB2* germline pathogenic variants (PVs) have an increased risk of developing various cancers, including breast, ovarian, and pancreatic cancers.[13] Particularly, *PALB2* is a high-penetrance breast cancer gene in the Caucasian population, similar to *BRCA1* and *BRCA2*.[14] The risk of developing breast cancer in individuals with *PALB2* germline PVs is ∼41%-60%, which is the highest following that of *BRCA1/2* and equivalent to that of *CDH1*, according to the National Comprehensive Cancer Network (NCCN) guidelines.[15]

Understanding the prevalence of *PALB2* germline variants across different populations is important, as genetic variations can exhibit distinct patterns among different ethnic groups, emphasizing the need for detailed analyses to inform risk assessment and management strategies.[16] Although some studies have reported *PALB2* germline PVs in the Asian population, to our knowledge, large-scale comprehensive investigation of *PALB2* germline variants in Korean patients with cancer have not yet been conducted.[17-19] Therefore, our study aimed to evaluate the prevalence of *PALB2* and the clinical characteristics of *PALB2* germline PVs in 3987 Korean patients with cancer. Moreover, we carried out the functional analysis of the *PALB2* splicing variant (c.3350+5G>A), which has been reclassified as pathogenic or likely pathogenic from uncertain significance by the ClinVar database in March 2023.

PATIENTS AND METHODS

Study population

We retrospectively reviewed data from 3987 patients who visited the National Cancer Center in Korea between April 2012 and April 2023. All included patients were suspected to have a hereditary cancer and underwent germline next-generation sequencing panel testing to identify cancer predisposition genes, including *PALB2*. Relaxed criteria were used to identify individuals at a high risk for hereditary cancer if they had multiple family histories of cancer or multiple primary organ cancers. Particularly for patients with breast and ovarian cancer, participants were selected based on the *BRCA1/2* variant testing insurance coverage criteria. In Korea, national insurance coverage has expanded since July 2020, before which the criteria were as follows: (i) breast cancer diagnosed <40 years old (patients aged 40 were also included by physician's decision); (ii) patients with breast cancer having a family history of breast or ovarian cancer among second-degree relatives; (iii) personal history of breast or ovarian cancer; (iv) triple-negative breast cancer; (v) male breast cancer, or (vi) epithelial ovarian cancer. After July 2020, the expanded criteria were as follows: (i) breast cancer diagnosed ≤40 years old; (ii) patients with breast cancer having a family history of breast, ovarian, male breast, metastatic prostate, or pancreatic cancer among third-degree relatives; (iii) personal history of breast cancer with ovarian or pancreatic cancer; (iv) triple-negative breast cancer diagnosed ≤60 years old; (v) male breast cancer, or (vi) epithelial ovarian cancer. Additionally, although there was no clinical evidence to suspect hereditary cancer, patients who voluntarily requested genetic testing were also included. Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2024.104132, illustrates the detailed frequency of patients according to each criterion before and after the change of criteria.

Detailed patient characteristics are presented in Table 1 (total patients, $n = 3987$), Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2024.104132 (patients with breast cancer, $n = 2186$), and Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2024.104132 (patients with ovarian, fallopian tube, and peritoneal cancer, $n = 1457$). Demographic data, including tumor location, tumor—node—metastasis staging, molecular subtype, and family history of cancer, were collected from the patients. In particular, for patients harboring the *PALB2* PVs, the status of family genetic testing and cancer screening history were also investigated.

Table 1. Clinicopathological characteristics of patients with and without germline *PALB2* pathogenic/likely pathogenic variants

Variable	Total ($n = 3987$) *n* (%)	PV/LPV ($n = 22$) *n* (%)	VUS/ND ($n = 3965$) *n* (%)	*P* value
Age at first cancer diagnosis				
Mean ± SD	49.4 ± 12.0	44.5 ± 10.1	50.2 ± 12.0	0.019[a]
<40	848 (21.3)	8 (36.4)	1145 (28.9)	
40-49	1150 (28.9)	5 (22.7)	1048 (26.4)	
50-59	1055 (26.5)	7 (31.8)	932 (23.5)	
≥60	934 (23.4)	2 (9.1)	840 (21.2)	
Sex				
Male	173 (4.3)	1 (4.5)	172 (4.3)	1.000[b]
Female	3814 (95.7)	21 (95.5)	3793 (95.7)	
Personal history of cancer				
Breast cancer	2186 (46.6)	19 (70.4)	2167 (46.5)	0.006[c]
Ovarian, fallopian tube, peritoneal cancer	1457 (31.1)	4 (18.2)	1453 (36.6)	0.006[c]
Pancreatic cancer	131 (2.8)	0 (0.0)	131 (3.3)	1.000[b]
Endometrial cancer	122 (2.6)	0 (0.0)	122 (3.1)	1.000[b]
Thyroid cancer	110 (2.3)	1 (3.7)	109 (2.7)	0.460[b]
Colorectal cancer	92 (2.0)	1 (3.7)	91 (2.3)	0.403[b]
Prostate cancer	67 (1.4)	0 (0.0)	67 (1.7)	1.000[b]
Lung cancer	38 (0.8)	1 (3.7)	37 (0.9)	0.190[b]
Gastric cancer	35 (0.7)	0 (0.0)	35 (0.9)	1.000[b]
Others	449 (9.6)	1 (3.7)	448 (11.3)	0.503[b]
Family history of cancer				
Yes	2691 (67.5)	18 (81.8)	2673 (67.4)	0.226[c]
No	1296 (32.5)	4 (18.2)	1292 (32.6)	
Multiple primary organ cancers				
Yes	334 (8.4)	5 (22.7)	329 (8.3)	0.032[b]
No	3653 (91.6)	17 (77.3)	3636 (91.7)	

