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Research Article/Özgün Araştırma

Investigation of the germline *PALB2* variants in cancer patients using the next-generation sequencing in Türkiye

Türkiye'deki kanser hastalarında kalıtsal *PALB2* gen varyantlarının yeni nesil dizileme yöntemiyle araştırılması

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Abstract

Aim: The study aimed to investigate germline *PALB2* gene variants in 1056 cancer patients in Türkiye, selected based on the National Comprehensive Cancer Network guidelines for genetic/familial high-risk assessment related to breast, ovarian, and pancreatic cancer.

Materials and Methods: The next-generation sequencing analysis of genomic DNA was performed using a Sophia Hereditary Cancer Solutions Panel for *PALB2* gene mutation screening.

Results: The *PALB2* genetic variants were detected in 48 patients, including 20 patients with pathogenic or likely pathogenic variants and 28 patients with variants of uncertain significance. The most common *PALB2* mutations were the frameshift mutations c.557dupA p.(Asn186Lysfs*4) and c.509_510del p.(Arg170Ilefs*14), found in 0.57% and 0.28% of patients, respectively.

Conclusion: The findings of the study emphasize the importance of *PALB2* gene analysis for breast cancer predisposition in Türkiye.

Keywords: *PALB2*, Germline mutations, Hereditary cancer risk factor.

Öz

Amaç: Çalışmada, meme, yumurtalık ve pankreas kanseri ile ilgili genetik/ailesel yüksek risk değerlendirmesi için Ulusal Kapsamlı Kanser Ağı kılavuzlarına göre seçilen, Türkiye'deki 1056 kanser hastasında germline *PALB2* geni varyantlarının araştırılması amaçlandı.

Gereç ve Yöntem: *PALB2* geni mutasyon taraması için Sophia Kalıtsal Kanser Çözümleri Paneli kullanılarak genomik DNA'nın yeni nesil dizileme analizi gerçekleştirildi.

Bulgular: *PALB2* genetik varyantları, 20 hastada patojenik veya muhtemel patojenik varyant ve 28 hastada belirsiz öneme sahip varyantlara sahip olmak üzere toplam 48 hastada tespit edildi. En yaygın *PALB2* mutasyonları, hastaların sırasıyla %0,57 ve %0,28'inde bulunan c.557dupA p.(Asn186Lysfs*4) ve c.509_510del p.(Arg170Ilefs*14) çerçeve kayması mutasyonlarıydı.

Sonuç: Araştırma bulguları, Türkiye'de meme kanseri yatkınlığı açısından *PALB2* gen analizinin önemini vurgulamaktadır.

Anahtar Kelimeler: *PALB2*; Germline mutasyonlar; Kalıtsal kanser risk faktörü.

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Introduction

Repairing of the DNA double-strand breaks (DSBs) by homologous recombination (HR) prevents cancer development. Hereditary pathogenic variants (PV) and likely pathogenic variants (LPV) of *BRCA1* and *BRCA2* are the major genetic causes of increased risk of breast, ovarian, and pancreatic cancers[1]. Genetic mutations in these genes are responsible for 20% of the inherited breast cancer[2]. *ATM, CHEK2,* and *PALB2* are involved in DNA damage response (DDR) which causes hereditary breast and ovarian cancer (HBOC)[2].

The *PALB2*, has 1186 amino acids, including a core coiled-coil motif and amino-terminal WD40 repeats[3], and is known as a BRCA-interacting protein[4]. It acts as a scaffold in forming the 'BRCA complex' involved in homologous recombination repair[5]. Cells with defective *BRCA1-PALB2* interaction display impaired homologous recombination[5]. Impaired homologous recombination repair causes genomic instability and carcinogenesis in *BRCA1, BRCA2,* and *PALB2* mutation carriers.

Biallelic mutations in *PALB2*, similar to *BRCA2*, are associated with Fanconi anemia[6], whereas monoallelic truncating mutations increase the risk of developing pancreatic, breast, and ovarian cancer[7]. Early research has indicated that individuals having pathogenic germline variants in the *PALB2* gene are at higher risk for breast cancer, with estimated penetrance up to 70% based on family history and diagnosis age[8,9]. Also, germline pathogenic variants (PVs) in *PALB2* have been detected in individuals with ovarian and pancreatic cancer[10,11].

The germline PV/LPV spectrum of the *PALB2* gene may differ among various global regions due to variations in ethnicity, lifestyle, and reproductive behaviors. These differences have sparked our curiosity to thoroughly comprehend the occurrence and diversity of *PALB2* gene variants within the Turkish cancer cohort. However, the range of *PALB2* mutations in Türkiye is still poorly understood. Therefore, in the present study, we aimed to investigate the PV/LPV and the variant of unsignificance (VUS) in *PALB2* genes in Turkish cancer patients which were selected based on the inclusion criteria established in the NCCN Guidelines for Genetic/Familial High-Risk Assessment concerning breast, ovarian, and pancreatic cancer[12]. The discovery of the recurrent *PALB2* PV/LPV and VUS may improve our understanding of their role in various cancer risks. This data can be used to develop optimal prevention and treatment strategies for *PALB2* mutation carriers in Türkiye.

