The PALB2 gene is a strong candidate for clinical testing in BRCA1 and BRCA2 negative hereditary breast cancer

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Running title: PALB2 mutations predispose for hereditary breast cancer

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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 germ-line 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 (LGRs) was performed by MLPA analysis.

Results: We have identified 13 different pathogenic alterations including 10 truncating mutations and three LGRs in 16 out of 409 patients (3.9%), while one truncating mutation was found in a group of 1226 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 to that previously reported for BRCA2) was found in a subgroup of hereditary breast cancer patients (13/235; 5.5%).

Impact: Our results show that mutation analysis of the PALB2 gene, including the analysis of LGRs, is primarily indicated in hereditary breast cancer patients in case of their BRCA1 and BRCA2 negativity.
Introduction

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

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 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 FA subtype N with a phenotype similar to that (FA-D1) caused by biallelic *BRCA2* mutations. Studies performed in the UK (11) and Finland (12) demonstrated that monoallelic mutations in *PALB2* predispose to BC. Five different *PALB2* mutations in ten of 923 (1.1%) individuals with familial BC were found in the UK. A founder *PALB2* c.1592delT mutation, associated with a roughly fourfold increased hereditary propensity for female BC, was detected in Finland in 3/113 (2.7%) familial BC patients and in 18/1918 (0.9%) unselected BC cases. Until now, about 50 truncating mutations have been detected in BC 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 considered a strong candidate BC susceptibility gene; however, due to the limited number of studies, estimation of penetrance of BC 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 high-risk *BRCA1* and *BRCA2*-negative breast/ovarian cancer patients. 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 BC patients, unselected BC patients, and controls

In total, 409 high-risk breast and/or ovarian cancer patients negatively tested for the presence of pathogenic mutations in *BRCA1* and *BRCA2* genes in our institute between 2000-2010 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 non-familial cancer cases). Familial cases met the following criteria in first or second degree relatives: two cases of either BC diagnosed before the age of 50 or ovarian cancer (OC) diagnosed at any age; and three or more cases of BC or OC diagnosed at...
any age. Non-familial cases included male BC cases, patients with bilateral BC with the first diagnosis before the age of 50 and patients with primary breast and ovarian cancer. The average BRCAPRO (38) risk estimates for each group of patients are given in Table 1.

The unselected BC group included DNA samples from 704 consecutive female BC patients. These patients were treated at the Department of Oncology, General University Hospital in Prague between 2004 – 2006 and were included into the study regardless of the age at diagnosis and BC family history. The average age of BC diagnosis in this group was 56.5 years (range 27 – 93 years) and the majority of samples involved patients with ductal and lobular histological subtypes (72% and 11%, respectively).

The control group consisted of DNA samples obtained from 1226 individuals involving 756 non-cancer 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, Mannheim, Germany) 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 μl reaction mixtures containing 1 μl 10 x PCR buffer, 0.2 mM of each dNTP, 0.4 μM of each primer, 1 μl of cDNA template, and 0.5 U of Fast Start Taq DNA polymerase (Roche). Following the initial denaturation (at 95°C for 4 min), 35 cycles (at 95°C for 30 sec, 60°C for 30 sec and 72°C for 2 min) and a final extension (at 72°C for 7 min) were performed. Primer sequences are listed in Supplementary Table S1. PCR products were purified with ExoSAP-IT (USB Corp., Cleveland, USA) according to the supplier’s instructions and sequenced using the BigDye Terminator v3.1 cycle sequencing kit on an ABI 3130 genetic analyzer (Applied Biosystems, Foster City, USA). All mutations were confirmed by the DNA sequencing analysis and re-confirmed from a new blood sample.

Each identified *PALB2* mutation that caused a truncation of the protein product was screened in 1226 control samples by a high resolution melting (HRM) analysis using an HRM Master kit and the Light Cycler 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 \textit{PALB2} c.172_175delTTGT mutation detected repeatedly in BC families was screened in a group of 704 unselected BC cases by HRM analysis of exon 3.

\textit{Detection of large genomic rearrangements}

The multiplex ligation-dependent probe amplification (MLPA) kit for \textit{PALB2} (probemix P260-A1; MRC-Holland, Amsterdam, The Netherlands) was used for a relative quantification of each of the \textit{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 identified by MLPA were determined 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 (41). Primer sequences are displayed in Supplementary Table S1.

\textit{Nomenclature of PALB2 mutations}

Mutations were described according to the recommended nomenclature system described by the Human Genome Variation Society (HGVS) (42) with nucleotides numbered from the A of the ATG translation initiation codon of the NCBI reference sequence NM_024675.3. Genomic and protein sequences for \textit{PALB2} are given by NG_007406.1, and NP_078951.2.