LPV, likely pathogenic variant; ND, not detected; PV, pathogenic variant; SD, standard deviation; VUS, variants of uncertain significance.
[a]Student's *t*-test.
[b]Fisher's exact test.
[c]Pearson's chi-squared test.

This study was approved by the Institutional Review Board (IRB) of the National Cancer Center of Korea (IRB No. NCC2021-0263, NCC2023-0136). The requirement for obtaining informed consent was waived owing to the retrospective nature of the study.

Multigene panel testing and classification

Genomic DNA was extracted from the patient's peripheral blood. We used customized panels from Celemics (Celemics, Seoul, South Korea), which included all coding sequences and intron−exon boundaries of the coding exons from >23 cancer predisposition genes (Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2024.104132). Library preparation followed the manufacturer's guidelines. Sequencing was carried out on Illumina platforms (Illumina, San Diego, CA): the MiSeqDX for some samples and the NextSeq for others, both generating 2 × 150 bp paired-end reads. DNA sequence reads were aligned to the reference human genome (UCSC GRCh37/hg19) using BWA-MEM (ver. 0.7.10).[20] Duplicate reads were marked with biobambam2 or Picard (ver. 1.138). Local alignment, base quality recalibration, and variant calling were carried out using a pipeline based on GATK Best Practices,[21] involving GATK (ver. 3.5),[22] samtools (ver. 0.1.19),[23] FreeBayes (v0.9.21-26-gbfd9832),[24] and Scalpel (ver. 0.5.3). Variant annotation was conducted using Variant Effect Predictor (VEP)[25] and dbNSFP.[26] Confirmatory Sanger sequencing was carried out to validate the PVs and multiplex ligation-dependent probe amplification to determine copy number variants.

Location and type of *PALB2* PVs were schematically presented as lollipops using the mutation mapper tool in cBioPortal. Based on the guidelines of the American College of Medical Genetics and Genomics, the detected variants were classified using a five-tier system as follows: pathogenic (PV), likely pathogenic (LPV), variants of uncertain significance (VUS), likely benign, and benign.[27]

Functional validation of PALB2 splicing variant

Total RNA was extracted from peripheral blood lymphocytes using the AllPrep DNA/RNA Mini Kit (Qiagen, #80204; Hilden, Germany) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized using the Transcriptor First Strand cDNA Synthesis Kit (Roche Life Science, #04896866001; Basel, Switzerland). RT−PCR was used to amplify the cDNA products, which were then separated using agarose gel electrophoresis, and cDNA fragments of different sizes were visualized. The transcriptional products were subsequently validated through Sanger sequencing to ensure accurate identification of the intronic variant in *PALB2*.

For RNA sequencing (RNA-seq), total RNA was isolated using the QIAGEN RNeasy Mini Kit following the manufacturer's instructions. A library was independently prepared with 0.5 μg of total RNA for each sample using the Illumina TruSeq Stranded Total RNA Library Prep Globin Kit (Illumina, #20020613; San Diego, CA). The cleaved RNA fragments are copied into first-strand cDNA using SuperScript II reverse transcriptase (Invitrogen, #18064014; Waltham, MA) and random primers. The cDNA libraries were quantified using KAPA Library Quantification kits for Illumina Sequencing platforms according to the quantitative PCR Quantification Protocol Guide (KAPA Biosystems, #KK4854; Wilmington, MA) and qualified using the TapeStation D1000 ScreenTape (Agilent Technologies, #5067-5582; Santa Clara, CA). Indexed libraries were then submitted to an Illumina NovaSeq X plus (Illumina, San Diego, CA), and paired-end (2 × 100 bp) sequencing was carried out.

