

## Research Article

The *PALB2* Gene Is a Strong Candidate for Clinical Testing in *BRCA1*- and *BRCA2*-Negative Hereditary Breast Cancer

Marketa Janatova<sup>1</sup>, Zdenek Kleibl<sup>1</sup>, Jana Stribrna<sup>1</sup>, Ales Panczak<sup>2</sup>, Kamila Vesela<sup>2</sup>, Martina Zimovjanova<sup>4</sup>, Petra Kleiblova<sup>1,2</sup>, Pavel Dundr<sup>3</sup>, Jana Soukupova<sup>1</sup>, and Petr Pohlreich<sup>1</sup>

## Abstract

**Background:** Several reports indicate that inherited mutations in the *PALB2* gene predispose to breast cancer. However, there is little agreement about the clinical relevance and usefulness of mutation screening in this gene. We analyzed the prevalence and spectrum of germline mutations in *PALB2* to estimate their contribution to hereditary breast and/or ovarian cancer in the Czech Republic.

**Methods:** The entire *PALB2* coding region was sequenced in 409 breast/ovarian cancer patients negative for *BRCA1* and *BRCA2* mutations. Testing for large genomic rearrangements (LGR) was performed by multiplex ligation-dependent probe amplification (MLPA) analysis.

**Results:** We have identified 13 different pathogenic alterations including 10 truncating mutations and three LGRs in 16 of 409 patients (3.9%), whereas one truncating mutation was found in a group of 1,226 controls (0.08%;  $P = 2.6 \times 10^{-9}$ ). Three novel LGRs included deletions involving exons 7–8 and 9–10, respectively, and a duplication spanning exons 9–11. Five frameshift and two nonsense mutations were novel, whereas three truncating mutations were described previously. The only recurrent mutation was the c.172\_175delTTGT detected in four unrelated breast cancer individuals.

**Conclusions:** Our analyses demonstrated the significant role of the *PALB2* gene in breast cancer susceptibility. The highest frequency of *PALB2* mutations (comparable with that previously reported for *BRCA2*) was found in a subgroup of patients with hereditary breast cancer (HBC) (13/235; 5.5%).

**Impact:** Our results show that mutation analysis of the *PALB2* gene, including the analysis of LGRs, is primarily indicated in patients with HBC in case of their *BRCA1* and *BRCA2* negativity. *Cancer Epidemiol Biomarkers Prev*; 22(12); 2323–32. ©2013 AACR.

## Introduction

Mutations in the *BRCA1* and *BRCA2* genes represent the most important genetic risk factors for hereditary breast cancer (HBC). In the Czech Republic, the germline mutations in these two breast cancer genes account for approximately 3% of unselected breast cancer cases and about 23% of familial breast cancer cases (1, 2). Mutations identified in other breast cancer-susceptibility genes, *ATM* (3), *CHEK2* (4), *TP53* (5), and *NBN* (6), were significantly less frequent and together were responsible for about 3% to 4% of patients with familial breast cancer. These results indi-

cate that mutations in other relevant genes may be involved in genetic susceptibility to breast cancer.

The *PALB2* protein (partner and localizer of *BRCA2*) was described as a binding partner of the *BRCA2* protein, essential for its function in DNA double-strand break repair (7). It also binds to *BRCA1* and mediates the interaction between *BRCA1* and *BRCA2* (8, 9). The *PALB2* gene (OMIM# 610355) was recently identified (10) as a Fanconi anemia (FA) gene (known as *FANCN*). Its biallelic mutations cause Fanconi anemia subtype N with a phenotype similar to that (FA-D1) caused by biallelic *BRCA2* mutations. Studies performed in the United Kingdom (11) and Finland (12) demonstrated that monoallelic mutations in *PALB2* predispose to breast cancer. Five different *PALB2* mutations in 10 of 923 (1.1%) individuals with familial breast cancer were found in the United Kingdom. A founder *PALB2* c.1592delT mutation, associated with a roughly 4-fold increased hereditary propensity for female breast cancer, was detected in Finland in 3 of 113 (2.7%) patients with familial breast cancer and in 18 of 1,918 (0.9%) unselected breast cancer cases. Until now, about 50 truncating mutations have been detected in breast cancer families worldwide (11–32) and mutations have also been reported in cases of inherited pancreatic (33–35) and ovarian cancer (18, 36). Therefore, *PALB2* has been

**Authors' Affiliations:** <sup>1</sup>Institute of Biochemistry and Experimental Oncology, First Faculty of Medicine, Charles University in Prague; <sup>2</sup>Institutes of Biology and Medical Genetics and <sup>3</sup>Pathology, and <sup>4</sup>Department of Oncology, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic

**Note:** Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (<http://cebp.aacrjournals.org/>).

**Corresponding Author:** Petr Pohlreich, Institute of Biochemistry and Experimental Oncology, First Faculty of Medicine, Charles University in Prague, U Nemocnice 5, 128 53, Prague 2, Czech Republic. Phone: 420-22496-5745; Fax: 420-22496-5732; E-mail: petr.pohlreich@lf1.cuni.cz

doi: 10.1158/1055-9965.EPI-13-0745-T

©2013 American Association for Cancer Research.

considered a strong candidate breast cancer–susceptibility gene; however, due to the limited number of studies, estimation of penetrance of breast cancer associated with *PALB2* mutation has not been precisely determined (37). The population of the Czech Republic and other countries in the Central Europe was not screened for *PALB2* mutations.

We sequenced the entire coding region of the *PALB2* gene in a group of patients with high-risk *BRCA1*- and *BRCA2*-negative breast/ovarian cancer. The purpose of the study was to characterize the spectrum of *PALB2* mutations and to estimate their contribution to the development of inherited breast and ovarian cancer.

## Materials and Methods

### Characteristics of high-risk breast cancer patients, unselected breast cancer patients, and controls

In total, 409 patients with high-risk breast and/or ovarian cancer negatively tested for the presence of pathogenic mutations in *BRCA1* and *BRCA2* genes in our institute between 2000 and 2010 were selected for this study (Table 1). The analyzed group included 330 familial cases and 79 cases without reported family history of cancer (referred to herein as nonfamilial cancer cases). Familial cases met the following criteria in first- or second-degree relatives: two cases of either breast cancer diagnosed before the age of 50 years or ovarian cancer diagnosed at any age; and three or more cases of breast cancer or ovarian cancer diagnosed at any age. Nonfamilial cases included male breast cancer cases, patients with bilateral breast cancer with the first diagnosis before the age of 50 years, and patients with primary breast and ovarian cancer. The average BRCA-PRO (38) risk estimates for each group of patients are given in Table 1.

The unselected breast cancer group included DNA samples from 704 consecutive female patients with breast cancer. These patients were treated at the Department of Oncology, General University Hospital in Prague (Prague, Czech Republic) between 2004 and 2006 and were

included into the study regardless of the age at diagnosis and breast cancer family history. The average age of breast cancer diagnosis in this group was 56.5 years (range, 27–93 years) and the majority of samples involved patients with ductal and lobular histologic subtypes (72% and 11%, respectively).