Materials and Methods

Selection/Description of the patients

The Clinical Research Ethics Committee of Istanbul University authorized the current research on 17.03.2023 with the approval number 2023/500 following the Declaration of Helsinki[13]. The pathology report evaluated for tumor parameters such as diagnosis, receptor status, and histological grades. Before the study, all patients signed an informed written consent form. The study included 1056 cancer patients and was presented by the Department of Cancer Genetics at Istanbul University, Türkiye. The NCCN Guidelines Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic[12] were used as inclusion criteria in the study.

Technical information

PALB2 mutation screening

The blood samples were first processed using the Ficoll (Sigma-Aldrich, Darmstadt, Germany) procedures for lymphocyte isolation. The DNA of lymphocyte pellets was assessed using the QIAamp DNA micro kit (Qiagen, Hilden, Germany) in accordance with the kit protocol. The DNA concentration was assessed using the NanoDrop 2000c Spectrophotometer (NanoDropT, DE, USA). Illumina's MiSeq® platform (Illumina, Ca, USA) was used to screen all coding exons of the *PALB2* gene to summarize the patterns of genetic variations and frequencies of the gene, and the Sophia Genetics DDM analysis (Illumina, CA, USA) was used. For library construction, the Sophia Hereditary Cancer Solutions 59 gene (Sophia Genetics, Boston, USA) kit was used in accordance with the

manufacturer's instructions. The next-generation sequencing (NGS) technique was applied via the MiSeq platform by Illumina.

The next generation sequencing

Illumina's MiSeq® platform (Illumina, Ca, USA) was used to screen all coding exons of the *PALB2* gene to summarize the patterns of genetic variations and frequencies of the gene and Sophia Genetics DDM analysis (Sophia Genetics, Boston, USA). During the research, the NGS pipeline utilized the Illumina MiSeq platform and Sophia Genetics DDM analysis, both of which were previously established methods (Illumina, San Diego, CA, USA)[14].

Sequencing

DNA libraries were prepared and subjected to NGS during the study using the Illumina MiSeq platform (San Diego, California, USA). The Illumina MiSeq Reagent Kit v3 (600-cycle) was used for the sequencing. For library construction, the Sophia Hereditary Cancer Solutions 59 gene (Sophia Genetics, Boston, USA) kit was used following the manufacturer's instructions. The DNA was denatured and diluted with 0.2 N NaOH at a concentration of 2 nM. The library was then further diluted to a final concentration of 10 pM using a Prechilled HT1 buffer. Additionally, 6% of PhiX Control v3 (Ilumina, San Diego, CA, USA) was added to create a spiked library.

Genetic analysis

The genetic analysis was performed using the Sophia DDM analysis program. For variant calling and alignment of sequences to the reference genome (GRCh37/hg19), the Sophia Genomic Alignment and Variant Calling software was utilized. Specifically, Sophia DDM software (Sophia Genetics, Ecublens, Switzerland) was employed for independent read alignment and variant calling. The variant call files generated were further analyzed and filtered using VariantStudio software by Illumina and Sophia DDM software.

Genome interpretation using in silico predictors

The web-based algorithms were employed to assess the potential impact of identified nonsynonymous *PALB2* germline variants on protein function. These algorithms included the databases such as dbSNP[15], G1000[16], GnomAD[17], SIFT[18], POLYPHEN2[19], MUTATION TASTER[20], ClinVar[21], and HGMD[22].

Variant classification

The classification of variants involved an assessment of findings from the ClinVar[21] and HGMD[22] databases, alongside adherence to the sequencing/sequence variants classification guidelines set forth by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP)[23]". In the ACMG/AMP guidelines, the only criterion designated with very strong strength level for pathogenicity is defined as "null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss-of-function (LoF) is a known mechanism of disease"[23]. In this study, the identified causal variants were categorized into three groups: variant of unsignificance, likely pathogenic, and pathogenic based on the ACMG criteria. The missense mutations obtained in the study were suggested to have disease-causing effects based on in silico analysis programs. However, they were classified as VUS due to insufficient evidence supporting their disease-causing effects according to the ACMG criteria. Conducting functional studies on the detected VUS and evaluating their impact in terms of benign or pathogenicity will significantly enhance the accuracy of variant classification.

Clinicopathologic features

We evaluated the clinicopathologic characteristics by referencing the pathology reports in the patient's clinical records. These reports provided data on the clinical stage and histologic grade of cancer patients.

