Human Genomics

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Two recurrent pathogenic/likely pathogenic variants in *PALB2* account for half of *PALB2* positive families in Slovenia

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Background

Cancer patients with suspected hereditary breast and ovarian cancer (HBOC) syndrome, along with their healthy relatives, can benefit from multigene panel testing. If they are identified as carriers of pathogenic/likely pathogenic variants (PV/LPVs) in high risk cancer genes, they may be offered prevention strategies such as enhanced cancer surveillance and discuss risk reducing surgeries to lower cancer burden [1]. Multigene panel testing may reveal not only PV/LPVs in *BRCA1* and *BRCA2* genes, but also in other HBOC-related genes.

For over a decade, it has been recognized that germline PV/LPVs in *PALB2* (partner and localizer of BRCA2) are associated with an increased risk of breast cancer (BC). Rahman et al. reported in 2007 the 2.3 fold increase in BC risk in carriers of *PALB2* PV/LPV in comparison to non-carriers [2]. Germline PV/LPVs in *PALB2* are reported in up to 1% of *BRCA1/2* negative breast cancer patients [3, 4]. Kotnik et al. performed a large population-based study in Slovenia between years 2014 and 2022. They identified *PALB2* PV/LPVs in 0.13% of 7091 individuals who were referred for exome sequencing for various rare genetic conditions other than cancer [5]. Other malignancies, such as male breast cancer [6], pancreatic cancer (PaC) [7], ovarian (OC), prostate, colorectal, and gastric cancer [8] were also reported in carriers of germline PV/LPVs in *PALB2*. The risk estimates are, however, based on analysis of small patients' cohorts, the existing literature presents conflicting data, and the statistical evidence remains weak. Notably, the largest study so far by Yang et al. on 524 families from 21 countries, demonstrated a substantial association between germline *PALB2* PV/LPVs and ovarian cancer (RR 2.91), pancreatic cancer (RR 2.37), and male breast cancer (RR 7.34) [9]. Nevertheless, further studies are necessary to enhance our understanding.

The *PALB2* gene encodes a protein that acts as a bridge between BRCA1 and BRCA2 proteins, playing a crucial role in homology-directed recombination DNA repair [10]. There is some evidence that new targeted treatments, which are effective in *BRCA1* and *BRCA2* PV/LPV carriers (such as poly-ADP-ribose polymerase (PARP) inhibitors), are also effective in individuals with *PALB2* PV/LPVs, which is unsurprising given the shared underlying biology [11]. Understanding an individual's *PALB2* status is therefore essential for personalised management, not only in preventive setting, but also when making treatment decisions for these cancer patients.

The prevalence and spectrum of PV/LPVs may vary across different regions due to ethnic differences. Quantifying cancer risks associated with specific PV/LPVs and understanding the biological characteristics of malignancies in carriers with these variants is important for establishment of targeted clinical guidelines.

Institute of Oncology Ljubljana (IOL), where the study was conducted, is the principal national institution that supervises programs on comprehensive cancer care in Slovenia, which is a central European country with

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a population of two million. IOL offers cancer genetic counselling and genetic testing of high-risk individuals at the national level and therefore serves as a referral tertiary centre for the whole country.

In the Slovene HBOC cohort the prevalence and spectrum of germline *PALB2* PV/LPVs have not yet been analysed and reported. Our study aimed to describe these PV/LPVs in *PALB2* and analyse the types of cancer and age of cancer diagnosis in *PALB2* PV/LPV carriers.

Methods

Patients

Multigene panel testing with next generation sequencing (NGS) for HBOC-related genes was introduced at the IOL in late 2014. The latest gene panel (in use since 2022) consists of nineteen genes: *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *EPCAM*, *MLH1*, *MSH2*, *MSH6*, *NF1*, *PALB2*, *PMS2*, *PTEN*, *RAD51C*, *RAD51D*, *STK11*, *TP53*. A proband (an index case) was defined as a family member (usually an affected individual) through whom a family with a PV/LPV is ascertained. If a PV/LPV was diagnosed in more than two seemingly unrelated families, it was considered a recurrent variant for the purpose of this study. Our retrospective study cohort encompassed 5099 individuals (4564 females and 535 males) from 4610 HBOC families who underwent genetic counselling in our Cancer Genetics Clinic at the Institute of Oncology Ljubljana and were referred to germline genetic testing between January 2015 and January 2022.