The control group consisted of DNA samples obtained from 1,226 individuals involving 756 noncancer individuals and 470 blood donors described previously (4, 39).

All patients and controls were of a Czech origin and were living in the Prague area. The study was approved by the Ethical Committee of the First Faculty of Medicine and the General University Hospital and all participants gave their written informed consent with the use of stored DNA/RNA samples for research purposes.

### Mutation analysis

Genomic DNA and total RNA from peripheral blood samples of high-risk individuals was isolated using standard extraction methods as described previously (40). The mutation analysis of the *PALB2* coding sequence was based on cDNA sequencing. Total RNA was reverse transcribed into cDNA using an Expand Reverse Transcriptase kit (Roche) and random hexamers (Roche) according to the manufacturer's instructions. The entire coding region corresponding to *PALB2* mRNA was divided into three overlapping fragments that were PCR-amplified. Amplifications were carried out in 10- $\mu$ L reaction mixtures containing 1  $\mu$ L 10 $\times$  PCR buffer, 0.2 mmol/L of each dNTP, 0.4  $\mu$ mol/L of each primer, 1  $\mu$ L of cDNA template, and 0.5 U of Fast Start Taq DNA polymerase (Roche). Following the initial denaturation (at 95°C for 4 minutes), 35 cycles (at 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 2 minutes) and a final extension (at 72°C for 7 minutes) were performed. Primer sequences are listed in Supplementary Table S1. PCR products were purified with ExoSAP-IT (USB Corp.) according to the supplier's instructions and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI 3130

**Table 1.** High-risk *BRCA1/BRCA2*-negative cancer patients tested for *PALB2* mutations

Group	Patients; N	Mean age at dg; y	BRCAPRO; mean value	Mutations; N (%)
Familial cases (N = 330)				
HBC families	235	47.2	0.16	13 (5.5)
HBOC families	90	47.8	0.35	0
HOC families	5	40.2	0.35	0
Nonfamilial cases (N = 79)				
Male breast cancer	22	64.7	0.14	1 (4.5)
Bilateral breast cancer before 50	39	43.7	0.30	1 (2.6)
Tumor duplicity, breast cancer + ovarian cancer	18	53.7	0.33	1 (5.6)
Total	409			16 (3.9)

Abbreviations: HBOC, hereditary breast and ovarian cancer; HOC, hereditary ovarian cancer.

genetic analyzer (Applied Biosystems). All mutations were confirmed by the DNA sequencing analysis and reconfirmed from a new blood sample.

Each identified *PALB2* mutation that caused a truncation of the protein product was screened in 1,226 control samples by a high-resolution melting (HRM) analysis using an HRM Master kit and the LightCycler 480 (Roche) according to the manufacturer's instructions. Primer sequences are listed in Supplementary Table S1. Mutations in samples with an aberrant melting profile were confirmed by sequencing from separate PCR reactions.

The *PALB2* c.172\_175delTTGT mutation detected repeatedly in breast cancer families was screened in a group of 704 unselected breast cancer cases by HRM analysis of exon 3.

### Detection of large genomic rearrangements

The multiplex ligation-dependent probe amplification (MLPA) Kit for *PALB2* (probemix P260-A1; MRC-Holland) was used for a relative quantification of each of the *PALB2* exons according to the manufacturer's protocol. The amplified products were separated on an ABI 3130 genetic analyzer and the MRC Coffalyser software (MRC Holland) was used for fragment analysis. Samples differing in a relative peak area by more than 30% were reanalyzed using an independent DNA sample. The breakpoints of large genomic rearrangements (LGR) identified by MLPA were determined by using long-range PCR (LR-PCR) and sequencing. LR-PCR was performed with primers flanking the breakpoints to amplify the junction fragment carrying the rearrangement. The amplified PCR fragments were analyzed on agarose gels, purified with ExoSAP-IT, and sequenced as described previously (41). Primer sequences are displayed in Supplementary Table S1.

### Nomenclature of *PALB2* mutations

Mutations were described according to the recommended nomenclature system described by the Human Genome Variation Society (HGVS; ref. 42) with nucleotides numbered from the A of the ATG translation initiation codon of the National Center for Biotechnology Information (NCBI) reference sequence NM\_024675.3. Genomic and protein sequences for *PALB2* are given by NG\_007406.1, and NP\_078951.2.

### Haplotype analysis

To define the origin of the recurrent *PALB2* mutation c.172\_175delTTGT, we genotyped all identified mutation carriers and 16 randomly selected samples from a non-cancer control group, negative for the presence of this mutation (27, 43). Haplotype analysis included marker (D16S412) flanking *PALB2* on the centromeric side and two markers (D16S417 and D16S420) flanking *PALB2* on the telomeric side. The positions of the markers and the *PALB2* gene were obtained from NCBI (NT\_010393.16). Each marker was amplified by PCR using FAM-labeled

forward primers as described previously (3, 44) and analyzed on an ABI 3130 genetic analyzer with a GeneMapper v4.0 Software.

### *In vitro* and *in silico* analyses

An *in vitro* protein truncation test (PTT) was performed to detect a premature termination of translation as a result of the dinucleotide change in the coding sequence of the gene (c.661\_662delinsTA), leading to the generation of a stop codon. A PCR fragment encompassing the alteration was amplified with a forward primer containing the T7 RNA polymerase promoter, the Kozak translation initiation sequence, and a start codon (Supplementary Table S1). The PTT was performed as described previously (40).

*BRCA1* and *BRCA2* gene mutation probabilities were calculated using the BRCAPRO BayesMendel model with the CancerGene software package available at <http://www4.utsouthwestern.edu/breasthealth/cagene/> (38). The pathogenicity of missense variants was assessed using the SIFT (sorts intolerant from tolerant; ref. 45), PolyPhen (46) and Align GVGD (Grantham Variation, Grantham Deviation) software (47).

### Statistical analysis

Frequencies of mutations in different subgroups were compared using Fisher exact test.

## Results

### Analysis of *PALB2* mutations in high-risk patients

To determine the prevalence of deleterious *PALB2* mutations in the group of patients with high-risk cancer, we analyzed the entire *PALB2* coding region by direct sequencing and MLPA screening. Ten different protein truncating mutations and three LGRs were identified in 16 of 409 (3.9%) participants (Table 2), whereas one mutation was observed in 1,226 controls (0.08%;  $P = 2.6 \times 10^{-9}$ ). In 330 familial cases, 13 mutations were found in a subgroup of 235 families (5.5%) with a history positive for breast cancer only (HBC families), whereas no mutation was detected in 95 cases from families with a history positive for breast cancer and ovarian cancer or for ovarian cancer only ( $P = 0.02$ ). In 79 high-risk nonfamilial patients, one carrier of the *PALB2* mutation was detected in a group of 22 male patients with breast cancer (4.5%), one in a group of 39 patients with bilateral breast cancer (2.6%), and one in a group of 18 patients with both breast and ovarian cancer (5.6%; Table 1).