The quality of raw reads in FASTQ format obtained from sequencing were evaluated using FastQC.[28] Reads were trimmed for adapters and low-quality bases using Trimmomatic.[29] Alignment of reads was carried out based on the reference genome (GRCh37) using HISAT2.[30] The resulting BAM files were sorted and indexed with SAMtools.[31] Finally, transcript assembly and estimation of the transcript abundance were conducted using StringTie.[32]

Statistical analysis

Clinicopathological variables were summarized using frequencies, percentages, and mean ± standard deviation. Age was compared using Student's *t*-test, while categorical variables between groups were compared using Pearson's chi-square test or Fisher's exact test. A two-sided *P* value of <0.05 was considered statistically significant. Statistical analyses were carried out using the R Statistical Software (v4.3.1; R Core Team 2023). In addition, *PALB2* PV/LPVs frequencies observed in this study were compared to data from other countries.

RESULTS

Frequency of PALB2 germline variants

A total of 104 patients (2.6%, 104/3987) presented *PALB2* germline variants with 8 (7.7%) PV, 14 (14.3%) LPV, and 82 (78.1%) VUS. Among these, *PALB2* PV/LPVs were detected at an overall frequency of 0.6% (22/3987) across all patients. The 13 germline *PALB2* PVs or LPVs identified in 22 patients are listed in Table 2, and schematic representations of variants are presented in Figure 1A using the mutation mapper tool in cBioPortal. Among these variants, nine (69.2%) were truncating variants and five (38.5%) were found in the WD40 domain, which directly binds with RAD51C, RAD51, and BRCA2 in HR and plays a crucial role in tumor suppression.[33]

Clinical characteristics of patients with PV

Among patients with *PALB2* PV/LPVs, 21 out of 22 carriers (95.5%) were women, with a median diagnosis age of 44 years (range 25-60 years). Overall, 18 carriers (81.8%) were diagnosed with breast cancer, 3 carriers (13.6%) with ovarian cancer, and 1 carrier (4.5%) with both cancers (Table 3).

Among 19 patients with breast cancer, 5 had bilateral breast cancer and 5 had multiorgan cancers (ovary, thyroid,

Table 2. Detected *PALB2* pathogenic/likely pathogenic variants in patients with cancer

No.	Nucleotide change	Protein change	Classification[a]	ClinVar[b]	Mutation	Frequency *n* (%)	Start[c]	End[c]	Ref	Alt
1	c.3350+5G>A	—	LPV	PV/LPV	Splice	6 (26.1)	23607859	23607859	C	T
2	c.454A>T	p.Lys152Ter	LPV	PV	Truncating	3 (13.1)	23636092	23636092	T	A
3	c.1048C>T	p.Gln350Ter	PV	PV	Truncating	2 (8.7)	23635498	23635498	G	A
4	c.2016dup	p.Glu673fs	PV	PV	Truncating	2 (8.7)	23630137	23630138	TT	TTT
5	c.902delA	p.Asp301fs	LPV	Not reported	Truncating	1 (4.4)	23635644	23635644	T	—
6	c.1424C>G	p.Ser475Ter	PV	PV/LPV	Truncating	1 (4.4)	23635122	23635122	G	C
7	c.1976_1977del	p.Leu659fs	LPV	PV	Truncating	1 (4.4)	23630177	23630178	CA	—
8	c.2095dup	p.Ser699fs	LPV	PV	Truncating	1 (4.4)	23630058	23630059	AAA	AAAA
9	c.2167_2168del	p.Met723fs	PV	PV	Truncating	1 (4.4)	23629986	23629987	AT	—
10	c.2834+2T>C	—	LPV	PV/LPV	Splice	1 (4.4)	23635328	23635328	A	G
11	c.2920_2921del	—	PV	PV	Truncating	1 (4.4)	23623044	23623045	TTT	T
12	Exon 11 deletion	—	PV	—	Exon deletion	1 (4.4)	—	—	—	—
13	c.3317delT	p.Met1106fs	LPV	Not reported	Missense	1 (4.4)	23607897	23607897	CA	C

LPV, likely pathogenic variant; PV, pathogenic variant.
[a]Classified by 2015 ACMG/AMP guideline.
[b]27/08/2024 accessed.
[c]Genomic position in hg38 coordinates.

colorectal, lung, and bladder cancer). Breast cancer phenotypes included 14 cases of intraductal carcinoma and 9 cases of ductal carcinoma *in situ*, with molecular subtypes comprising 3 cases of Luminal A, 12 cases of Luminal B, 5 cases of triple-negative, and 2 cases of human epidermal growth factor receptor 2 (HER2)-positive. Moreover, 16 carriers (72.7%) had first-degree relatives with 8 types of cancer (breast, ovarian, pancreatic, lung, gastric, liver, thyroid, and colorectal cancer), 12 carriers (54.6%) had second-degree relatives with 11 types of cancer (breast, pancreatic, lung, gastric, liver, prostate, leukemia, laryngeal, colorectal, head and neck, and uterine cancer), and 4 carriers (18.2%) did not have a family history of cancer (Figure 1B, Table 3).