Family history data was collected from all tested families and all cancer diagnoses were verified in the Slovenian Cancer Registry. The registry contains data of all cancer diagnoses since 1950, when compulsory reporting of cancer diagnosis started in Slovenia. Positive family history for HBOC syndrome was defined as a family history of at least one first- or second-degree relative with breast, ovarian, prostate, or pancreatic cancer. We disregarded cases of non-melanoma skin cancer and cervical cancer in the analysis.

Family members of carriers of PV/LPVs were offered either cascade genetic testing for known PV/LPV in the family or NGS panel testing in case they fulfilled the inclusion criteria for panel testing. If the tested individuals gave their consent to report secondary findings (reporting the finding of a PV/LPV not initially suspected), secondary findings were also reported according to the ACMG criteria [12]. Patients who harboured variants of unknown significance were not included in the analysis.

All participants provided written informed consent. The present study was approved by the National Ethics Committee and the Institutional Ethics Committee of the Institute of Oncology Ljubljana (0120–591/2020/3 on the 20th of January 2021). Research was conducted according to the 1975 Helsinki Declaration as revised in 1983 and the procedures used met the ethical standards of these bodies.

DNA extraction

DNA was isolated from blood samples according to the established laboratory protocol as previously published [13].

Next generation sequencing

Next generation sequencing (NGS) was performed on Illumina MiSeqDx Sequencing System using TruSight Cancer Panel or TruSight Hereditary Panel (Illumina, San Diego, CA, USA) to enrich and sequence all translated exons and ±25 bp flanking intronic regions of all HBOC panel genes. Bioinformatics and copy number analysis were performed as described by our group previously [14, 15]. Germline variants were classified for their clinical importance according to ACMG/AMP guidelines [12, 16]. Variants were described according to HGVS v20.05 nomenclature [17]. All PV/LPV germline variants in HBOC genes detected by NGS were additionally confirmed by Multiplex Ligation-dependent Probe Amplification analysis or Sanger sequencing as described by our group previously [13].

Haplotype analysis

All together 21 patients were tested for the presence of a common haplotype, 11 patients from 8 families with *PALB2*:c.509_510delGA p.(Arg170Ilefs*14) variant and 12 patients from 7 families with *PALB2*:c.1451T>A p.(Leu484*) variant. Seven STR markers (21732AC23, 23037GT23, D16S412, 23622TCTA14, 23749TATG14, D16S417, D16S401) spanning a region of 3 Mb on chromosome 16 in the vicinity of *PALB2* gene, were genotyped using FAM labelled forward primers. PCR products were separated by capillary electrophoresis (ABI3500, ThermoFisher Scientific, Waltham, MA, USA). Detailed description of the primers and PCR conditions were published previously by Catucci and colleagues [18]. Haplotypes were puzzled together manually based on the PCR product length.

Copy number variant detection

Detection of copy number variants (deletion of single or multiple exons) from NGS data was performed using SeqNext v4.4.0 – v.5.2.0 (JSI medical systems, Ettenheim, Germany) as previously described by Klančar et al. [15].

PMS2 variant detection

Variants detected in the *PMS2* gene were confirmed using long-range PCR (LongAmp Taq 2X Master Mix, New England Biolabs, Ipswich, MA, USA) followed by Sanger sequencing. To avoid amplifying the highly

homologous *PMS2* pseudogenes, primers for long-range PCR were specifically designed to amplify only the *PMS2* gene. The method is described in detail by Clendenning and colleagues [19].

Statistical analysis

Statistical analysis was performed using SPSS software (version 25). We used descriptive statistics to describe patients' clinical, pathological, and genetic characteristics.

Results

Study cohort

From January 1st 2015 to January 31st 2022, 5099 individuals (535 males and 4564 females) from 4610 families were tested for germline PV/LPVs in HBOC-related genes. The median age of individuals at the time of testing was 54 years. The characteristics of the cohort are shown in Supplementary Table 1.