The only recurrent mutation was the c.172\_175delTTGT deletion found in four unrelated families. Other mutations distributed widely across the coding sequence of the gene were observed only once and included six frameshift mutations (c.509\_510delGA, c.901\_907del7, c.1227\_1231del5, c.1924delA, c.1942\_1945delCTTAinsAAC, and c.3362delG) and two nonsense mutations (c.73A>T and c.1240C>T). The c.661\_662delinsTA variant represented the dinucleotide change. A shorter protein product obtained from the patient's DNA by a PTT analysis confirmed that bases GT 661-662 substituted

**Table 2.** Truncating mutations and large rearrangements in *PALB2* gene

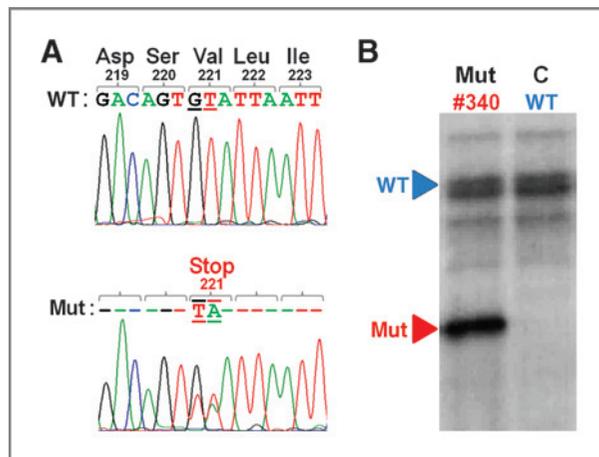
Patient No.	Exon	Nucleotide change	Protein change	Mut. in ctris	BC/other cancers age at dg	Cancers in the family (age at dg)	BRCAPRO <sup>®</sup>	Receptors
High-risk patients: N = 409 (truncating mutations)								
509 <sup>a,b</sup>	2	c.73A>T	p.K25*	0	BC 75/75 Bladder 78	Breast, D (40) Uterus, M (47)	0.05	N.A.
451	3	c.172_175delTTGT	p.L58fs	0	BC 34	Breast, PGM (36) Endometrial, PS (?)	0.11	N.A.
738					BC 39	Breast, S (41) Breast, MGM (70)	0.29	ER <sup>-</sup> , PR <sup>-</sup> HER2 <sup>+</sup>
1,026					BC 38	Colon, MS (50) Breast, M (56) Breast, MGM (40)	0.21	ER <sup>+</sup> , PR <sup>+</sup> HER2 <sup>-</sup>
1,120 <sup>a</sup>					BC 42/52	Colon, MGF (77) Breast, S (55) Breast, MGM (?)	0.39	N.A.
579	4	c.509_510delGA	p.R170fs	1	BC 59	Colon, B (56) Kidney, B (58) Pancreas, M (46)	0.07	N.A.
340	4	c.661_662delinsTA	p.V221*	0	BC 46	Breast, S (50) Breast, MGM (60) Gallbladder, F (68)	0.07	ER <sup>+</sup> , PR <sup>+</sup> HER2 <sup>-</sup>
1260 <sup>c</sup>	4	c.901_907del7	p.D301fs	0	BC 36	None	0.14	ER <sup>+</sup> , PR <sup>+</sup> HER2 <sup>-</sup>
1320	4	c.1227_1231del5	p.Y409fs	0	BC 37	Breast, S (38) Breast, M (65)	0.70	ER <sup>-</sup> , PR <sup>-</sup> HER2 <sup>+</sup>
379 <sup>d</sup>	4	c.1240C>T	p.R414*	0	BC 38	Breast, M (50)	0.09	N.A.
413	5	c.1924delA	p.M642fs	0	BC 46	Breast, PGM (37)	0.08	N.A.
211 <sup>a</sup>	5	c.1942_1945delCTTAinsAAC	p.L648fs	0	BC 41/42	None	0.39	N.A.
1436	13	c.3362delG	p.G1121fs	0	BC 45	Breast, MGM (49)	0.08	ER <sup>-</sup> , PR <sup>-</sup> HER2 <sup>-</sup>

*(Continued on the following page)*

**Table 2.** Truncating mutations and large rearrangements in PALB2 gene (Cont'd)

Patient No.	Exon	Nucleotide change	Protein change	Mut. in ctrls	BC/other cancers age at dg	Cancers in the family (age at dg)	BRCAPRO <sup>e</sup>	Receptors
High-risk patients: N = 409 (large rearrangements)								
1647	Del ex 7-8	<b>g.19557_22659del3103</b>	<b>p.N863fs</b>	N.A.	BC 34	Breast, S (30) Gallbladder, MGM (73)	0.44	N.A.
1507 <sup>b</sup>	Del ex 9-10	<b>g.22947_26370del3424, 26372C&gt;G</b>	<b>p.A946_W1038del</b>	N.A.	BC 42 Bladder 42	Breast, M (52) Breast, MGM (40)	0.15	N.A.
79 <sup>b</sup>	Dup ex 9-11	<b>g.22904_36589dup13686</b>	<b>p.G1068fs</b>	N.A.	BC 59 OC 52 Colon 52	Gallbladder, M (?) Colon, F (?)	0.31	ER <sup>+</sup> , PR <sup>+</sup> HER2 <sup>+</sup>
Unselected BC patients: N = 704								
A210	3	c.172_175delTTGT	p.L58fs	0	BC 65	Lung, F (55) Kidney, M (53)	0.01	ER <sup>-</sup> , PR <sup>-</sup> HER2 <sup>-</sup>
A361					BC 46	None	0.06	ER <sup>-</sup> , PR <sup>-</sup> HER2 <sup>-</sup>

NOTE: Novel mutations are marked in bold.  
Abbreviations: B, brother; BC, breast cancer; D, daughter; ER, estrogen receptor; F, father; M, mother; MGF, maternal grandfather; MGM, maternal grandmother; MS, maternal sister; N.A., not available; OC, ovarian cancer; PGM, paternal grandmother; PR, progesterone receptor; PS, paternal sister; S, sister.  
<sup>a</sup>Bilateral BC.  
<sup>b</sup>Tumor duplicity or triplicity.  
<sup>c</sup>Male BC.  
<sup>d</sup>Medullary BC.  
<sup>e</sup>BRCAPRO scores (37) in mutation carriers. Position in mRNA and genomic DNA is according to the NCBI reference sequence NM\_024675.3 and NG\_007406.1.