Furthermore, the first cancer diagnosis tended to occur at a younger age among *PALB2* PV/LPVs, compared with those without these variants (44.6 ± 10.1 versus 50.2 ± 12.0, $P = 0.019$) (Table 1). Although family history of cancer (81.8% versus 67.4%, $P = 0.226$) was not significantly different between *PALB2* PV/LPVs carriers and non-carriers, the occurrence of multiple primary organ cancer diagnoses was significantly higher in *PALB2* PV/LPVs carriers (22.7% versus 8.3%, $P = 0.032$).

Neither of the two patients with ovarian cancer but without breast cancer had undergone risk-reducing mastectomy (RRM) or breast cancer screening. Additionally, among 18 patients with non-ovarian cancer, 9 (50.0%) had undergone risk-reducing salpingo-oophorectomy (RRSO). Furthermore, 7 out of 22 patients (31.8%) had undergone computed tomography (CT) scans of the pancreas, and 4 patients (18.2%) were undergoing annual follow-up for pancreatic cancer. Only 1 out of the 22 patients (4.5%) had undergone genetic testing for family members; the daughter of PT17 underwent family genetic testing for the same *PALB2* variant (c.2095dup), but the variant was not detected (Figure 1B).

Functional analysis of the PALB2 c.3350+5G>A

We found *PALB2* c.3350+5G>A variants in six patients. SpliceAI[34] predicted that this variant affects the acceptor splice sites (Figure 2A). Some studies have reported natural alternative splicing events resulting in exon 12 skipping.[35] To elucidate the functional implications of these intronic variants, we conducted exon splicing analysis using RT—PCR and Sanger sequencing using lymphocyte DNA from a patient (PT01). As a result, we observed two distinct amplicons in lymphocytes with *PALB2* c.3350+5G>A, including transcripts with exon 12 skipping and exon 11 and 12 skipping (Figure 2B). Compared with their corresponding wild-type transcripts, the exon 11 and 12 skipping transcripts were abnormal, and their translational prediction suggested an in-frame deletion of 78 amino acids (N1039-G1116) within the WD40 repeats domain. Additionally, we used RNA-seq to assess exon coverage of the *PALB2* transcripts (Figure 2C). Compared with RNA-seq results of a patient with breast cancer without *PALB2* variants, one with the *PALB2* c.3350+5G>A showed alternative splicing transcripts. Our findings indicated a correlation between the identified abnormal transcripts lacking exon 11 and 12 and the results obtained from Sanger sequencing.

DISCUSSION

PALB2 germline PV/LPVs were detected at a frequency of 0.6% (22/3987) in all patients with cancer, 0.9% (19/2186) in patients with breast cancer, and 0.3% (4/1457) in patients with ovarian, fallopian tube, and peritoneal cancer. These rates are similar to those found in studies conducted in other East Asian countries, China, and Japan (0.20%-1.66%). Consistently, *PALB2* germline PV/LPVs were observed more frequently in patients with breast cancer than in patients with ovarian cancer.

Generally, the prevalence of germline PV in *PALB2* varies depending on the target population, cancer type, and study design. For example, in cohorts including patients with cancer, the approximate prevalence was 0.10%-2.65%, whereas that of the general population was 0.02%-1.80% (Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2024.104132). Moreover, in terms of geography, a comparison of the location and frequency of *PALB2* PV/LPVs from studies in