PV/LPV detection rate among 4610 tested probands/families

In 19.1% (883/4610) of tested families a germline PV/LPV in HBOC-related genes was detected. *BRCA1* PV/LPVs were detected in 8.4% (386/4610). Additionally, PV/LPVs were detected in *BRCA2* in 4.9% (224/4610) of all probands, in *CHEK2* in 1.8% (83/4610), in *ATM* in 1.5% (69/4610) and in *PALB2* in 0.9% (40/4610). The frequency of all PV/LPVs in HBOC-related genes is presented in Supplementary Fig. 1.

PALB2 PV/LPVs were detected in 1.0% of all *BRCA1/2* negative families (40/4000). In 22 out of 883 (2.5%) families, probands were diagnosed with two PV/LPVs and in one family (0.1%) one proband was diagnosed with three PV/LPVs in the HBOC-related genes.

PALB2 study cohort

We identified *PALB2* PV/LPVs carriers in 40 HBOC families. Within these 40 families, a total of 60 family members were identified as carriers of a *PALB2* PV/LPV.

Spectrum of *PALB2* PV/LPVs in the Slovenian cohort of 40 *PALB2* positive families

We identified 13 different *PALB2* PV/LPVs, which are shown in Table 1; Fig. 1.

PALB2 c.912 del p.(Val305*) is a novel variant and had previously not been reported in the literature. It was found in one proband (Table 1).

Four PV/LPVs were recurrent. The two most frequent were c.509_510del and c.1451T>A, detected in 10 different families each, together encompassing half (20/40, 50.0%) of all PV/LPVs detected in our population.

Haplotype analysis

Haplotype analysis was performed for both *PALB2* c.509_510delGA and c.1451T>A variants. All 7 unrelated variant c.509_510delGA carriers share a common core haplotype of approximately 0.61 Mb in length. Recombination event presumably occurred between hg19 genomic coordinates chr16:23037671-chr16:23162662 (23037GT23 - D16S412) and chr16:23777202-chr16:24686016 (D16S417 - D16S401). Additionally, three of those unrelated carriers (Family 5, 7, 8) also share a larger common haplotype of at least 3 Mb (spanning over all 7 STR markers), that overlaps with a core haplotype. Neither the 3 Mb haplotype nor the 0.61 Mb core haplotype was detected in non-carriers, suggesting a common ancestor. Data is shown in Supplementary Fig. 2.

Additionally, all tested carriers of pathogenic variant c.1451T>A share a distinct haplotype spanning across 0.15 Mb. The recombination events occurred between coordinates chr16:23162662-chr16:23622400 (D16S412–23622TCTA14) and chr16:23777202-chr16:24686016 (D16S417 - D16S401), which is shown in Supplementary Fig. 3. This common haplotype suggests that the *PALB2* c.1451T>A variant originated from a single ancestor.

Cancer types diagnosed in *PALB2* PV/LPV carriers

Out of the 60 *PALB2*-positive patients, 42 (70.5%) were diagnosed with at least one type of cancer (2 males, 40 females). The distribution of cancer types and ages at diagnosis are illustrated in Fig. 2. The median age at the diagnosis of the first malignancy was 47 years (range 32 years – 69 years).

Among 36 *PALB2* positive BC (invasive and in situ) patients, 35 were females and one was male. The median age at BC diagnosis was 46.5 years, ranging from 32 years to 69 years. The median age at genetic testing was 51 years, ranging from 21 years – 79 years. Twelve different PV/LPVs were identified in *PALB2* positive BC patients.

Clinical characteristics of double heterozygotes

Among *PALB2* PV/LPV carriers, three patients were classified as double heterozygotes (DH). Their clinical characteristics are presented in Table 2.

Multiple primary cancers

In total, 12 out of 42 (28.6%) *PALB2* positive patients with cancer were diagnosed with more than one malignant tumor. Among them, 7/12 had negative family history and 3/7 had their first cancer diagnosed after the age of 50 years. Median interval between first diagnosis and a new primary cancer was 8 years (range 1–18 years). Characteristics of patients with multiple primary malignancies are presented in Table 3.