Results

NGS analysis

In the present study, *PALB2* variant analysis was conducted among *BRCA1/2* non-mutant 1056 patients who presented to our clinic for a genetic testing (828 breast cancer patients, 97 ovarian cancer patients, 19 endometrial cancer

patients, 26 pancreatic cancer patients, 56 colon cancer patients and 30 prostate cancer patients).

Among the investigated patients, PV/LPV or VUS were detected in the *PALB2* gene in forty-one breast cancer patients (41/828), four ovarian cancer patients (4/97), one endometrial cancer patient (1/19), one pancreatic cancer patient (1/26), one prostate cancer patient (1/30).

The causal variants found in the study were classified following the variant classification guidelines determined by the ACMG. In total, 20 patients (20/1056; ~1.9%) had at least one PV/LPV variant (13 different mutations) (Table 1), and 28 patients (28/1056; ~2.7%) had a VUS (23 different variants) (Table 2). In terms of *PALB2* VUS, we found 28 patients with 23 different *PALB2* VUSs.

Table 1. Pathogenic and likely pathogenic *PALB2* variants and their risk assessment among cancer patients in Türkiye in this study **(***PALB2* / TRANSCRIPT: NM_024675.3 / REFERENCE GENOM: GRCh37/hg19 Chromosome:16)

Nucleotide substitution	Amino acid change	Impact	dbSNP number	GnomAD Freq.	POLYPHEN2	SIFT	Mut. Tast.	ClinVar Clinical Sig.	HGMD
c.1692_1698 dup	p.(His567Lysfs*13)	frameshift	N/A	N/A	N/A	N/A	N/A	No Data	Not reported
c.1704_1707 delAAAA	p.(Lys569Argfs*29)	frameshift	rs1060502759	N/A	N/A	N/A	N/A	pathogenic	Disease causing mutation Breast cancer risk
c.172_175 delTTGT	p.(Gln60Argfs*7)	frameshift	rs180177143	0.000036	N/A	N/A	N/A	pathogenic	Disease causing mutation Pancreatic cancer risk
c.1960_1961 insC	p.(Ile654Thrfs*9)	frameshift	N/A	N/A	N/A	N/A	N/A	No Data	Not reported
c.1967dupC	p.(Glu657Argfs*6)	frameshift	N/A	N/A	N/A	N/A	N/A	No Data	Not reported
c.211+1G>T	p.(?)	splice_donor +1	rs1555462026	N/A	N/A	N/A	1.0	likely pathogenic	Disease causing mutation Breast cancer risk
c.2368C>T	p.(Gln790*)	nonsense	rs886039480	N/A	N/A	N/A	1.0	pathogenic	Not reported
c.2587-1G>C	p.(?)	splice_acceptor-1	rs761214886	0.000004	N/A	N/A	1.0	likely pathogenic	Disease causing mutation Breast and/or ovarian cancer risk
c.3256 C>T	p.(Arg1086*)	nonsense	rs587776527	0.00002	N/A	N/A	1.0	pathogenic	Disease causing mutation? Pancreatic cancer risk
c.390_391insT	p.(Arg131*)	nonsense	N/A	N/A	N/A	N/A	N/A	likely pathogenic	Disease causing mutation Breast cancer risk
c.481_482del	p.(Asp161Leufs*6)	frameshift	rs1597099149	N/A	N/A	N/A	N/A	pathogenic	Disease causing mutation Breast and/or ovarian cancer risk
c.509_510del	p.(Arg170Ilefs*14)	frameshift	rs515726123	0.000014	N/A	N/A	N/A	pathogenic	Disease causing mutation Breast Cancer Risk
c.557dupA	p.(Asn186Lysfs*4)	frameshift	rs1555461727	N/A	N/A	N/A	N/A	pathogenic	Disease causing mutation Breast Cancer risk

Freq: Frequency, Mut.Tast: Mutation Taster, Sig: Significance, HGMD: The Human Gene Mutation Database, N/A: Not Applicable

Table 2. *PALB2* variants of unsignificance (VUS) and their risk assessment among cancer patients in Türkiye in this study.