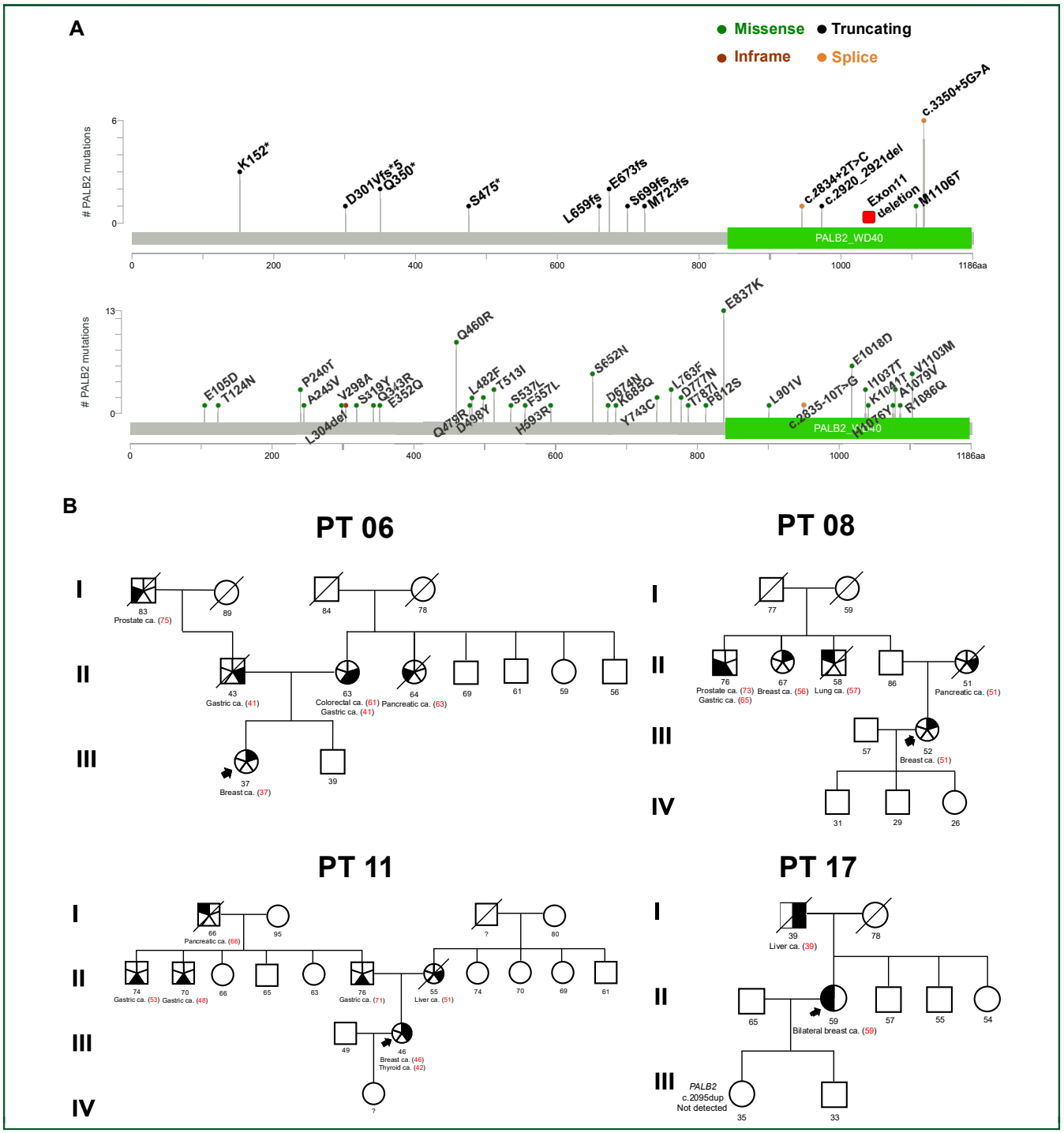

Figure 1. Pedigrees and schematic representation of the *PALB2* germline variants. (A) Location and frequency of 13 pathogenic variants (upper) and 35 variants of uncertain significance (low) indicated in lollipop plot generated by cBioPortal (http://www.cbioportal.org/). The *x*-axis represents the number of amino acid residues. (B) Pedigrees of four patients with *PALB2* germline pathogenic variants. Genetic testing for family member was conducted only for PT17.

China, Japan, United States, and Germany (Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2024.104132) revealed an apparent heterogeneity across various countries and ethnicities. Also, previous international studies have identified *PALB2* PV/LPVs in the WD40 domain at frequencies of 26.3% (5/19) in Japan,[18] 26.6% (21/79) in China,[17] 36.4% (12/33) in the United States,[36] and 21.9% (7/32) in Germany.[37] These percentages were marginally lower than that reported in our study (38.5%). No significant differences were observed in the clinicopathological characteristics of patients with *PALB2* PV/LPVs in and out of the WD40 domain (Supplementary Table S5, available at https://doi.org/10.1016/j.esmoop.2024.104132).

Among *PALB2* PV/LPVs, the c.3350+5G>A intronic variant, which is the most common in East Asia, according to gnomAD v4.1.0, was detected at a high rate of 27.3%.

Table 3. Clinical characteristics of patients with *PALB2* pathogenic variants or likely pathogenic variants ($n = 22$)