Table 1 Spectrum of *PALB2* PV/LPVs in the Slovenian population

***PALB2* PV/LPV type**	**Previously reported**	**ACMG/AMP and Variant class**	**Variant Type**	***N* of carriers**	***N* of families (% of all families)**
c.1451T>A p.(Leu484*)	yes	PV Class 5	nonsense	19	10 (24.5%)
c.509_510del p.(Arg170Ilefs*14)	yes	PV Class 5	frameshift	14	10 (24.5%)
c.1027 C>T p.(Gln343*)	yes	PV Class 5	nonsense	8	6 (14.7%)
c.172_175del p.(Gln60Argfs*7)	yes	PV Class 5	frameshift	5	4 (9.8%)
c.3549 C>G p.(Tyr1183*)	yes	LPV Class 4	nonsense	2	2 (4.9%)
c.1317del p.(Phe440Leufs*12)	yes	PV Class 5	frameshift	3	1 (2.4%)
c.1240 C>T p.(Arg4*)	yes	PV Class 5	nonsense	3	1 (2.4%)
c.48G>A p.Cys11*	yes	PV Class 5	synonymous, splicing	1	1 (2.4%)
c.1676_1677delinsG p.(Gln559Argfs*2)	yes	PV Class 5	frameshift	1	1 (2.4%)
c.3164dup p.(Tyr1055*)	yes	PV Class 5	nonsense	1	1 (2.4%)
deletion of exons 11–12 c.(3113+1_3114-1)_(3350+1_3351-1)del p.?	yes	PV Class 5	multiple exon deletion	1	1 (2.4%)
c.395del p.(Val132Alafs*45)	yes	PV Class 5	frameshift	1	1 (2.4%)
c.912del p.(Val305*)	no	PV Class 5	nonsense	1	1 (2.4%)
All *PALB2* PV/LPVs				**60**	**40 (100%)**

Legend: PV/LPV=pathogenic variant/likely pathogenic variant, ACMG/AMP classification [12], classification system proposed by Plon et al. [16]

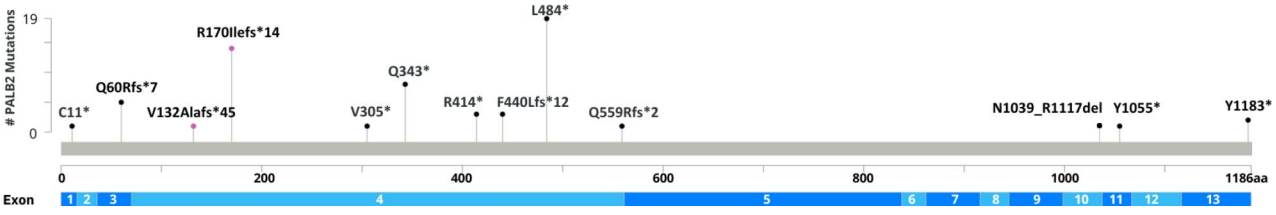

Fig. 1 A lollipop plot showcasing all PV/LPV in the *PALB2* gene identified within our cohort. The plot was created using MutationMapper [20, 21]

As shown in Table 3, one carrier of *PALB2* PV/LPV, who also harboured a *BRCA1* PV/LPV, was diagnosed with four primary cancers: twice with BC (at 42 and 67 years), a high-grade serous carcinoma of the ovary (age at diagnosis 57) and a pancreatic adenocarcinoma (age at diagnosis 69). Additionally, two *PALB2* positive patients both developed BC (aged 48 and 56, respectively) and melanoma (aged 47 and 53, respectively).

PALB2 positive carriers without cancer diagnosis

Our cohort of *PALB2* PV/LPV carriers without a cancer diagnosis consisted of 18 individuals from 11 families. Thirteen were female and five were male. Two of them were identified through panel testing and 16 by cascade testing. Median age at genetic testing was 44 years (range 21 – to 75 years).