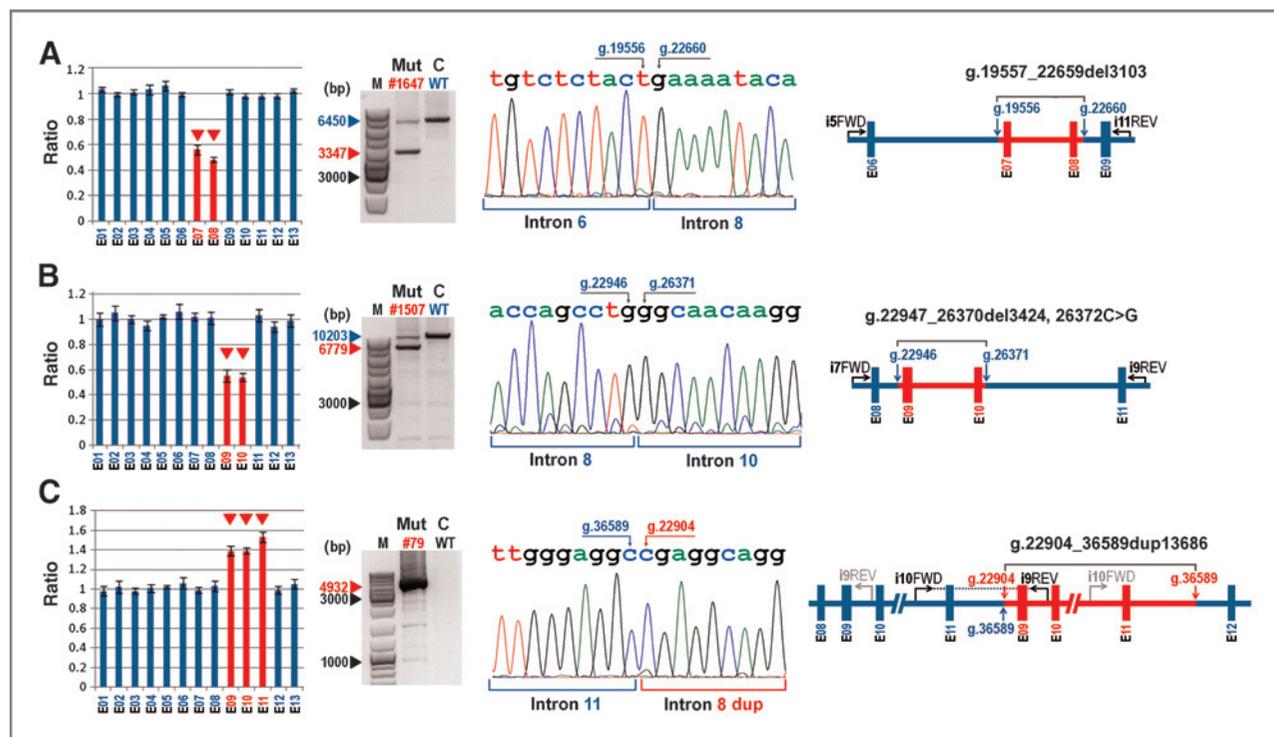
**Figure 1.** The truncating mutation c.661\_662delGTinsTA that introduced a TAA stop codon into position 221 in the *PALB2* gene identified by sequencing (left) and PTT (right). Truncated polypeptide is indicated by a red arrowhead; wild-type (WT) translation product is indicated by a blue arrowhead; C—WT control.

to TA were located on the same allele of the *PALB2* gene and a dinucleotide change replaced valine at position 221 with a termination codon (Fig. 1). The mutations c.172\_175delTTGT, c.509\_510delGA, and c.1240C>T belong to alterations that have already been described in the United States and different European countries

(15, 17–19, 33, 36, 48), whereas seven other identified gene alterations are novel (Table 2).

An MLPA-based analysis identified three undescribed rearrangements within the *PALB2* gene, two large deletions involving exons 7–8 and 9–10, respectively, and one duplication spanning exons 9–11 (Table 2). A deletion involving exons 7–8 (Fig. 2A) was detected in an HBC family (#1647) with two early-onset breast cancer cases. A specific junction fragment of 3347 bp was amplified from the patient's DNA with a forward primer located in intron 5 and a reverse primer located in intron 9 (Supplementary Table S1). A sequence analysis of this fragment revealed a 3103 bp deletion (g.19557\_22659del3103). The deletion breakpoints occurred in a region of perfect identity of 10 bp within two *AluSx1* sequences located in intron 6 and 8. The deletion causes a shift in the reading frame and substantial truncation of the *PALB2* protein (p.N863Gfs\*7).

A deletion comprising exons 9–10 (Fig. 2B) was identified in an HBC family (#1507) with three cases of breast cancer. With PCR primers located in introns 7 and 11 (Supplementary Table S1), an extra fragment of 6779 bp was amplified from the patient's DNA but not from control DNA. The sequencing of this PCR product demonstrated a deletion of 3424 bp and a substitution of C to G at position the g.26372 of intron 10 (g.22947\_26370del3424, 26372C>G). Breakpoints were located in a sequence



**Figure 2.** MLPA-based detection of LGRs at the *PALB2* locus and breakpoint analysis. Deletion of exons 7–8 is shown in (A), deletion of exons 9–10 in (B), and duplication of exons 9–11 in (C). The figures (from left to right) contain MLPA results, PCR amplification of junction fragments, breakpoints identification by a sequence analysis, and schemes of the rearrangements showing the breakpoints (vertical arrows) with coordinates and used amplification primers (horizontal arrows). The mutation carriers are indicated by a number, the junction fragments are marked by a red arrowhead, wild-type (WT) PCR products by a blue arrowhead; lane C, negative control; lane M, size standards.

containing 16 identical nucleotides within *AluSx3* and *AluSz6* repeats in intron 8 and 10, respectively. Although the deletion of exons 9–10 maintains the *PALB2* reading frame, an elimination of the gene region coding for 93 amino acids (p.A946\_W1038del) that interfere with WD-40 domains essential for BRCA2 binding (residues 850–1186; ref. 49) may be considered pathogenic.

The duplication of the sequence containing exons 9–11 (Fig. 2C) was found in a patient (#79) who was affected by breast, ovarian, and colon cancer. With duplication-specific primers in introns 10 (forward) and 9 (reverse; Supplementary Table S1), a fragment of 4932 bp was amplified from the patient's DNA but not from control DNA. A sequence analysis of this fragment revealed a duplicated region of 13686 bp (g.22904–36589dup) containing exons 9–11. Breakpoints were mapped to a 26-bp region of an identical sequence within two *Alu* elements, namely *AluSx3* in intron 8 and *AluY* located in intron 11. The duplication of exons 9–11 results in an out-of-frame translation leading to the truncation of the *PALB2* protein (p.G1068Gfs\*7).