No.	Sex	Age	Nucleotide change	Protein change	Personal history of cancer	Molecular subtype[a]	Family history
PT01	F	52	c.3350+5G>A	—	Breast cancer (DCIS) Thyroid cancer	LA	2 FDR (lung cancer, gastric cancer, and liver cancer) 5 SDR (leukemia, breast cancer, gastric cancer, 2 liver cancer)
PT02	F	52	c.3350+5G>A	—	Ovarian cancer (HGSC)	—	2 FDR (ovarian cancer, colorectal cancer), 1 SDR (laryngeal cancer)
PT03	F	54	c.3350+5G>A	—	Breast cancer (IDC)	LB	2 FDR (breast cancer, gastric cancer)
PT04	F	39	c.3350+5G>A	—	Bilateral breast cancer (Rt: IDC, Lt: IDC)	Rt: LA/Lt: LB	1 FDR (breast cancer)
PT05	F	38	c.3350+5G>A	—	Breast cancer (DCIS)	LB	None
PT06	F	37	c.3350+5G>A	—	Breast cancer (DCIS)	LB	2 FDR (1 gastric cancer, gastric cancer and colorectal cancer) 2 SDR (pancreatic cancer, prostate cancer)
PT07	F	41	c.454A>T	p.Lys152Ter	Ovarian cancer (adenocarcinoma)	—	None
PT08	F	51	c.454A>T	p.Lys152Ter	Breast cancer (IDC)	LB	1 FDR (pancreatic cancer) 3 SDR (lung cancer, prostate cancer, and gastric cancer, breast cancer)
PT09	F	43	c.454A>T	p.Lys152Ter	Breast cancer (IDC)	LB	1 FDR (liver cancer) 2 SDR (breast cancer, gastric cancer)
PT10	F	60	c.1048C>T	p.Gln350Ter	Bilateral breast cancer (Rt: IDC, Lt: IDC)	Rt: TNBC/Lt: TNBC	2 FDR (thyroid cancer, lung cancer) 2 SDR (colorectal cancer, H&N cancer)
PT11	F	43	c.1048C>T	p.Gln350Ter	Breast cancer (IDC), thyroid cancer	LB	2 FDR (gastric cancer, liver cancer) 3 SDR (2 gastric cancer, pancreatic cancer)
PT12	F	56	c.2016dup	p.Glu673fs	Breast cancer (IDC)	TNBC	1 FDR (lung cancer) 3 SDR (laryngeal cancer, gastric cancer, uterine cancer)
PT13	F	27	c.2016dup	p.Glu673fs	Ovarian cancer (serous borderline)	—	None
PT14	F	46	c.902delA	p.Asp301fs	Bilateral breast cancer (Rt: DCIS, Lt: UK)	Rt: HER2/Lt: UK	1 FDR (gastric cancer) 1 SDR (breast cancer)
PT15	M	25	c.1424C>G	p.Ser475Ter	Breast cancer (DCIS)	LB	1 FDR (breast cancer) 1 SDR (lung cancer)
PT16	F	33	c.1976_1977del	p.Leu659fs	Bilateral breast cancer (Rt: DCIS, Lt: DCIS)	Rt: LB/Lt: HER2	None
PT17	F	59	c.2095dup	p.Ser699fs	Bilateral breast cancer (Rt: DCIS, Lt: IDC)	Rt: LB/Lt: LB	1 FDR (liver cancer)
PT18	F	45	c.2167_2168del	p.Met723fs	Breast cancer (DCIS)	TNBC	1 FDR (breast cancer)
PT19	F	35	c.2834+2T>C	—	Breast cancer (IDC)	LA	3 SDR (2 lung cancer, breast cancer)
PT20	F	35	c.2920_2921del	—	Breast cancer (IDC), colorectal cancer, Lung cancer	LB	2 FDR (liver cancer, colorectal cancer)
PT21	F	50	Exon 11 deletion	—	Breast cancer (IDC), ovarian cancer (UK)	TNBC	1 SDR (breast cancer)
PT22	F	60	c.3317delT	p.Met1106fs	Breast cancer (IDC), bladder cancer	TNBC	2 FDR (2 gastric cancer)

DCIS, ductal carcinoma *in situ*; FDR, first-degree relatives; H&N, head and neck; HER2, human epidermal growth factor receptor 2; HGSC, high-grade serous carcinoma; IDC, invasive ductal carcinoma; LA, Luminal A; LB, Luminal B; LPV, likely pathogenic variant; PV, pathogenic variant; SDR, second-degree relatives; TNBC, triple-negative breast cancer.

[a]Applicable only to breast cancer.

A

Gene	Variant	SpliceAI score				
		Acceptor loss (AL)	Donor loss (DL)	Acceptor gain (AG)	Donor gain (DG)	*P* (Aberrant splicing)
PALB2	c.3350+5G>A	0.44	0.14	0.00	0.00	0.52