Among these individuals, *PALB2* c.1451T>A p.(Leu484*) PV/LPV was found in 11 individuals from six families, *PALB2* c.509_510del (p.(Arg170Ilefs14)) was found in two individuals from two different families, *PALB2* c.1317del p.(Phe440Leufs12) was found in two individuals from the same family, and one individual was identified with each of the following PV/LPVs:

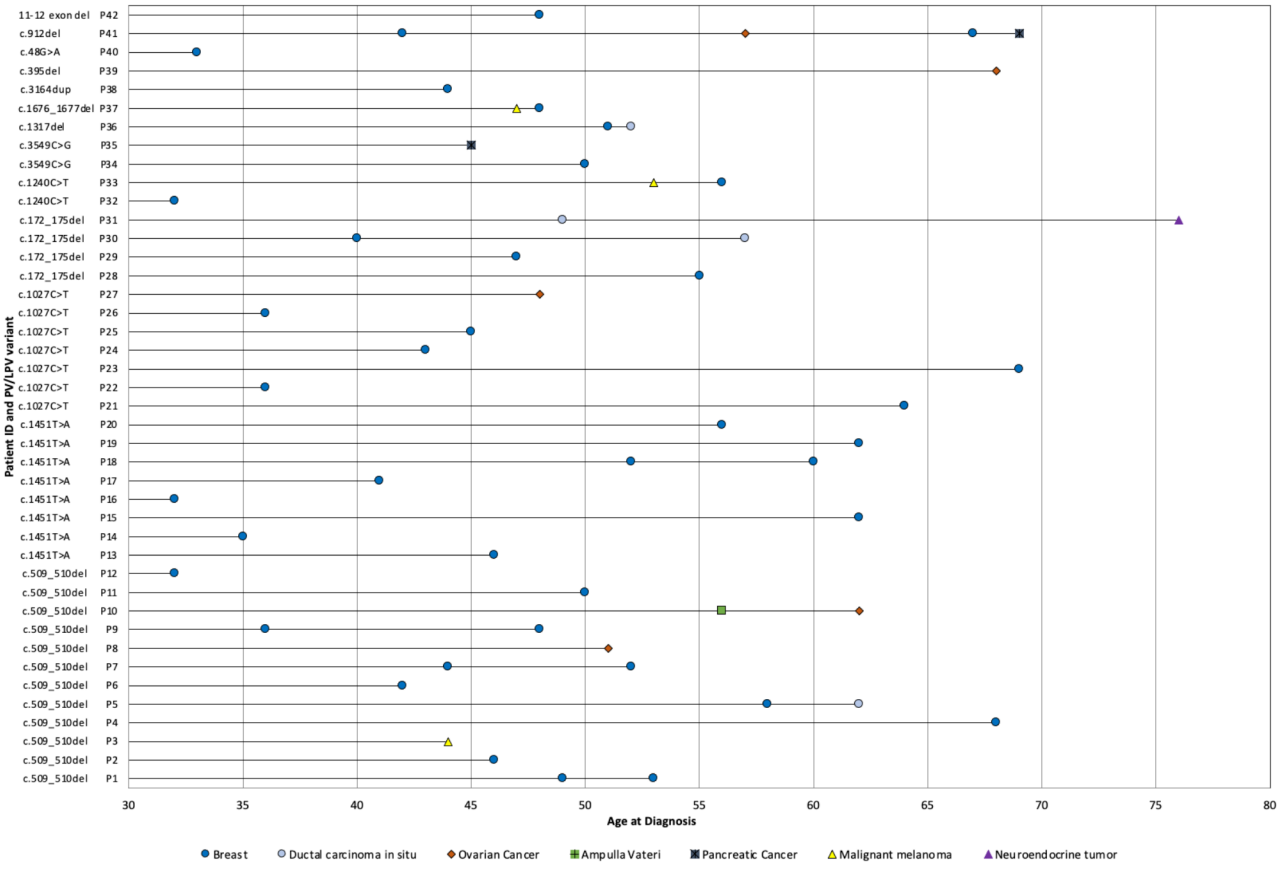

Fig. 2 PV/LPV variant, type of cancer and age at diagnosis in *PALB2* PV/LPV carriers