Nucleotide substitution	Amino acid change	Impact	dbSNP number	GnomAD Freq.	POLYPHEN2	SIFT	Mut. Tast.	ClinVar Clinical Sig.	HGMD
c.1001A>G	p.(Tyr334Cys)	missense	rs200620434	0.00006	0.03	0.83	0.0	uncertain sig.	Disease-causing mutation? Colorectal cancer suscept.
c.1163C>T	p.(Pro388Leu)	missense	rs1597096898	N/A	0.04	0.9	0.0	uncertain sig.	Not reported
c.121G>A	p.(Ala41Thr)	missense	N/A	N/A	1.0	1.0	0.76	No Data	Not reported
c.1298T>C	p.(Leu433Ser)	missense	rs1597096465	N/A	0.797	0.999	0.094	uncertain sig.	Not reported
c.13C>T	p.(Pro5Ser)	missense	rs377085677	0.00004	0.027	0.423	0.0	uncertain sig.	Disease-causing mutation? Breast cancer risk
c.1408A>G	p.(Thr470Ala)	missense	rs150636811	0.00001	0.006	0.551	0.0	uncertain sig.	Not reported
c.1448C>T	p.(Ser483Leu)	missense	rs1057520736	0.00001	0.999	0.883	0.004	uncertain sig.	Disease causing mutation? Cancer pred. syndrome
c.1867A>G	p.(Lys623Glu)	missense	rs1966864669	N/A	0.927	1.0	0.125	uncertain sig.	Not reported
c.194C>T	p.(Pro65Leu)	missense	rs62625272	0.00004	0.003	0.505	N/A	uncertain sig.	Disease causing mutation? Breast cancer risk
c.2113T>A	p.Tyr705Asn	missense	N/A	N/A	0.253	1.0	0.022	uncertain sig.	Not reported
c.2974A>C	p.(Met992Leu)	missense	rs1555459522	N/A	0.013	0.755	0.04	uncertain sig.	Not reported
c.307G>C	p.(Gly103Arg)	missense	N/A	N/A	0.053	0.973	0.0	No Data	Not reported
c.3073G>A	p.(Ala1025Thr)	missense	rs746872839	0.00001	0.403	0.953	0.999	uncertain sig.	Disease causing mutation? Cancer pred. syndrome
c.3122A>C	p.(Lys1041Thr)	missense	rs781663559	N/A	0.17	0.986	0.9954	uncertain sig.	Disease causing mutation? Cancer pred. syndrome
c.315G>C	p.(Glu105Asp)	missense	rs515726108	N/A	0.027	0.978	0.0	uncertain sig.	Disease causing mutation? Breast cancer risk, male
c.3201+4del	p.(?)	splice_donor +4	rs1555458807	N/A	N/A	N/A	0.0	uncertain sig.	Not reported
c.3203G>A	p.(Gly1068Glu)	missense	rs759587160	N/A	1.0	0.997	0.999	uncertain sig.	Not reported

c.3306C>G	p.(Ser1102Arg)	missense	rs515726112	N/A	0.609	0.989	0.0	uncertain sig.	Disease causing mutation? Breast cancer risk
c.3529G>A	p.(Asp1177Asn)	missense	N/A	N/A	0.252	0.753	0.94	No Data	Not reported
c.758T>C	p.(Leu253Pro)	missense	N/A	N/A	0.0	0.939	0.0	uncertain sig.	Not Reported
c.814G>A	p.(Glu272Lys)	missense	rs515726127	0.00001	0.107	0.646	0.0	uncertain sig.	Disease causing mutation? Breast and/or ovarian cancer risk
c.833_834 delinsAT	p.(Leu278His)	missense	rs587778582	N/A	N/A	N/A	N/A	uncertain sig.	Disease causing mutation? Cancer pred. syndrome
c.91A>G	p.(Thr31Ala)	missense	rs1967110664	N/A	0.997	1.0	0.585	uncertain sig.	Not Reported

Freq: Frequency, Mut.Tast: Mutation Taster, Sig: Significance, HGMD: The Human Gene Mutation Database, N/A: Not Applicable, pred: predisposition, sust: susceptibility

All breast cancer patients with *PALB2* mutation, had invasive-ductal breast cancer (100%), with 85% being hormone receptor-positive. Triple-negative histology was 15% among PV/LPV carriers. In terms of tumor grade, patients had grade 1 (5%) or grade III (30%) tumors, and the majority were at stage II (65%). Except for one patient, all investigated patients who were found to contain the PV/LPV had cancer in the first/second/third-degree relatives. The clinical features of *PALB2* mutant breast cancer patients are presented in Table 3. Additionally, 95% of *PALB2* mutation-carrier breast cancer patients had at least one relative diagnosed with cancer (Table 4).

Table 3. Clinico-pathologic features of Turkish breast cancer patients with *PALB2* PV/LPV detected in this study.