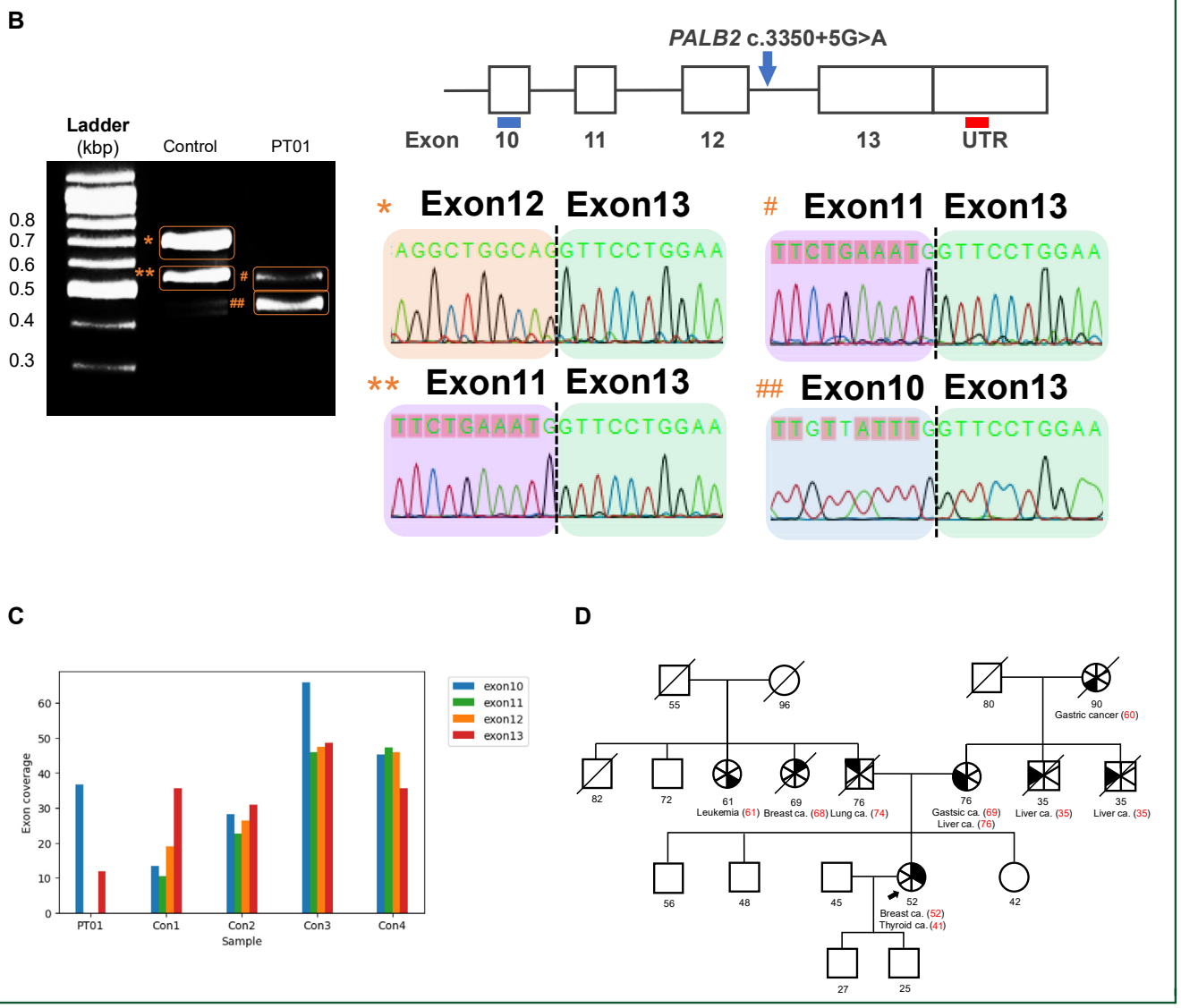

Figure 2. Functional analysis of the *PALB2* c.3350+5G>A variant. (A) Alternative splicing prediction using the SpliceAI score of *PALB2* c.3350+5G>A. The probability of aberrant splicing (P aberrant splicing) is calculated as 1−((1−AG) ×(1−AL)×(1−DG)×(1−DL)), which represent the probabilities of acceptor gain, acceptor loss, donor gain, and donor loss, respectively. (B) RT−PCR of lymphocyte-derived RNA. PCR primers were visualized with the blue and red bars. Two distinct PCR products were observed in both the control and patient samples. Vertical lines in the chromatograms indicate the exonic junction in the transcripts. In both the control and patient samples, skipping of exon 12 (88 bp) between exon 11 and exon 13 transcripts was identified. However, skipping of exon 11 (149 bp) and exon 12 between exon 10 and exon 13 transcripts was specifically observed in the patient sample. (C) Exon coverage in the transcript usage based on RNA-seq data. Exon 11 and 12 in the red box were not utilized in the *PALB2* transcript of the patient with *PALB2* c.3350+5G>A variant. (D) Pedigree of PT01.

Functional analysis of c.3350+5G>A revealed that its variant causes the skipping of exon 11 and 12. *PALB2* c.3350+5G>A was observed in several cohorts of various ethnicity, but in our study, its prevalence was higher in Korea than in other populations, including China and Japan.[38-41] Specifically, in a previous study of Korean patients with *BRCA1/2* mutation-negative breast cancer, c.3350+5G>A was observed at a high frequency among *PALB2* PV/LPVs (2/17).[42] Thus, c.3350+5G>A may potentially be a founder variant in Korea. However, c.3350+5G>A was recently reclassified as PV/LPV; hence, it may not have been well investigated.

