The Breast Cancer Susceptibility Mutation PALB2 1592delT Is Associated with an Aggressive Tumor Phenotype

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Abstract Purpose: To determine the effect of the breast cancer susceptibility mutation PALB2 1592delT on tumor phenotype and patient survival.

Experimental Design: We defined the PALB2 mutation status in 947 familial and 1,274 sporadic breast cancer patients and 1,079 population controls, and compared tumor characteristics and survival in mutation carriers relative to other familial and sporadic cases and to 79 BRCA1 and 104 BRCA2 mutation carrier cases.

Results: The PALB2 1592delT mutation was found in 19 familial (2.0%; odds ratio, 11.03; 95% confidence interval (CI), 2.65-97.78; \( P < 0.0001 \)) and eight sporadic patients (0.8%; odds ratio, 3.40; 95% CI, 0.68-19.95; \( P = 0.1207 \)) compared with two (0.2%) control individuals. Tumors of the PALB2 mutation carriers presented triple negative (estrogen receptor negative/progesterone receptor negative/Human epidermal growth factor receptor-2 negative) phenotype more often (54.5%; \( P < 0.0001 \)) than those of other familial (12.2%) or sporadic (9.4%) breast cancer patients. They were also more often of higher grade (\( P = 0.0027 \) and \( P = 0.0017 \), respectively) and had higher expression of Ki67 (\( P = 0.0004 \) and \( P = 0.0490 \), respectively). Carrying a PALB2 mutation was also associated with reduced survival, especially in familial cases (hazard ratio, 2.30; 95% CI, 1.01-5.24; \( P = 0.0466 \)) and among familial patients with HER2-negative tumors (hazard ratio, 4.57; 95% CI, 1.96-9.80; \( P = 0.0004 \)). Carrying a BRCA2 mutation was also found to be an independent predictor of poor survival at 10-year follow-up (\( P = 0.04 \)).

Conclusions: The PALB2 1592delT mutation has a strong effect on familial breast cancer risk. The tumors rising in patients carrying this mutation manifest a phenotype associated with aggressive disease. Our results also suggest a significant impact of carrying a BRCA2 mutation on long-term breast cancer survival.

Breast cancer, the most common cancer among women in the world, has been estimated to have an inherited component of up to 27% (1). Mutations in the two high-penetrance predisposing genes, BRCA1 and BRCA2, are estimated to cause ~15% of familial predisposition to breast cancer (2). Based on a meta-analysis, individuals with an inactivating germline mutation in BRCA1 have ~57% risk for developing breast cancer and 40% risk for ovarian cancer by the age of 70 years, and individuals with a BRCA2 mutation have 49% risk for breast cancer and 18% risk for ovarian cancer (3). However, the risks may vary depending on family history or other modifying risk alleles (4, 5). The proteins encoded by these genes have an essential role in the maintenance of genomic integrity by homologous recombination repair of DNA double-strand breaks. They carry out these functions through interactions with a diverse network of other proteins (6). One of the most important interaction partners for BRCA2 is PALB2.

PALB2 (partner and localizer for BRCA2) has an important function in the regulation and localization of BRCA2 (7). PALB2 protein colocalizes with BRCA2 in nuclear foci, strongly increasing the stability of BRCA2. PALB2 participates in the regulation of the nuclear functions of BRCA2, particularly homologous recombination–based DNA double-strand break repair, and in the regulation of the involvement of BRCA2 in the S phase checkpoint (7). Biallelic inactivating mutations in PALB2 (also known as FANCN) cause Fanconi anemia subtype N, which resembles the Fanconi anemia phenotype caused by biallelic mutations in BRCA2 (FANCDD1; refs. 8, 9). PALB2 has been shown to act as a breast cancer susceptibility gene (10), and large studies on the role of PALB2 mutations in breast cancer susceptibility have been conducted in British (8), Finnish (11), French Canadian (12), Ashkenazi Jewish (13),

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Survival among mutation-positive cases. We also studied breast cancer BRCA2 mutation carriers and compared those with those of other breast thusfar. In this study, we have comprehensively investigated the on the survival of breast cancer patients has not been studied (18), but the effects of BRCA2 mutations on tumor characteristics and histopathologic features have thus far been investigated in only a few tumors (11–15). The effect PALB2 may have on the survival of breast cancer patients has not been studied thus far. In this study, we have comprehensively investigated the histopathologic characteristics of breast tumors from PALB2 mutation carriers and compared those with those of other breast cancers in extensive series of familial cases negative for mutations in BRCA1 and BRCA2, sporadic cases, and BRCA1 and BRCA2 mutation–positive cases. We also studied breast cancer survival among PALB2, BRCA1, and BRCA2 mutation carriers.