\textit{Haplotype analysis}

To define the origin of the recurrent \textit{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 \textit{PALB2} on the centromeric side and two markers (D16S417 and D16S420) flanking \textit{PALB2} on the telomeric side. The positions of the markers and the \textit{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 Gene Mapper v4.0 Software.

\textit{In vitro and in silico analyses}

An \textit{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 (45), PolyPhen (46) and Align GVGD software (47).

Statistical analysis

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

Results

Analysis of PALB2 mutations in high-risk patients

To determine the prevalence of deleterious PALB2 mutations in the group of high-risk cancer patients, we analyzed the entire PALB2 coding region by direct sequencing and MLPA screening. Ten different protein truncating mutations and three large genomic rearrangements were identified in 16 out of 409 (3.9%) participants (Table 2), whereas one mutation was observed in 1226 controls (0.08%; P = 2.6×10⁻⁹). In 330 familial cases, 13 mutations were found in a subgroup of 235 families (5.5%) with a history positive for BC only (HBC families), whereas no mutation was detected in 95 cases from families with a history positive for BC and OC or for OC only (P = 0.02). In 79 high-risk non-familial patients, one carrier of the PALB2 mutation was detected in a group of 22 male BC patients (4.5%), one in a group of 39 patients with bilateral BC (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_1231del15, c.1924delA, c.1942_1945delCTTAinsAAC, c.3362delG) and two nonsense mutations (c.73A>T, 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 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 USA and different European countries (15, 17-19, 33, 36, 48), while 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 BC 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 *Alu*Sx1 sequences located in intron 6 and intron 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 BC. 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 g.26372 of intron 10 (g.22947_26370del3424, 26372C>G). Breakpoints were located in a sequence containing 16 identical nucleotides within *Alu*Sx3 and *Alu*Sz6 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) (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 *Alu*Sx3 in intron 8 and *Alu*Y 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 non-pathogenic (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 BC 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 BC patients (data not shown). Two cases of bilateral BC (15.4%, 2/13) occurred in patients with a PALB2 mutation, whereas 14 bilateral cases (6.3%, 14/222) 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, while 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 the 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 BC phenotype. Segregation was demonstrated in four families (Fig. 3A–D), while in family #1120 the pedigree analysis did not show segregation of the recurrent mutation c.172_175delTTGT with the cancer phenotype (Fig. 3E).

**Analysis of the recurrent \(PALB2\) mutation c.172_175delTTGT in unselected BC cases**

The presence of the recurrent mutation c.172_175delTTGT was further analyzed in an independent group of 704 unselected consecutive BC patients 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 BC patients) and on 16 randomly selected samples from non-cancer controls using three microsatellite markers flanking the \(PALB2\) gene. Four of six unrelated carriers shared a common \(~1.0\) 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% (4/16) 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 BC patients (0.3%) was lower compared to the frequency of the recurrent mutation c.1592delT reported by Erkko et al. from Finland (18/1918; 0.9%) (12).

We have identified large rearrangements at the \(PALB2\) locus in three families, which represents 18.8% (3/16) of all detected mutations and suggests that this type of gene alteration may play a notable role in predisposition to BC. 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 BC family in Spain (28) and in a Canadian patient affected with breast and pancreatic cancer (35).

The risk of BC associated with \(PALB2\) mutations was not calculated in our study because we were able to perform an extensive segregation analysis in only five mutation-positive families (Fig. 3A-E). Rahman et al. estimated initially that \(PALB2\) mutations were associated with a 2.3-fold increased risk.
of BC (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 BC penetrance may be similar in PALB2 and BRCA2 mutation carriers. This assumption support reports of Southey et al. (30) and Erkko et al. (50). The PALB2 c.3113G>A mutation identified by Southey et al. (30) in an Australian study was associated with 49% BC risk by the age of 50 and 91% risk (95% CI, 44%–100%) by the age of 70. The authors concluded that the hazard ratio (HR) of BC for their recurrent PALB2 mutation was similar to the age-specific HRs reported for mutations in the BRCA2 gene. Erkko et al. (50) estimated that the recurrent Finnish c.1592delT mutation is associated with a 40% (95% CI, 17%–77%) risk of BC to the age of 70 years. Similarly to BRCA2, defects in the PALB2 gene are also associated with hereditary predisposition to male BC (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 22 male BC cases, which may support the view of BC predisposition in men with mutations in PALB2.

A negative steroid receptor status predominated in breast tumors (reported in 5/9 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 high-risk breast/ovarian cancer patients 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 BC-susceptibility genes (ATM, CHEK2, 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 to that previously reported for mutations in BRCA2 (Table 1, Supplementary Table S3).

The presented results thus suggest that screening for protein-truncating alterations and large genomic rearrangements 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 BC 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 e.g. male BC cases.