Table 2 Characteristics of double heterozygotes

Patient (sex)	*PALB2* PV/LPV	Other HBOC gene PV/LPV	Cancer type (age at diagnosis)	Histopathological Characteristics
Patient 43 (f)	deletion of exons 11–12 c.(3113 + 1_3114-1)_(3350 + 1_3351-1)del p.?	*ATM* c.2413 C > T p.(Arg805*)	BC (48)	poorly differentiated, bifocal, luminal B IDC
Patient 17 (f)	c.1451T > A p.(Leu484*)	*CHEK2* c.1100del p.(Thr367Metfs*15)	BC (32)	poorly differentiated, triple negative IDC
Patient 42 (f)	c.912del p.(Val305*)	*BRCA1* c.181T > G p.(Cys61Gly)	BC (42)	atypical medullary
			OC (57)	HGSC
			BC (67)	unknown histology
			PaC (69)	adenocarcinoma

Legend: PV/LPV = pathogenic variant/likely pathogenic variant, BC = breast cancer, OC = ovarian cancer, PaC = pancreatic cancer, HGSC = high-grade serous carcinoma, FIGO = the International Federation of Gynecology and Obstetrics, f = female, m = male

PALB2 c.1240 C > T p.(Arg414*), *PALB2* c.1027 C > T p.(Gln343*), and *PALB2* c.172_175del p.(Gln60Argfs*7).

Discussion

Having epidemiological data on the frequency and spectrum of germline PV/LPVs associated with different hereditary cancers is of the utmost importance for every country aiming to organize an optimal cancer prevention programme and optimize cancer patients' management. Very little is known about the characteristics of carriers of *PALB2* PV/LPVs and the clinicopathological characteristics of tumors diagnosed in these patients. This gap in knowledge underscores the importance of further research into *PALB2*-associated cancers, not only for specialized institutions but also for primary care physicians.

Table 3 Characteristics of *PALB2* positive patients with multiple primary malignancies

Patient (sex)	Patient with PV/LPVs	Type of Cancer (laterality, age at diagnosis)	Family history (number of affected family members)
Patient 33 (f)	*PALB2* c.1240 C > T p.(Arg414*)	melanoma (53), BC (left, 56)	positive (1)
Patient 10 (f)	*PALB2* c.509_510del p.(Arg170Ilefs*14)	papilla Vateri (56), fallopian tube (62)	negative
Patient 37 (f)	*PALB2* c.1676_1677delinsG p.(Gln559Argfs*2)	melanoma (47), BC (right, 48)	positive (1)
Patient 31 (f)	*PALB2* c.172_175del p.(Gln60Argfs*7)	DCIS (right, 49), NET origo ignota (67)	negative
Patient 42 (f)	*PALB2* c.912del p.(Val305*) *BRCA1* c.181T > G p.(Cys61Gly)	BC (right, 42), (left, 67), OC (57), PaC (69)	positive (1)
Patient 1 (f)	*PALB2* c.509_510del p.(Arg170Ilefs*14)	BC (left, 49, (left, 53)	positive (3)
Patient 7 (f)	*PALB2* c.509_510del p.(Arg170Ilefs*14)	BC (left, 44), (right, 52)	negative
Patient 5 (f)	*PALB2* c.509_510del p.(Arg170Ilefs*14)	BC (left, 58), (right, 62)	negative
Patient 9 (f)	*PALB2* c.509_510del p.(Arg170Ilefs*14)	BC (left, 36), (right, 48)	negative
Patient 18 (f)	*PALB2* c.1451T > A p.(Leu484*)	BC (right, 52), (left, 60)	negative
Patient 36 (f)	*PALB2* c.1317del p.(Phe440Leufs*12)	BC (right, 51), BC (right, 52), DCIS (left, 52)	negative
Patient 30 (f)	*PALB2* c.172_175del p.(Gln60Argfs*7)	BC (right, 40), (right, 57)	positive (2)

Legend: PV/LPV = pathogenic variant/likely pathogenic variant, BC = breast cancer, OC = ovarian cancer, DCIS = ductal carcinoma in situ, NET = neuroendocrine tumor, PaC = pancreatic cancer; f - female

Detection rate and spectrum of *PALB2* PV/LPVs

PV/LPVs in *PALB2* were diagnosed in 0.9% of all individuals tested, making *PALB2* the fifth most commonly mutated gene in our cohort. This was expected as it had previously been reported that 0.2–0.9% of women with BC who undergo genetic testing will carry germline PV/LPV in *PALB2* [22].