Other detected sequence variants of the *PALB2* gene including silent and missense substitutions have been previously reported as nonpathogenic (11); one novel substitution has been predicted as probably damaging and four have been assessed as tolerable (Supplementary Table S2).

#### Characteristics of *PALB2* mutation carriers from HBC families

The mean age of breast cancer diagnosis in HBC families was lower in 13 *PALB2* heterozygotes (44.3 years) than in 222 patients not carrying a mutation in the *PALB2* gene (47.4 years), but the difference was not statistically significant ( $P = 0.12$ ). However, it should be noted that the age at diagnosis in all high-risk families and therefore also in *PALB2* mutation carriers is significantly lower from that in unselected patients with breast cancer (data not shown). Two cases of bilateral breast cancer (15.4%; 2 of 13) occurred in patients with a *PALB2* mutation, whereas 14 bilateral cases (6.3%; 14 of 222) were registered in *PALB2*-negative patients ( $P = 0.22$ ). An analysis of cancer profiles in 13 HBC families with *PALB2* mutations (Table 2) revealed two cases of pancreatic cancer, whereas four cases of this cancer were reported in 222 families without a *PALB2* mutation. Invasive ductal carcinomas predominated among *PALB2*-associated tumors; medullary carcinoma was reported in one carrier (#379). Five of nine *PALB2*-associated tumors were negative for the expression of estrogen and progesterone receptors and three of these also lacked the expression of a HER2 receptor (Table 2).

In five *PALB2* families, we obtained DNA samples from other family members to assess the segregation of *PALB2* mutations with the breast cancer phenotype. Segregation was demonstrated in four families (Fig. 3A–D), whereas in family #1120 the pedigree analysis did not show segre-

gation of the recurrent mutation c.172\_175delTTGT with the cancer phenotype (Fig. 3E).

#### Analysis of the recurrent *PALB2* mutation c.172\_175delTTGT in unselected breast cancer cases

The presence of the recurrent mutation c.172\_175delTTGT was further analyzed in an independent group of 704 unselected patients with consecutive breast cancer and two other carriers (0.3%) were found (Table 2).

#### Haplotype analysis of c.172\_175delTTGT mutation carriers

A haplotype analysis was performed on all six identified mutation carriers (four from high-risk patients and two from unselected breast cancer patients) and on 16 randomly selected samples from noncancer controls using three microsatellite markers flanking the *PALB2* gene. Four of six unrelated carriers shared a common approximately 1.0 Mb haplotype defined by the D16S412, D16S417, and D16S420 markers, suggesting the unique origin of the c.172\_175delTTGT mutation (Table 3). The detected disease-associated haplotype was not found in 16 control DNA samples (data not shown) and also in two identified mutation carriers. Interestingly, one of them (#1120) comes from the previously mentioned family with a deficient segregation of the mutation with the disease.

#### Discussion

We have identified 16 pathogenic mutations in the whole series of 409 high-risk patients (3.9%) and 13 mutations in a group of 235 HBC families (5.5%). This frequency of *PALB2* mutations is markedly higher than in other analyzed populations (15). The majority of truncating mutations was unique to individual families; seven of them have not yet been described. The only recurrent mutation found in 25% (4 of 16) of carriers was the c.172\_175delTTGT. This alteration is not restricted to the Czech region but has also been detected in families of German and Irish ancestry (13, 15). Its frequency observed in a large population of unselected patients with breast cancer (0.3%) was lower compared with the frequency of the recurrent mutation c.1592delT reported by Erkkö and colleagues from Finland (18 of 1,918; 0.9%; ref. 12).

We have identified large rearrangements at the *PALB2* locus in three families, which represents 18.8% (3 of 16) of all detected mutations and suggests that this type of gene alteration may play a notable role in predisposition to breast cancer. An analysis of a larger group of patients is needed to obtain statistically relevant results. *Alu* sequences were involved in all detected rearrangements. To date, a large rearrangement in *PALB2* was detected only in a breast cancer family in Spain (28) and in a Canadian patient affected with breast and pancreatic cancer (35).

The risk of breast cancer associated with *PALB2* mutations was not calculated in our study because we were able to perform an extensive segregation analysis in only five