Authors’ Contributions

Conception and design: P. Pohlreich, M. Janatova, Z. Kleibl
Analysis and interpretation of data (e.g. genotyping, MLPA, statistics): M. Janatova, J. Stribrna, P. Kleiblova, J. Soukupova, P. Dundr

Acquisition of data: (e.g. patients’ characteristics, statistics): A. Panczak, M. Zimovjanova, K. Vesela


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Tables:

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Patients; N</th>
<th>Mean age at dg: years</th>
<th>BRCAPRO; mean value</th>
<th>Mutations; N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Familial cases (N=330)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“HBC families”</td>
<td>235</td>
<td>47.2</td>
<td>0.16</td>
<td>13 (5.5)</td>
</tr>
<tr>
<td>“HBOC families”</td>
<td>90</td>
<td>47.8</td>
<td>0.35</td>
<td>0</td>
</tr>
<tr>
<td>“HOC families”</td>
<td>5</td>
<td>40.2</td>
<td>0.35</td>
<td>0</td>
</tr>
<tr>
<td><strong>Non-familial cases (N=79)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male BC</td>
<td>22</td>
<td>64.7</td>
<td>0.14</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>Bilateral BC before 50</td>
<td>39</td>
<td>43.7</td>
<td>0.30</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>Tumor duplicity, BC + OC</td>
<td>18</td>
<td>53.7</td>
<td>0.33</td>
<td>1 (5.6)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>409</strong></td>
<td></td>
<td></td>
<td><strong>16 (3.9)</strong></td>
</tr>
</tbody>
</table>

“HBC, hereditary breast cancer; “HBOC, hereditary breast and ovarian cancer; “HOC, hereditary ovarian cancer
Table 2. Truncating mutations and large rearrangements in PALB2 gene

<table>
<thead>
<tr>
<th>Pat. No.</th>
<th>Exon</th>
<th>Nucleotide change</th>
<th>Protein change</th>
<th>Mut. inctrls</th>
<th>BC/other cancers age at dg</th>
<th>Cancers in the family (age at dg)</th>
<th>BRCA-PRO</th>
<th>Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIGH-RISK PATIENTS: N=409 (TRUNCATING MUTATIONS)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>509b</td>
<td>2</td>
<td>c.73A&gt;T</td>
<td>p.K25*</td>
<td>0</td>
<td>BC 75/75 Bladder 78</td>
<td>Breast, D (40) Uterus, M (47)</td>
<td>0.05</td>
<td>N.A.</td>
</tr>
<tr>
<td>451</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Breast, PGM (36) Endometrial, PS (?)</td>
<td></td>
<td>0.11</td>
<td>N.A.</td>
</tr>
<tr>
<td>738</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Breast, S (41) Breast, MGM (70) Colon, MS (50)</td>
<td></td>
<td>0.29</td>
<td>ER, PR, HER2</td>
</tr>
<tr>
<td>1026</td>
<td>3</td>
<td>c.172_175delTTGT</td>
<td>p.L58fs</td>
<td>0</td>
<td>BC 38</td>
<td>Breast, M (56), Breast, MGM (40) Colon, MGF (77)</td>
<td>0.21</td>
<td>ER*, PR, HER2</td>
</tr>
<tr>
<td>1120c</td>
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<td></td>
<td></td>
<td></td>
<td>BC 42/52</td>
<td>Breast, S (55), Breast, MGM (7); Colon, B (56); Kidney, B (58) Pancreas, M (46)</td>
<td>0.39</td>
<td>N.A.</td>
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<tr>
<td>579</td>
<td>4</td>
<td>c.509_510delGA</td>
<td>p.R170fs</td>
<td>1</td>
<td>BC 59</td>
<td>Breast, S (40), Breast, PGM (50) Breast, MGM (72) Uterus, MS (?) Pancreas, PGM (?)</td>
<td>0.07</td>
<td>N.A.</td>
</tr>
<tr>
<td>340</td>
<td>4</td>
<td>c.661_662delinsTA</td>
<td>p.V221*</td>
<td>0</td>
<td>BC 46</td>
<td>Breast, S (50) Breast, MGM (60) Gallbladder, F (68)</td>
<td>0.07</td>
<td>ER*, PR, HER2</td>
</tr>
<tr>
<td>1260b</td>
<td>4</td>
<td>c.901_907del7</td>
<td>p.D301fs</td>
<td>0</td>
<td>BC 36</td>
<td>None</td>
<td>0.14</td>
<td>ER*, PR, HER2</td>
</tr>
<tr>
<td>1320</td>
<td>4</td>
<td>c.1227_1231delI5</td>
<td>p.Y409fs</td>
<td>0</td>
<td>BC 37</td>
<td>Breast, S (38) Breast, M (65)</td>
<td>0.70</td>
<td>ER, PR, HER2</td>
</tr>
<tr>
<td>379a</td>
<td>4</td>
<td>c.1240C&gt;T</td>
<td>p.R414*</td>
<td>0</td>
<td>BC 38</td>
<td>Breast, M (50)</td>
<td>0.09</td>
<td>N.A.</td>
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<tr>
<td>413</td>
<td>5</td>
<td>c.1924delA</td>
<td>p.M642fs</td>
<td>0</td>
<td>BC 46</td>
<td>Breast, PGM (37)</td>
<td>0.08</td>
<td>N.A.</td>
</tr>
<tr>
<td>211*</td>
<td>5</td>
<td>c.1942_1945delCTTAinsAAC</td>
<td>p.L648fs</td>
<td>0</td>
<td>BC 41/42</td>
<td>None</td>
<td>0.39</td>
<td>N.A.</td>
</tr>
<tr>
<td>1436</td>
<td>13</td>
<td>c.3362delG</td>
<td>p.G1121fs</td>
<td>0</td>
<td>BC 45</td>
<td>Breast, MGM (49)</td>
<td>0.08</td>
<td>ER, PR, HER2</td>
</tr>
<tr>
<td><strong>HIGH-RISK PATIENTS: N=409 (LARGE REARRANGEMENTS)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1647</td>
<td>Del ex 7-8</td>
<td>g.19557_22659del3103</td>
<td>p.N863fs</td>
<td>N.A.</td>
<td></td>
<td>Breast, S (30) Gallbladder, MGM (73)</td>
<td>0.44</td>
<td>N.A.</td>
</tr>
<tr>
<td>1507a</td>
<td>Del ex 9-10</td>
<td>g.22947_26370del13424, 26372C&gt;G</td>
<td>p.A946_W1038del</td>
<td>N.A.</td>
<td></td>
<td>Breast, M (52) Breast, MGM (40)</td>
<td>0.15</td>
<td>N.A.</td>
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<tr>
<td>79a</td>
<td>Dup ex 9-11</td>
<td>g.22904_36589dup13686</td>
<td>p.G1068fs</td>
<td>N.A.</td>
<td></td>
<td>Breast, M ( ?) Colon, F (?) Gallbladder, M (?)</td>
<td>0.31</td>
<td>ER*, PR, HER2</td>
</tr>
<tr>
<td><strong>UNSELECTED BC PATIENTS: N=704</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A210</td>
<td>3</td>
<td>c.172_175delTTGT</td>
<td>p.L58fs</td>
<td>0</td>
<td>BC 65</td>
<td>Lung, F (55) Kidney, M (53)</td>
<td>0.01</td>
<td>ER, PR, HER2</td>
</tr>
<tr>
<td>A361</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BC 46</td>
<td>None</td>
<td>0.06</td>
<td>ER, PR, HER2</td>
</tr>
</tbody>
</table>