Nucleotide substitution	Age at Diag.	St.	Gr.	His. Sub.	ER	PR	HER2	Node Inv.	TNBC	Met.	Status
c.1692_1698dup	38	III	3	IDC	Pos.	Neg.	Neg.	Yes	No	Yes	Alive
c.1704_1707del	42	II	2	IDC	Pos.	Pos.	Neg.	Yes	No	No	Alive
c.172_175del	39	II	2	IDC	Pos.	Pos.	Neg.	No	No	No	Alive
c.1960_1961insC	43	III	3	IDC	Pos.	Pos.	Neg.	No	No	No	Alive
c.1967dupC	42	II	2	IDC	Pos.	Pos.	Pos.	Yes	No	No	Alive
c.211+1G>T	67	II	2	IDC	Neg.	Neg.	Neg.	Yes	Yes	No	Alive
c.2368C>T	44	II	2	IDC	Pos.	Pos.	Neg.	No	No	No	Alive
c.2587-1G>C	40	III	3	IDC	Neg.	Neg.	Neg.	No	Yes	No	Alive
c.3256 C>T	40	II	2	IDC	Pos.	Pos.	Neg.	No	No	No	Alive
c.390_391insT	51	II	3	IDC	Pos.	Neg.	Pos.	No	No	No	Alive
c.481_482del	39	III	3	IDC	Pos.	Pos.	Neg.	No	No	Yes	Alive
c.509_510del	50	II	2	IDC	Pos.	Pos.	Pos.	Yes	No	Yes	Alive
c.509_510del	36	II	2	IDC	Pos.	Pos.	Neg.	Yes	No	Yes	Alive
c.509_510del	24	I	1	IDC	Pos.	Pos.	Pos.	No	No	No	Alive
c.557dupA	45	II	1	IDC	Pos.	Pos.	Pos.	No	No	No	Alive
c.557dupA	31	III	3	IDC	Neg.	Neg.	Neg.	Yes	Yes	Yes	Alive

c.557dupA	41	II	2	IDC	Pos.	Pos.	Neg.	No	No	No	Alive
c.557dupA	51	II	2	IDC	Pos.	Pos.	Pos.	Yes	No	Yes	Alive
c.557dupA	36	II	2	IDC	Pos.	Pos.	Neg.	No	No	Yes	Alive
c.557dupA	40	III	3	IDC	Pos.	Pos.	Neg.	Yes	No	Yes	Alive

Diag: Diagnosis, St: Stage, Gr: Grade, His Sub: Histologic Subtype, ER: Estrogen Receptor, PR: Progesterone Receptor, HER2: Human Epidermal Growth Factor Receptor 2, Node Inv: Node Involvement, TNBC: Triple-negative Breast Cancer, Met: Metastasis, Pos: Positive, Neg: Negative

Table 4. Frequency of PV/LPV and family history of tested individuals in this study.

Exon	Nucleotide substitution	Amino acid change	Age at Diagnosis & cancer type	Family history
5	c.1692_1698dup	p.(His567Lysfs*13)	38y/44y Bilateral Breast Ca	Esophageal Ca Ovarian Ca
5	c.1704_1707delAAAA	p.(Lys569Argfs*29)	42y Unilateral Breast Ca	Breast Ca Stomach Ca
3	c.172_175delTTGT	p.(Gln60Argfs*7)	39y Unilateral Breast Ca	Breast Ca
5	c.1960_1961insC	p.(Ile654Thrfs*9)	43y Unilateral Breast Ca	Breast Ca Cervix Ca
5	c.1967dupC	p.(Glu657Argfs*6)	42y Bilateral Breast Ca	Lung Ca Breast Ca
3	c.211+1G>T	p.(?)	67y Breast Ca	Ovarian Ca Cervix Ca Stomach Ca Endometrial Ca
5	c.2368C>T	p.(Gln790*)	44y Unilateral Breast Ca	Thyroid Ca Stomach Ca
7	c.2587-1G>C	p.(?)	40y Bilateral Breast Ca	None
12	c.3256 C>T	p.(Arg1086*)	40y/56y Bilateral Breast Ca	Bladder Ca Breast Ca
4	c.390_391insT	p.(Ala1025Thr)	51y Unilateral Breast Ca	Prostate Ca Ovarian Ca Breast Ca
4	c.481_482del	p.(Asp161Leufs*6)	39y/53y Bilateral Breast Ca	Cervix Ca Breast Ca Prostat Ca
4	c.509_510del	p.(Arg170Ilefs*14)	50y Unilateral Breast Ca	Breast Ca Cervix Ca Non-Hodgkin lymphoma. Prostat Ca Uterus Ca

4	c.509_510del	p.(Arg170Ilefs*14)	24y Unilateral Breast Ca	Breast Ca
4	c.509_510del	p.(Arg170Ilefs*14)	36y/52y Bilateral Breast Ca	Breast Ca Cervix Ca
4	c.557dupA	p.(Asn186Lysfs*4)	36y Unilateral Breast Ca	Breast Ca Lung Ca
4	c.557dupA	p.(Asn186Lysfs*4)	40y Unilateral Breast Ca	Pancreas Ca Thyroid Ca Lung Ca Breast Ca Cervix Ca
4	c.557dupA	p.(Asn186Lysfs*4)	45y/57y Bilateral Breast Ca	Prostat Ca Lung Ca Cervix Ca
4	c.557dupA	p.(Asn186Lysfs*4)	41y Unilateral Breast Ca	Breast Ca
4	c.557dupA	p.(Asn186Lysfs*4)	31y Unilateral Breast Ca	Breast Ca Uterus Ca
4	c.557dupA	p.(Asn186Lysfs*4)	51y Unilateral Breast Ca	Pancreas Ca