In 40 families 13 different *PALB2* PV/LPVs were detected. *PALB2* c.912 del p.(Val305*) had not been reported previously and was found in one proband. Newly described *PALB2* PV/LPVs in patients with BC are important since they can contribute to international databases and patients may benefit from prevention and treatment options.

Recurrent PV/LPVs in *PALB2* in the Slovenian population

Four PV/LPVs in *PALB2* were recurrent in our population, with *PALB2* c.509_510del and *PALB2* c.1451T > A being the two most frequent, detected in 10 different families each, and together encompassing half (20/40 or 50.0%) of all detected PV/LPVs in our population. Different recurrent *PALB2* PV/LPVs have been reported in populations around the world, such as those from Argentina [23], Finland [24], Greece [25], and Poland [26]. *PALB2* c.509_510del has been described by Noskowitz et al. as being present in about 1 in 400 unselected breast cancer patients from Central Europe (Germany) and Eastern Europe (Belarus, Russia) [26]. *PALB2* c.509_510del has also been described as a recurrent variant in BC and OC patients from Poland [27]. We found no reports on the presence of *PALB2* c.509_510del in Western European, Asian or American populations or no genotype-phenotype correlation studies for this specific PV. Of note, three patients from our cohort were diagnosed with a metachronous contralateral BC, one additional with a metachronous contralateral and ipsilateral BC. We found two cases of OC with a median age at diagnosis 56.5 years (range 51 years – 62 years), one case of carcinoma of the papilla Vateri and one malignant melanoma. Based on our data BC patients harbouring *PALB2* c.509_510del are at high risk of developing a second BC and OC, making them high-risk group among *PALB2* PV carriers, therefore enhanced surveillance is warranted.

PALB2 c.1451T > A has not yet been described as a recurrent variant in any population in the literature, however it has been found in nineteen individuals from ten different families in our cohort, which makes it a unique recurrent variant in the Slovenian population. Among nineteen carriers of the above-mentioned PV, eight were diagnosed with BC, with a median age at diagnosis 49 years. While it is challenging to establish genotype-phenotype correlation, this information could still be valuable in everyday clinical practice and may be included in the studies with bigger sample sizes.

Malignancies among *PALB2* PV/LPV carriers

It has been known for more than a decade that *PALB2* PV/LPVs increase BC risk [28]. The risk is 2–30 times higher than in the general population, depending on the type of PV/LPV, age, and family history and *PALB2* is considered a high-penetrance susceptibility gene for BC [29]. As expected, the most common malignancy diagnosed in our cohort was BC in 36 patients, which confirms this association. Recent research has strengthened the correlation between *PALB2* PV/LPVs and OC. Yang et al. demonstrated a substantial association between germline *PALB2* PV/LPVs and OC with a risk ratio of

around 3 and the lifetime risk of OC estimated to be around 3–5% [9]. OC with 5 cases was indeed the second most prevalent malignancy in our study. Two *PALB2* PV/LPV carriers in our cohort (2/60, 3.3%) were diagnosed with PaC and additional carrier with carcinoma of Papilla Vateri. It is estimated that 3–4% of patients with familial PaC are expected to harbour *PALB2* PV/LPV. In the newest version of the National Comprehensive Cancer Network (NCCN) Guidelines enhanced screening not only for BC (with possible risk reducing surgeries), but also for OC (risk reducing surgery is offered as an option) and PaC is recommended [30].

Double heterozygotes

The increased use of multigene panels in the recent years has led to identification of individuals harbouring more than one PV/LPV in cancer susceptibility genes, although the data is still scarce [31]. The results of the studies on DH PV in HBOC-related genes in populations other than Ashkenazi Jews have been conflicting and inconclusive. To the best of our knowledge no research has been conducted on DH with one of the variants being *PALB2* PV/LPV. There is emerging evidence of multiplicative effect of presence of PV/LPVs in more than one cancer susceptibility gene. Heidemann et al. showed that Caucasian female DH for *BRCA1/2* seem to develop BC at a younger age and have more severe disease than carriers of a single *BRCA1/2* PV/LPV [32]. Similarly, Sokolenko et al. pointed out that the presence of additional gene defect in female *BRCA1* PV carriers may further increase their chances for cancer [33]. On the other hand, Lavie et al. suggest that DH PV in *BRCA1/2* in females of Ashkenazi Jewish heritage does not seem to cause a more severe phenotype than in cases where only one of the genes is implicated [31].