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According to the systematic review of 115 studies examining *PALB2* germline PV found in patients with breast, ovarian, and pancreatic cancers, the five most frequently reported PVs—c.509_510del (p.Arg170IlefsTer14), c.3113G>A (p.Trp1038Ter), c.1592del (p.Leu531CysfsTer30), c.172_175del (p.Gln60ArgfsTer7), and c.1240C>T (p.Arg414Ter)—accounted for 57.3% of the PVs identified in these patients.[43] In contrast, in our cohort, none of these five variants were detected.

Out of 22 patients with *PALB2* PV/LPVs, 18 patients (81.8%) had a family history of various cancers other than breast, ovarian, or pancreatic cancer, while four patients (18.2%) had no family history. Hence, even in the absence of a family history, individuals with clinical features suggestive of hereditary cancer should undergo genetic testing, including that for *PALB2*, to assess their susceptibility to hereditary cancers. Patients with *PALB2* PV/LPVs were younger at the first cancer diagnosis with a significantly higher frequency of multiple primary organ cancers than those without these variants.

The latest NCCN guideline recommends that carriers of *PALB2* PV should undergo breast cancer screening similar to or equivalent to that for *BRCA1/2* PV carriers.[15] For these individuals, annual breast magnetic resonance imaging and mammography are advised, and RRM may be considered. Moreover, NCCN recommends that *PALB2* PV carriers may consider RRSO starting at ages 45-50 and screened for pancreatic cancer in case they have a family history of pancreatic cancer. In our study, half of the *PALB2* PV/LPVs carriers underwent RRSO, while 31.8% underwent CT scan of the pancreas, among whom two had a family history of pancreatic cancer and three had a family history of gastrointestinal cancers. In addition, 18.2% of the patients were being followed-up annually for pancreatic cancer. Notably, none of the carriers had undergone RRM, and only one had undergone family testing. The proportion of carriers who chose to undergo RRSO was similar to the rate observed in a previous study conducted by the same institution among women with *BRCA1/2* PV (52.4%).[44] However, the adherence rates for RRM and genetic testing for family members, as outlined by the NCCN guidelines, raise concerns. Although the number of non-breast cancer patients was limited, the observed differences in screening adherence among various cancer types may be attributed to the challenges associated with early detection of ovarian cancer and its comparatively poor prognosis relative to other malignancies. Hence, clinicians should essentially prioritize referrals for patients with PV to genetic counseling specialists. This approach ensures that patients receive thorough explanations regarding the significance of the variant, the importance of family testing, and personalized cancer screening strategies. In a previous study conducted in Korea, it was found that multigene panel testing combined with genetic counseling helped decrease concerns about the risk of additional cancer development. Additionally, genetic counseling positively impacted mood, daily functioning, and conjunction with improving the general public's understanding of hereditary cancer syndrome.[42]

Furthermore, patients with metastatic breast cancer and the *PALB2* PV can consider poly (ADP-ribose) polymerase (PARP) inhibitors to aid in systemic treatment decisions, and high-risk patients with HER2-negative breast cancer can consider olaparib to aid in adjuvant treatment decisions, according to the NCCN guideline. Recently, some clinical trials using PARP inhibitors have demonstrated efficacy in treating patients with breast, pancreatic, and prostate cancer carrying the germline *PALB2* PVs.[45-47] Therefore, the clinical relevance of genomic profiling for *PALB2* offers a robust basis for the determination of treatment options.

To the best of our knowledge, this study included the largest prevalence of *PALB2* in Korea. However, there are some limitations in this study. Firstly, recall bias may exist in identifying the family history, as some of the patients did not visit the genetic counseling clinic. Secondly, there may be constraints in collecting clinical data since some patients only underwent genetic testing at our center but received treatment in other institutions. Thirdly, given that most patients in our cohort were women and had been diagnosed with breast and ovarian cancers, the outcomes of our study should be generalized to other cancer types with caution.

In conclusion, our study revealed germline *PALB2* PV frequencies of 0.6%, 0.9%, and 0.3% in Korean patients with total cancer, breast cancer, and epithelial ovarian, fallopian tube and peritoneal cancers, respectively. These rates are consistent with previous study findings. Age at first cancer diagnosis and the presence of multiple primary organ cancers were significantly different variables between patients with and without *PALB2* PV/LPVs. Among the *PALB2* PV/LPVs, c.3350+5G>A may be a founder variant in the Korean population considering its high prevalence in Korean patients with cancer. Notably, a low rate of screening and family genetic testing among carriers was observed according to the NCCN guidelines, indicating a need for solutions to improve guideline adherence.

Although our study includes a variety of cancer types compared with previous research, most of the cases we focused on were breast and ovarian cancers. Therefore, further studies are needed to investigate the characteristics of germline *PALB2* variants across a broader range of cancer types.

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DISCLOSURE

The authors have declared no conflicts of interest.

DATA SHARING

All the data generated or analyzed during this study are available from the corresponding author on reasonable request within the range of IRB approval.

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