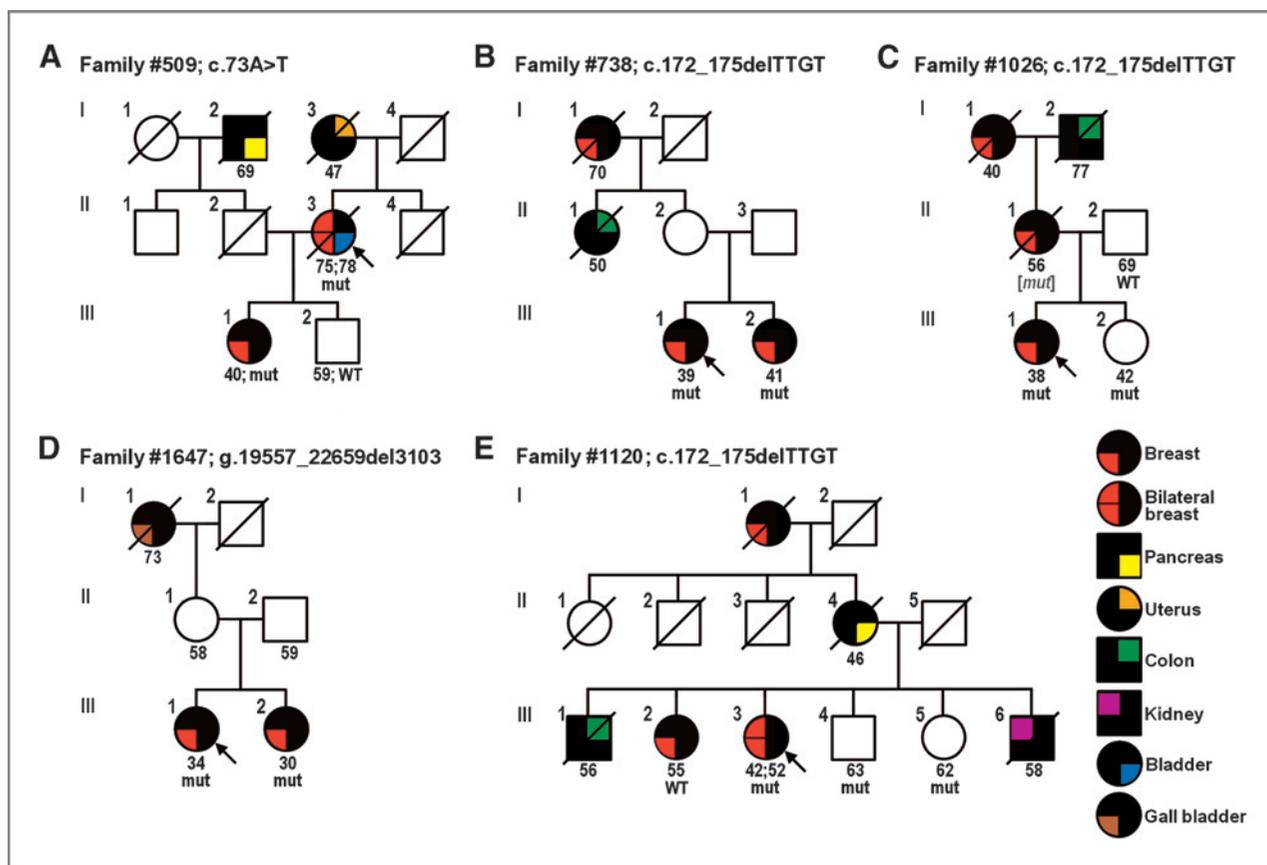


Figure 3. Pedigrees of the five breast cancer families with *PALB2* mutations. Proband is indicated by arrows, slashed symbols denote deceased individuals. Age at diagnosis is included when known. Individuals genotyped for *PALB2* mutations are marked either as carriers (mut) or noncarriers (WT). A note: in family C, mutation status in deceased mother (II/1) was assigned on the basis of the negativity of mutation analysis in proband's father (II/2).

mutation-positive families (Fig. 3A–E). Rahman and colleagues estimated initially that *PALB2* mutations were associated with a 2.3-fold increased risk of breast cancer (11), but following studies have reported a higher risk (15, 25, 30, 50). A tight interaction between the *PALB2* and *BRCA2* proteins in DNA double-strand break repair (8, 9) indicates that also the disease phenotype and breast cancer penetrance may be similar in *PALB2* and *BRCA2*

mutation carriers. This assumption support reports of Southey and colleagues (30) and Erkko and colleagues (50). The *PALB2* c.3113G>A mutation identified by Southey and colleagues (30) in an Australian study was associated with 49% breast cancer risk by the age of 50 years and 91% risk [95% confidence interval (CI), 44%–100%] by the age of 70 years. The authors concluded that the HR of breast cancer for their recurrent *PALB2* mutation was similar to the age-specific HRs reported for mutations in the *BRCA2* gene. Erkko and colleagues (50) estimated that the recurrent Finnish c.1592delT mutation is associated with a 40% (95% CI, 17%–77%) risk of breast cancer to the age of 70 years.

Similar to *BRCA2*, defects in the *PALB2* gene are also associated with hereditary predisposition to male breast cancer (15, 31) and pancreatic cancer (33–35) and according to some results they may also predispose to ovarian cancer (18, 36). A comparison of cancer profiles in mutation-positive and -negative families in our study indicated only a weak association of *PALB2* mutations with pancreatic and ovarian cancer. Further information from a larger population of *PALB2* families will be necessary for a more precise evaluation of these data. One *PALB2* mutation was detected in a small group of

**Table 3.** Microsatellite genotyping in c.172\_175delTTGT mutation carriers

Patient no	Microsatellite marker (sizes in bp)		
	D16S412	D16S417	D16S420
451	<b>151/151</b>	<b>260/246</b>	255/259
738	<b>151/151</b>	<b>260/246</b>	<b>245/255</b>
1,026	<b>151/151</b>	<b>260/260</b>	<b>245/261</b>
1,120	<b>151/153</b>	246/258	<b>245/243</b>
A210	<b>151/149</b>	<b>260/242</b>	<b>245/255</b>
A361	<b>151/151</b>	<b>260/246</b>	<b>245/255</b>

NOTE: Alleles of identical size are highlighted in bold numbers.

22 male breast cancer cases, which may support the view of breast cancer predisposition in men with mutations in *PALB2*.

A negative steroid receptor status predominated in breast tumors (reported in 5 of 9 tumors) from heterozygous mutation carriers (Table 2). Receptor negativity has also been observed in some other studies (23, 27). In contrast, the positivity of steroid receptors in most *PALB2*-positive tumors has been reported by other groups (12, 16).

Pathogenic mutations of the *BRCA1* and *BRCA2* genes detected in our patients with high-risk breast/ovarian cancer were more frequent than mutations identified in the *PALB2* gene (Supplementary Table S3). However, protein truncating mutations in *PALB2* strongly prevailed over alterations in other candidate breast cancer-susceptibility genes (*ATM*, *CHEK2*, and *NBN*) we examined in *BRCA1*- and *BRCA2*-negative patients (3, 4, 6). In familial cases, mutations in *PALB2* were found exclusively in a subgroup of HBC families with a frequency comparable with that previously reported for mutations in *BRCA2* (Table 1 and Supplementary Table S3).

The presented results thus suggest that screening for protein-truncating alterations and LGRs at the *PALB2* locus is relevant at least in HBC families and support the recently published suggestions that screening for *PALB2* mutations may be clinically useful (15, 37) in addition to screening for mutations in *BRCA1*, *BRCA2*, and c.1100delC in the *CHEK2* gene (51). Further analyses of a larger series of patients are highly desirable for accurate estimation of breast cancer penetrance to improve clinical management in *PALB2* mutation carriers, and to reveal whether genetic testing for the *PALB2* gene may also be

recommended to other risk groups, including, for example, male breast cancer cases.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### Authors' Contributions

**Conception and design:** M. Janatova, Z. Kleibl, P. Pohlreich  
**Development of methodology:** M. Janatova, Z. Kleibl, P. Pohlreich  
**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** A. Panczak, K. Vesela, M. Zimovjanova, P. Dundr  
**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** M. Janatova, J. Stribrna, P. Kleiblova, J. Soukupova  
**Writing, review, and/or revision of the manuscript:** M. Janatova, Z. Kleibl, J. Stribrna, A. Panczak, K. Vesela, P. Kleiblova, J. Soukupova, P. Pohlreich  
**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** M. Janatova, A. Panczak, K. Vesela, P. Dundr  
**Study supervision:** P. Pohlreich

#### Acknowledgments

The authors thank Stanislav Kormunda for help with statistical analyses, Marie Epsteinova and Marketa Dostalova for their excellent technical assistance, and all patients for cooperation in this project.

#### Grant Support

This study was supported by Internal Grant Agency of the Ministry of Health of the Czech Republic number NT13343 (to M. Janatova, Z. Kleibl, J. Stribrna, A. Panczak, K. Vesela, M. Zimovjanova, P. Kleiblova, J. Soukupova, and P. Pohlreich) and number NT14006 (to M. Janatova, Z. Kleibl, P. Kleiblova, and P. Pohlreich), and Charles University in Prague project PRVOUK-P27/LF1/1 (to M. Janatova, Z. Kleibl, A. Panczak, P. Dundr, and P. Pohlreich).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 24, 2013; revised September 17, 2013; accepted September 17, 2013; published OnlineFirst October 17, 2013.