Translational Relevance

We have shown that the PALB2 1592delT breast cancer susceptibility mutation is associated with an increasing breast cancer risk by increasing family history. The tumors rising in the patients carrying this mutation manifest a phenotype associated with an aggressive disease, particularly the triple-negative and basal-like subtype, and mutation carriers have reduced survival, especially those with a family history and those with HER2-negative tumors. These results may warrant consideration of genetic testing for the 1592delT mutation and more careful cancer surveillance, as well as more aggressive adjuvant treatment of carriers in breast cancer families. Our results also suggest a significant negative impact of BRCA2 mutation on long-term breast cancer survival.

Materials and Methods

Subjects. The breast cancer cases genotyped in this study consisted of two unselected patient series (1,870 patients in total) and additional 542 familial patients collected at the Helsinki University Central Hospital. The first unselected series of 884 patients was collected at the Department of Oncology in 1997 to 1998 and 2000, (including 79% of all consecutive, newly diagnosed breast cancer cases during the collection periods; refs. 19, 20) and the second series of 986 unselected patients at the Department of Surgery at 2001 to 2004 (87% of all consecutive, newly diagnosed breast cancer cases; ref. 21), with 1,706 samples (92% of all) genotyped for the PALB2 1592delT mutation. Familial breast cancer cases were collected at the Helsinki University Central Hospital, as described (22). Familial cases consisted of patients with strong familial background, defined as three or more breast or ovarian cancers among the first- or second-degree relatives, including the proband, who had been tested negative for BRCA1 and BRCA2 mutations, as previously described (23–25). The rest of the familial cases had one first-degree family member affected with breast cancer and had been screened negative for Finnish BRCA1/2 founder mutations (24, 25). The genealogies were confirmed through population registries, and cancer diagnoses were confirmed through the Finnish Cancer Registry and hospital records. For statistical analyses, all patients and tumors (including contralateral breast cancers) from the different patient series were stratified by family history status, resulting in 947 familial cases (426 cases from the families with three or more cases and 521 from the smaller two-case families) with information on 996 invasive tumors and 1,274 sporadic patients (not fulfilling the above family history criteria) with 1,325 invasive tumors. Population controls consisted of 1,079 healthy females collected from the same geographic region. Seventy-nine BRCA1 mutation carriers from 40 different families (with information on 75 invasive tumors) and 104 BRCA2 mutation carriers from 41 different families (with information on 78 invasive tumors) were also included in this study.

Information on tumor histology, grade, size, nodal status, and distant metastases were collected from pathology reports (26). In addition, a breast cancer pathologist rereviewed 1,423 tumors (the tumors included in the tumor microarrays indicated below; 56% of the total number) for tumor histology and grade. Grading was done according to Scarff-Bloom-Richardson modified by Elston and Ellis (27). Estrogen receptor and progesterone receptor status were abstracted from pathology reports. Evaluation of the results and the staining methods used in routine diagnostics has been described by Eerola et al. (26). For estrogen receptor and progesterone receptor status, samples with >10% of the cancer cells stained positive were considered as positive. HER2 status was based on immunohistochemical staining (samples with <10% of the cells stained were considered negative and >90% positive) and gene amplification with chromogenic in situ hybridization (more than six replications was considered positive and zero to five replications was considered negative) on tumor microarrays, as described (28, 29). p53 protein expression was studied by immunohistochemical staining, also as previously described (30), and samples were defined as positive for p53 when >20% of the cancer cells were positive for the staining. Ki67 status was defined as described (31), with strong positive expression considered when ≥30%, intermediate when 20% to 29%, weak positive when 5% to 19%, and negative when ≤5% of the cancer cells were stained with Ki67 antibody (32). Cytokeratin expression had been determined by immunohistochemical analysis of tumor microarrays with antibodies to cytokeratins 5/6, 14, and 17. Specimens were considered negative if immunopositivity was found in 0% to 10% and positive if 11% to 100% of the cancer cells showed immunopositivity (33). Cyclin D1 status was defined as described (34). The samples with more positively stained cells than the mean value of all samples (9.1%) were considered as high-expression tumors and the samples with less than mean value as low expression. The tumors were further divided into the subtypes of luminal A, luminal B, HER2-positive, and triple negative using their estrogen receptor/progesterone receptor and HER2 status as surrogate markers for these subtypes, as has been previously described (35, 36). Luminal A was defined as having positive estrogen receptor or progesterone receptor expression and no HER2 overexpression/amplification, luminal B as having positive estrogen receptor or progesterone receptor expression and HER2 overexpression/amplification, and the HER2-positive subtype as having negative expression of estrogen receptor and progesterone receptor but HER2 overexpression/amplification. Triple-negative tumors were negative for all three markers. The basal-like subtype was differentiated from the triple-negative group by the expression of any of the basal cytokeratins 5/6, 14, or 17 (36).