Among *PALB2* PV/LPV carriers, three double heterozygotes were identified, with the second PV/LPV located in one of the other hereditary BC genes. Although all three patients had early-onset BC (<50 years), the contribution of each PV/LPV likely varied in terms of causality. PV/LPVs in *BRCA1* and *PALB2* are highly penetrant, associated with a strong genetic effect, and significantly increase the risk of BC and OC. In contrast, PV/LPVs in *CHEK2* are considered moderately penetrant, with a much lower genetic effect compared to *PALB2* and *BRCA1*.

In our cohort, one double heterozygote also carried the *CHEK2* variant c.1100del together with *PALB2* PV. For females with this variant, the odds ratio (OR) for breast cancer risk is 2.66, indicating a moderate risk. Hinić et al. suggest that individuals with biallelic *CHEK2* PVs display a more severe phenotype than heterozygous carriers, with earlier onset, more cancer cases, and more instances of multiple cancers [34].

Strengths and limitations of our study

There are several limitations of our study. The expected population burden of *PALB2* PV/LPVs in the Slovenian population is 0.13% [5]. The absolute number of included PV/LPV carriers was small (60). 42 (70.0%) had a cancer diagnosis. However, our cohort represents 2% of the expected population of *PALB2* PV/LPV carriers in Slovenia. Also, in comparison to the literature, where mostly case series are described, this is a large cohort of *PALB2* PV/LPV carriers. The subgroups of patients with different PV/LPV were very small, therefore we were not able to analyse them separately. The retrospective collection of data is always unfavourable, since data can be missing or inappropriately understood, however as it can be seen from our data, the information regarding patients' and tumours' characteristics was complete for patients in our study.

Its strength lies in a reliable family history, with information obtained from the Slovenian National Cancer Registry. It is one of the oldest Registries in Europe, where the diagnoses are cross-checked with histopathological reports.

In cases of rare genetic diseases large multicentric studies are required to achieve a substantial number of patients and we hope these will be able to benefit from our cohort of *PALB2* PV/LPV carriers.

Conclusions

This report provides the first comprehensive insight into the genotype and phenotype of *PALB2* PV/LPV carriers in Slovenia. The frequency of *PALB2* PV/LPV in Slovenia is consistent with rates reported in other countries, accounting for 1.0% of all individuals tested for PVs in HBOC-related genes. Notably, we identified four recurrent *PALB2* PV/LPVs within our population, collectively encompassing more than half of all affected families. Of particular interest is the *PALB2* c.1451T>A variant, which has not been previously documented as a recurrent variant in any population, rendering it unique to Slovenia. The most common malignancy in our cohort was as expected BC, followed by OC and PaC, which adds to the existing evidence of *PALB2* involvement in the pathogenesis of these cancer types. Despite the rarity of *PALB2* carriers, who also carry PV/LPVs in other HBOC-related genes, we have identified three such individuals, and studying their disease characteristic can help elucidate the biological effect of being a DH. Overall, the results of our study provide valuable genotype and phenotype data from *PALB2* positive patients which may already be utilized in a population specific assessment.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40246-024-00706-5.

Supplementary material 1

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Author contributions
Conceptualization: VAM, MK, Methodology: MK, KDS, SH, VŠD, VS, SN; Formal analysis and investigation: VŠD, PŠ, SN, VS, VAM, MK; Writing - original draft preparation: VAM; Writing - review and editing: MK, AB, KS, KDS, VŠD, VS, SN. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval
All participants provided written informed consent. The present study was approved by the National Ethics Committee and the Institutional Ethics Committee of the Institute of Oncology Ljubljana (0120–591/2020/3 on the 20th of January 2021). Research was conducted according to the 1975 Helsinki Declaration as revised in 1983 and the procedures used met the ethical standards of these bodies.

Competing interests
The authors declare no competing interests.

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