#### References

- Mateju M, Stribrna J, Zikan M, Kleibl Z, Janatova M, Kormunda S, et al. Population-based study of *BRCA1/2* mutations: family history based criteria identify minority of mutation carriers. *Neoplasma* 2010;57:280-5.
- Pohlreich P, Zikan M, Stribrna J, Kleibl Z, Janatova M, Kotlas J, et al. High proportion of recurrent germline mutations in the *BRCA1* gene in breast and ovarian cancer patients from the Prague area. *Breast Cancer Res* 2005;7:R728-36.
- Soukupova J, Dundr P, Kleibl Z, Pohlreich P. Contribution of mutations in *ATM* to breast cancer development in the Czech population. *Oncol Rep* 2008;19:1505-10.
- Kleibl Z, Novotny J, Bezdickova D, Malik R, Kleiblova P, Foretova L, et al. The *CHEK2* c.1100delC germline mutation rarely contributes to breast cancer development in the Czech Republic. *Breast Cancer Res Treat* 2005;90:165-7.
- Ticha I, Stribrna J, Soukupova J, Pohlreich P. Mutations in *CHEK2* and *TP53* genes in high-risk hereditary breast and ovarian cancer patients in the Czech Republic. *Eur J Cancer Suppl* 2011;47(Suppl 1):S132.
- Mateju M, Kleiblova P, Kleibl Z, Janatova M, Soukupova J, Ticha I, et al. Germline mutations 657del5 and 643C>T (R215W) in *NBN* are not likely to be associated with increased risk of breast cancer in Czech women. *Breast Cancer Res Treat* 2012;133:809-11.
- Xia B, Sheng Q, Nakanishi K, Ohashi A, Wu J, Christ N, et al. Control of *BRCA2* cellular and clinical functions by a nuclear partner, *PALB2*. *Mol Cell* 2006;22:719-29.
- Sy SM, Huen MS, Chen J. *PALB2* is an integral component of the *BRCA* complex required for homologous recombination repair. *Proc Natl Acad Sci U S A* 2009;106:7155-60.
- Zhang F, Fan Q, Ren K, Andreassen PR. *PALB2* functionally connects the breast cancer susceptibility proteins *BRCA1* and *BRCA2*. *Mol Cancer Res* 2009;7:1110-18.
- Reid S, Schindler D, Hanenberg H, Barker K, Hanks S, Kalb R, et al. Biallelic mutations in *PALB2* cause Fanconi anemia subtype FA-N and predispose to childhood cancer. *Nat Genet* 2007;39:162-4.
- Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A, et al. *PALB2*, which encodes a *BRCA2*-interacting protein, is a breast cancer susceptibility gene. *Nat Genet* 2007;39:165-7.
- Erkko H, Xia B, Nikkila J, Schleutker J, Syrjakoski K, Mannermaa A, et al. A recurrent mutation in *PALB2* in Finnish cancer families. *Nature* 2007;446:316-9.
- Hellebrand H, Sutter C, Honisch E, Gross E, Wappenschmidt B, Schem C, et al. Germline mutations in the *PALB2* gene are population specific and occur with low frequencies in familial breast cancer. *Hum Mutat* 2011;32:E2176-88.
- Peterlongo P, Catucci I, Pasquini G, Verderio P, Peissel B, Barile M, et al. *PALB2* germline mutations in familial breast cancer cases with personal and family history of pancreatic cancer. *Breast Cancer Res Treat* 2011;126:825-8.
- Casadei S, Norquist BM, Walsh T, Stray S, Mandell JB, Lee MK, et al. Contribution of inherited mutations in the *BRCA2*-interacting protein *PALB2* to familial breast cancer. *Cancer Res* 2011;71:2222-9.