Ca: cancer

The eight frame-shift *PALB2* pathogenic mutations: c.1692_1698dup, c.1704_1707delAAAA, c.172_175delTTGT, c.1960_1961insC, c.1967dupC, c.481_482del, c.509_510del, c.557dupA were among breast cancer patients. The mutation median age of mutation carriers of breast cancer was 39.8 years (Table4).

The most common *PALB2* PV/LPV found in the study were: The c.557dupA p.(Asn186Lysfs*4) was identified in six breast cancer patients. c.509_510del p.(Arg170Ilefs*14) in three breast cancer patients.

The frameshift pathogenic mutations were more frequent compared to missense genetic alterations here in our study cohort. The frameshift, non-sense, splice donor and spice acceptor variant frequencies were 61.5%, 23.1%, 7.7%, and 7.7%, respectively (Figure1). Surprisingly, no missense pathogenic genetic alterations were found in our patient groups. All identified PV/LPV resulted in a loss-of-function of the *PALB2* gene.

The two most common PV were PALB2, c.509_510del p.(Arg170Ilefs*14) and c.557dupA p.(Asn186Lysfs*4) variant.

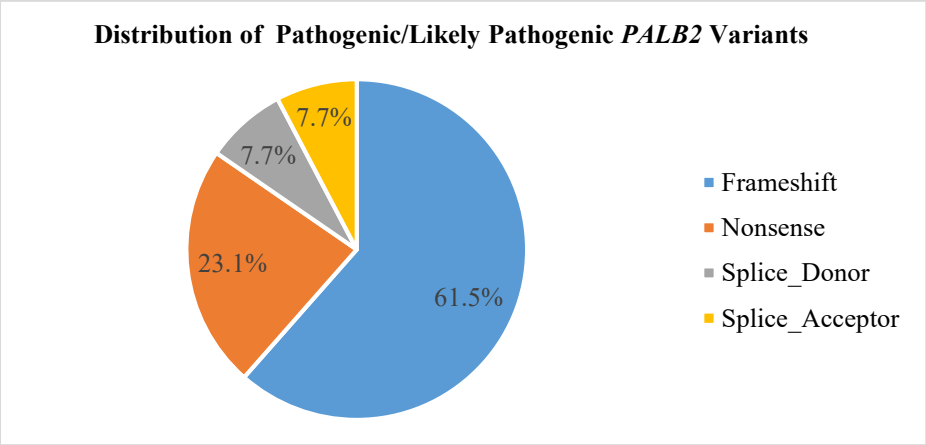

Figure 1. The prevalence of pathogenic/likely pathogenic *PALB2* variant types in cohort of cancer investigated in this study.

Discussion

In this study, we analyzed the *PALB2* PV/LPV and VUS frequencies in the Turkish population. We investigated 1056 cancer patients selected based on the inclusion criteria established in the NCCN Guideline. We found 13 different PV/LPV in 20 patients (20/1056; ~1.9%) and 23 different VUS in 28 patients (28/1056; ~2.7%) in the *PALB2* gene in the entire cohort. We found that the *PALB2* PV/LPV ratio was ~1.9% among patients. In the literature, the detection rate varied from 0.36% to 4.8% overall[24]. The higher prevalence was detected in Finland, which was attributed to the presence of a founder mutation[25] and low incidence was noted in the Jewish Ashkenazi population, in the Netherlands Japan and Ireland studies [26].

Women with germline *PALB2* mutations are at risk of up to 58% for developing breast cancer when they have a positive family history, approximately five-fold higher than the general population[27]. Yang et al. reported that individuals with inherited pathogenic variants in *PALB2* face an increased risk of 7.18 times for breast cancer in women, 2.91 times for ovarian cancer, 2.37 times for pancreatic cancer, and 7.34 times for male breast cancer[28].

The *PALB2* PV/LPV and VUS were identified in forty-one breast cancer patients (41/828), four ovarian cancer patients (4/97), one endometrial cancer patient (1/19), one pancreatic cancer patient (1/26), one prostate cancer patient (1/30).

However, this result should be taken with caution because the number of patients in the endometrial, pancreatic, and prostate cancer cohort is relatively small compared with the breast cancer cohort that was part of this study.