aBilateral BC; b tumor duplicity or triplicity; c male BC; d medullary BC. 1 BRCA-PRO 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. Novel mutations are marked in bold. Abbreviations: D, daughter; M, mother; F, father; S, sister; B, brother; MS, maternal sister; PS, paternal sister; MGMT, maternal grandmother; PGM, paternal grandmother; MGF, maternal grandfather; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; N.A., not available.
Table 3. Microsatellite genotyping in c.172_175delTTGT mutation carriers

<table>
<thead>
<tr>
<th>Patient no</th>
<th>Microsatellite marker (sizes in bp)</th>
<th>D16S412</th>
<th>D16S417</th>
<th>D16S420</th>
</tr>
</thead>
<tbody>
<tr>
<td>451</td>
<td>151/151</td>
<td>260/246</td>
<td>255/259</td>
<td></td>
</tr>
<tr>
<td>738</td>
<td>151/151</td>
<td>260/246</td>
<td>245/255</td>
<td></td>
</tr>
<tr>
<td>1026</td>
<td>151/151</td>
<td>260/260</td>
<td>245/261</td>
<td></td>
</tr>
<tr>
<td>1120</td>
<td>151/153</td>
<td>246/258</td>
<td>245/243</td>
<td></td>
</tr>
<tr>
<td>A210</td>
<td>151/149</td>
<td>260/242</td>
<td>245/255</td>
<td></td>
</tr>
<tr>
<td>A361</td>
<td>151/151</td>
<td>260/246</td>
<td>245/255</td>
<td></td>
</tr>
</tbody>
</table>

Note: Alleles of identical size are highlighted in bold numbers.
Figure Legends:

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 translation product is indicated by a blue arrowhead; C – wt control.

Figure 2. MLPA-based detection of large genomic rearrangements 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 PCR products by a blue arrowhead; lane C – negative control; lane M – size standards.

Figure 3. Pedigrees of the five breast cancer families with PALB2 mutations. Probands are 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 non-carriers (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).
Cancer Epidemiology, Biomarkers & Prevention

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Cancer Epidemiol Biomarkers Prev  Published OnlineFirst October 17, 2013.