16. Tischkowitz M, Xia B, Sabbaghian N, Reis-Filho JS, Hamel N, Li G, et al. Analysis of PALB2/FANCN-associated breast cancer families. *Proc Natl Acad Sci U S A* 2007;104:6788–93.
17. Adank MA, van Mil SE, Gille JJ, Waisfisz Q, Meijers-Heijboer H. PALB2 analysis in BRCA2-like families. *Breast Cancer Res Treat* 2011;127:357–62.
18. Dansonka-Mieszkowska A, Kluska A, Moes J, Dabrowska M, Nowakowska D, Niwinska A, et al. A novel germline PALB2 deletion in Polish breast and ovarian cancer patients. *BMC Med Genet* 2010;11:20.
19. Bogdanova N, Sokolenko AP, Iyevleva AG, Abyshva SN, Blaut M, Bremer M, et al. PALB2 mutations in German and Russian patients with bilateral breast cancer. *Breast Cancer Res Treat* 2011;126:545–50.
20. Sluiter M, Mew S, van Rensburg EJ. PALB2 sequence variants in young South African breast cancer patients. *Fam Cancer* 2009;8:347–53.
21. Cao AY, Huang J, Hu Z, Li WF, Ma ZL, Tang LL, et al. The prevalence of PALB2 germline mutations in BRCA1/BRCA2 negative Chinese women with early onset breast cancer or affected relatives. *Breast Cancer Res Treat* 2009;114:457–62.
22. Zheng Y, Zhang J, Niu Q, Huo D, Olopade OI. Novel germline PALB2 truncating mutations in African American breast cancer patients. *Cancer* 2012;118:1362–70.
23. Garcia MJ, Fernandez V, Osorio A, Barroso A, Llorc G, Lazaro C, et al. Analysis of FANCB and FANCN/PALB2 Fanconi anemia genes in BRCA1/2-negative Spanish breast cancer families. *Breast Cancer Res Treat* 2009;113:545–51.
24. Balia C, Sensi E, Lombardi G, Roncella M, Bevilacqua G, Caligo MA. PALB2: a novel inactivating mutation in a Italian breast cancer family. *Fam Cancer* 2010;9:531–36.
25. Tischkowitz M, Capanu M, Sabbaghian N, Li L, Liang X, Vallee MP, et al. Rare germline mutations in PALB2 and breast cancer risk: a population-based study. *Hum Mutat* 2012;33:674–80.
26. Papi L, Putignano AL, Congregati C, Piaceri I, Zanna I, Sera F, et al. A PALB2 germline mutation associated with hereditary breast cancer in Italy. *Fam Cancer* 2010;9:181–5.
27. Foulkes WD, Ghadirian P, Akbari MR, Hamel N, Giroux S, Sabbaghian N, et al. Identification of a novel truncating PALB2 mutation and analysis of its contribution to early-onset breast cancer in French-Canadian women. *Breast Cancer Res* 2007;9:R83.
28. Blanco A, de la Hoya M, Balmaña J, Ramón y Cajal T, Teulé A, Miramar MD, et al. Detection of a large rearrangement in PALB2 in Spanish breast cancer families with male breast cancer. *Breast Cancer Res Treat* 2012;132:307–15.
29. Hofstatter EW, Domchek SM, Miron A, Garber J, Wang M, Compomeschi K, et al. PALB2 mutations in familial breast and pancreatic cancer. *Fam Cancer* 2011;10:225–31.
30. Southey MC, Teo ZL, Dowty JG, Odefrey FA, Park DJ, Tischkowitz M, et al. A PALB2 mutation associated with high risk of breast cancer. *Breast Cancer Res* 2010;12:R109.
31. Ding YC, Steele L, Kuan CJ, Greilac S, Neuhausen SL. Mutations in BRCA2 and PALB2 in male breast cancer cases from the United States. *Breast Cancer Res Treat* 2011;126:771–8.
32. Teo ZL, Park DJ, Provenzano E, Chatfield CA, Odefrey FA, Nguyen-Dumont T, et al. Prevalence of PALB2 mutations in Australasian multiple-case breast cancer families. *Breast Cancer Res* 2013;15:R17.
33. Jones S, Hruban RH, Kamiyama M, Borges M, Zhang X, Parsons DW, et al. Exomic sequencing identifies PALB2 as a pancreatic cancer susceptibility gene. *Science* 2009;324:217.
34. Slater EP, Langer P, Niemczyk E, Strauch K, Butler J, Habbe N, et al. PALB2 mutations in European familial pancreatic cancer families. *Clin Genet* 2010;78:490–4.
35. Tischkowitz MD, Sabbaghian N, Hamel N, Borgida A, Rosner C, Taherian N, et al. Analysis of the gene coding for the BRCA2-interacting protein PALB2 in familial and sporadic pancreatic cancer. *Gastroenterology* 2009;137:1183–6.
36. Prokofyeva D, Bogdanova N, Bermisheva M, Zinnatullina G, Hillemanns P, Khusnutdinova E, et al. Rare occurrence of PALB2 mutations in ovarian cancer patients from the Volga-Ural region. *Clin Genet* 2012;82:100–1.
37. Southey MC, Teo ZL, Winship I. PALB2 and breast cancer: ready for clinical translation! *Appl Clin Genet* 2013;6:43–52.
38. Parmigiani G, Berry D, Aguilar O. Determining carrier probabilities for breast cancer-susceptibility genes BRCA1 and BRCA2. *Am J Hum Genet* 1998;62:145–158.
39. Kleibl Z, Havranek O, Hlavata I, Novotny J, Sevcik J, Pohreich P, et al. The CHEK2 gene I157T mutation and other alterations in its proximity increase the risk of sporadic colorectal cancer in the Czech population. *Eur J Cancer* 2009;45:618–24.
40. Pohreich P, Stribrna J, Kleibl Z, Zikan M, Kalbacova R, Petruzalka L, et al. Mutations of the BRCA1 gene in hereditary breast and ovarian cancer in the Czech Republic. *Med Princ Pract* 2003;12:23–9.
41. Ticha I, Kleibl Z, Stribrna J, Kotlas J, Zimovjanova M, Mateju M, et al. Screening for genomic rearrangements in BRCA1 and BRCA2 genes in Czech high-risk breast/ovarian cancer patients: high proportion of population specific alterations in BRCA1 gene. *Breast Cancer Res Treat* 2010;124:337–47.
42. den Dunnen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. *Hum Mutat* 2000;15:7–12.
43. Gunnarsson H, Arason A, Gillanders EM, Agnarsson BA, Johannesdottir G, Johannsson OT, et al. Evidence against PALB2 involvement in Icelandic breast cancer susceptibility. *J Negat Results Biomed* 2008;7:5.
44. Janatova M, Zikan M, Dunder P, Matous B, Pohreich P. Novel somatic mutations in the BRCA1 gene in sporadic breast tumors. *Hum Mutat* 2005;25:319.
45. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc* 2009;4:1073–81.
46. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. *Nat Methods* 2010;7:248–9.
47. Tavtigian SV, Byrnes GB, Goldgar DE, Thomas A. Classification of rare missense substitutions, using risk surfaces, with genetic- and molecular-epidemiology applications. *Hum Mutat* 2008;29:1342–54.
48. Walsh T, Lee MK, Casadei S, Thornton AM, Stray SM, Pennil C, et al. Detection of inherited mutations for breast and ovarian cancer using genomic capture and massively parallel sequencing. *Proc Natl Acad Sci U S A* 2010;107:12629–33.
49. Sy SM, Huen MS, Zhu Y, Chen J. PALB2 regulates recombinational repair through chromatin association and oligomerization. *J Biol Chem* 2009;284:18302–10.
50. Erkkö H, Dowty JG, Nikkila J, Syrjäkoski K, Mannermaa A, Pylkas K, et al. Penetrance analysis of the PALB2 c.1592delT founder mutation. *Clin Cancer Res* 2008;14:4667–71.
51. Narod SA. Testing for CHEK2 in the cancer genetics clinic: ready for prime time? *Clin Genet* 2010;78:1–7.

# Cancer Epidemiology, Biomarkers & Prevention

## The *PALB2* Gene Is a Strong Candidate for Clinical Testing in *BRCA1*- and *BRCA2*-Negative Hereditary Breast Cancer

Marketa Janatova, Zdenek Kleibl, Jana Stribrna, et al.

*Cancer Epidemiol Biomarkers Prev* 2013;22:2323-2332. Published OnlineFirst October 17, 2013.

<b>Updated version</b>	Access the most recent version of this article at: doi: <a href="https://doi.org/10.1158/1055-9965.EPI-13-0745-T">10.1158/1055-9965.EPI-13-0745-T</a>
<b>Supplementary Material</b>	Access the most recent supplemental material at: <a href="http://cebp.aacrjournals.org/content/suppl/2013/10/17/1055-9965.EPI-13-0745-T.DC1">http://cebp.aacrjournals.org/content/suppl/2013/10/17/1055-9965.EPI-13-0745-T.DC1</a>

<b>Cited articles</b>	This article cites 51 articles, 8 of which you can access for free at: <a href="http://cebp.aacrjournals.org/content/22/12/2323.full#ref-list-1">http://cebp.aacrjournals.org/content/22/12/2323.full#ref-list-1</a>
-----------------------	---

<b>Citing articles</b>	This article has been cited by 4 HighWire-hosted articles. Access the articles at: <a href="http://cebp.aacrjournals.org/content/22/12/2323.full#related-urls">http://cebp.aacrjournals.org/content/22/12/2323.full#related-urls</a>
------------------------	---

<b>E-mail alerts</b>	<a href="#">Sign up to receive free email-alerts</a> related to this article or journal.
----------------------	--

<b>Reprints and Subscriptions</b>	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at <a href="mailto:pubs@aacr.org">pubs@aacr.org</a> .
-----------------------------------	--

<b>Permissions</b>	To request permission to re-use all or part of this article, use this link <a href="http://cebp.aacrjournals.org/content/22/12/2323">http://cebp.aacrjournals.org/content/22/12/2323</a> . Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.
--------------------	--