In the current study, the most common recurrent frameshift mutation c.557dupA p.(Asn186Lysfs*4) was detected in six unrelated breast cancer patients 30% (6 of 20 among PV/LPV carriers) diagnosed with early onset cancer breast cancer. The prevalence of *PALB2* germline mutations in patients with early-onset breast cancer has been reported, indicating its potential contribution to hereditary breast cancer similar to our results. The c.557dupA p.(Asn186Lysfs*4) variant in the *PALB2* gene has been extensively studied in the context of cancer susceptibility, particularly in relation to breast cancer. The evidence suggests that *PALB2* plays a significant role in cancer predisposition and has clinical implications for genetic testing and cancer risk assessment. It was determined that a mutation c.557dupA p.(Asn186Lysfs*4) in the *PALB2* gene created a non-sense codon (stop codon) at position 186, leading to a shortening in the length of the protein. In the literature, the effect of this mutation on protein function and cancer risk prediction is yet unknown. The second common recurrent frameshift mutation c.509_510del p.(Arg170Ilefs*14) was detected in 3 unrelated breast cancer patients 15% (3 of 20 among PV/LPV carriers) two were diagnosed at an early age and all patients had familial breast cancer). This mutation is anticipated to result in a significant alteration in the protein structure, potentially affecting its binding sites with *BRCA2*[29] causing an activation of HR for repair of double-strand DNA breaks[8]. *PALB2* gene the PV c.509_510del p.(Arg170Ilefs*14) appears to be a prevalent mutation that has also been observed in other groups[30]. Dansonka-Mieszkowsa discovered the *PALB2*: c.509_510del p.(Arg170Ilefs*14) pathogenic variant in breast/ovarian cancer patients from the southern Polish population[10]. They detected this mutation in 0.6% (4 out of 648) of familial breast cancer patients and 0.08% (1 out of 1310) in the control group, which was s statistically significant. We detected *PALB2*: c.509_510del p.(Arg170Ilefs*14) mutation in 3 breast cancer patients (3/828; 0.36%). More research is necessary to evaluate whether it may be regarded as a founder mutation in the Turkish population.

The most prevalent pathogenic genetic variations among our patients were frameshift, nonsense, and splice variants; and pathogenic missense genetic alterations were not detected. The higher frequency of pathogenic or likely pathogenic loss-of-function mutations, such as frameshift, nonsense, splice, deletions/duplications, compared to missense variants may be attributed to the greater

difficulty in functionally validating missense variants. This difficulty in validating missense variants could lead to a higher number of reported pathogenic or likely pathogenic loss-of-function mutations. In order to address this issue, advanced functional assays such as protein-protein interaction or proficiency testing in homologous recombination repair should be utilized. Despite these efforts, a considerable number of missense variants are still not categorized. As a result, the ClinVar[21] and HGMD[22] have endeavored to offer expert curation on pathogenic/likely pathogenic *PALB2* variants.

The research on *PALB2* has predominantly concentrated on identifying the truncating mutations; however, there were also the documented cases of VUS in patients [31], the presence of these variants poses a challenge for genetic counselors, clinicians, and patients. Although no distinctions were observed in the clinicopathological parameters of *PALB2* VUS carriers in this study, additional functional characterization of *PALB2* VUS could help to differentiate particular VUS with potential pathogenicity, thereby contributing to clinical practice. Until the role of VUS in *PALB2* is elucidated, the ACMG advises against utilizing *PALB2* VUSs to inform the clinical management[32]. However, the functional characterization of *PALB2* VUS have revealed their potential to disrupt DNA repair and lead to functional defects in homologous recombination repair in some studies [33].

Various cancer types were observed among the family members of the patients, carrying this variant in the Turkish cohort. Studies conducted in other populations have reported varying prevalence rates of *PALB2* mutations. Studies have highlighted the significance of *PALB2* as a tumor suppressor gene[33] and its interaction with *BRCA2* in breast cancer susceptibility[34] and the impact of this variant on DNA repair and cancer predisposition[35]. These studies collectively emphasize the importance of investigating the functional consequences of this variant in the context of cancer predisposition and DNA repair mechanisms. To exemplify the *PALB2* mutations accounted for 0.9% of breast cancer cases in the Chinese population[36]. They were similarly truncated *PALB2* mutations detected in 3 out of 96 American patients with familial pancreatic cancer[7]. These findings suggest that *PALB2* mutations may contribute to a small but significant proportion of cancer cases in different populations.

According to the clinicopathologic features of *PALB2* mutation-carrier breast cancer patients detected in this study, all patients had invasive-type ductal cancer. Most cancer patients were classified from intermediate to high-grade types and mostly had hormone receptor-positive expression. Notably, all individuals carrying *PALB2* pathogenic variants were diagnosed at a younger age, most of them aged below 50 years, including six younger than 40 years.

There is no specific study on *PALB2* mutations in a large number of Turkish cancer patients using NGS as in our study. However, researchers in a study aimed to identify the prevalence of *PALB2* variants in *BRCA1/2* and *PALB2*-negative early-onset breast and ovarian cancer patients in a Turkish population[37]. Although the study did not focus solely on *PALB2* mutations, it provides valuable insights into the genetic landscape of hereditary breast and ovarian cancers in Türkiye. Also, in 2016, Cecener et al. investigated all *PALB2* exons in 223 Turkish women with early-onset breast cancer who tested negative for *BRCA1/2* mutations and identified 18 distinct variants by heteroduplex analysis (HDA) and DNA sequencing in Türkiye [38]. However, only a limited number of variants and no conclusively pathogenic variants were detected. Also, Bilen et al. investigated the effects of three different single nucleotide polymorphisms (rs249954, rs249935, and rs16940342) of the *PALB2* gene on Turkish breast cancer predisposition in 2020[39]. Their research aimed only to explore the association between specific single-nucleotide polymorphisms (SNPs) and their impact on breast cancer risk. This study contributes to the growing body of research on the genetic factors influencing breast cancer predisposition and provides valuable insights into the potential role of *PALB2* variants in breast cancer susceptibility.

PALB2 mutations have important clinical implications, particularly regarding cancer risk assessment and genetic testing. It was reported that pathogenic large genomic rearrangements (LGRs) in *PALB2* accounted for 10.3% of pathogenic *PALB2* variants detected in Australian families with familial breast cancer[40], highlighting the importance of considering LGRs in genetic testing for *PALB2* mutations.

Furthermore, *PALB2* mutations have been associated with an increased risk of breast cancer similar to *BRCA2* mutations[41]. Therefore, the inclusion of the *PALB2* in genetic testing panels for high-risk breast and ovarian cancer patients is crucial, as demonstrated in a study on Chinese patients[42].

Of the entire cohort, we identified 13 different PV/LPV in 20 patients, accounting for ~1.9%(20/1056) and 23 different VUS in 28 patients, accounting for ~2.7% (28/1056). However, this result should be taken with caution because the number of patients in the endometrial, pancreatic, and prostate cancer cohort is relatively small compared with the breast cancer cohort that was the part of this study. Overall, the incidence of *PALB2* variants is typically between 0.1% and 1.5%, influenced by the factors such as the study population, the size of the cohort, and the testing methods[43]. The pathogenic *PALB2* variants detected in this study in the Turkish population is about ~1.9% (20/1056 patients), which is slightly higher than the reported frequencies worldwide.

Study Limitations

Firstly, the selection of cancer patients from one hospital for the study may cause bias, and limit the generalizability of the results. Secondly, the small size of the prostate, pancreatic, and colon cancer patients makes it challenging to definitively conclude the non-PALB2 pathogenic variant carriers of cancer patients from the population-based group investigation.

Conclusion

This study successfully determined the *PALB2* variants in cancer patients in Türkiye. The ratio of the *PALB2* variants in cancer patients seems to be slightly higher than the ratio in other populations.

Notably, the recurrent *PALB2* c.557dupA p.(Asn186Lysfs*4) and c.509_510del p.(Arg170Ilefs*14) mutations should be considered as a significant portion of *PALB2* mutation carriers. Recently, the efficacy of PARP inhibitors in *PALB2*-mutated breast cancer patients has been shown, suggesting a possible avenue for targeted therapy that may be helpful for breast cancer patients. Therefore, we recommend that genetic testing for *PALB2* could be integrated into the genetic evaluation of breast cancer patients in Türkiye. This approach might have the potential to make a valuable understanding of breast cancer risks and facilitate the development of prevention and treatment strategies in Türkiye.

Although the number of specific studies on *PALB2* mutations in Turkish cancer patients is scarce, the available evidence from other populations suggests that *PALB2* mutations may contribute to a small but significant proportion of hereditary breast, ovarian, and pancreatic cancers. Further research is needed to determine the prevalence and clinical implications of *PALB2* mutations in the Turkish population.

Ethics Committee Approval

This study was approved by the Clinical Research Ethics Committee of Istanbul Faculty of Medicine in Istanbul University with the decision number of 2023/500 dated 17.03.2023. The study was in compliance with the Helsinki Declaration.

Informed Consent

The written informed consent was obtained from all patients before the study was commenced.

Author Contributions

Seref Bugra Tuncer: Conceptualization, Formal analysis, Investigation, Methodology, Writing-original draft. Seda Kılıc Erciyas: Formal analysis and Investigation. Ozge Sukruoglu Erdogan: Formal analysis, investigation; Betul Celik: Investigation,

Writing-original-draft; Zubeyde Yalnız Kayım; Busra Kurt Gultaslar: Formal analysis.

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Conflict of Interest

There is no conflict of interest to declare.

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