Information on death due to breast cancer was obtained from the Finnish Cancer Registry, which collects diagnostic as well as death information on all cancer patients in Finland. Survival was calculated as 10-y breast cancer–specific survival: the time from the date of primary surgery to the date of death due to breast cancer within 10 y. A total of 2,342 patients with invasive breast cancer were included in the survival analysis. The median follow-up time was 75 mo (1-488 mo). Of all patients in the survival analysis, 284 died from breast cancer within 10 y from diagnosis.
This study was done with informed consent from the patients as well as permission from the ethics committee of the Helsinki University Central Hospital and from the Ministry of Social Affairs and Health in Finland.

**Genotyping.** The 1592delT mutation was genotyped on genomic DNA isolated from the patients' blood samples using Amplifluor fluorescent genotyping (KBiosciences) or conformation sensitive gel electrophoresis heteroduplex conformation analysis (37). All detected mutations were verified by direct sequencing using ABI BigDye Terminator 3.1 Cycle Sequencing kit and ABI Prism 310 genetic analyzer (Applied Biosystems).

**Statistical analyses.** The statistical analyses were done using the SPSS 15.0 for Windows (SPSS, Inc.). P values for comparisons of patient groups by family history and for the evaluation of the differences in tumor characteristics were calculated using Pearson's $\chi^2$ or Fisher's exact test, the latter when the expected number of cell count was less than five. All P values were two-sided. The P values for comparisons of the age of onset among different patient groups were calculated using Student's $t$ test. Survival analyses were done by calculating the Kaplan-Meier survival curves and comparing the 10- and 15-year breast cancer-specific survival of the separate groups of patients with log-rank test. The estimates of relative risks for breast cancer death between different patient groups of PALB2 1592delT, BRCA1, BRCA2 mutation carriers versus other familial or sporadic cases were calculated using univariate Cox's regression analysis. We also constructed Cox's proportional hazards regression models using the forward conditional algorithm of SPSS v15.0 to evaluate independent effects of the mutations on survival when adjusted for other known prognostic factors (tumor size, nodal status, primary metastasis, estrogen receptor, progesterone receptor, grade, Ki67, p53, Her2) among all cases and HER2-negative cases. The models were also applied to invasive familial cases only.

**Results**

**Mutation frequency in patient groups and risk effects.** Table 1 shows the frequencies of the 1592delT mutation in PALB2 among patients with different familial background compared with population controls. Only two control individuals of 1,079 were found to be mutation carriers (0.2%). The mutation was considerably more common in patients with a family history of breast cancer [2.0%; odds ratio relative to controls, 11.03; 95% confidence interval (95% CI), 2.65-97.78] than in sporadic cases (0.6%; odds ratio, 3.40; 95% CI, 0.68-32.95). The mutation frequency was highest for patients with the strongest breast cancer family history (2.6%; odds ratio, 14.27; 95% CI, 3.09-132.8; $P = <0.0001$), which was also significantly higher than among sporadic breast cancer patients (odds ratio, 4.19; 95% CI, 1.52-12.09; $P = 0.0022$). The PALB2 1592delT mutation was found in two unrelated BRCA2 carriers of the 104 screened but not in any of the 79 BRCA1 mutation carriers.

The average age of first breast cancer among 1592delT carriers was 53.1 years (with variation between 33.4 and 79.9 years), which is not significantly lower than the mean age of diagnosis among all familial PALB2/BRCA1/BRCA2-negative breast cancer patients (54.8 years; 22.3-95.4; $P = 0.4722$; $n = 872$) or among sporadic patients (58.0 years; 27.8-95.6; $P = 0.1515$; $n = 1,268$). The mean age of diagnosis among BRCA1 carriers in our data set was 45.2 years (22.2-79.8) and among BRCA2 carriers 47.4 years (27.3-80.3), both of which are significantly lower ($P < 0.0001$) than other familial or sporadic breast cancer patients. The incidence of contralateral breast cancer was similar in PALB2 carriers (3 of 28; 10.7%) and among other familial non-BRCA1/2 breast cancer cases (94 of 859; 10.9%; $P = 0.99$).

**Tumor characteristics.** The histopathologic features of the PALB2 1592delT mutation carriers and noncarriers are presented in Table 2. Compared with familial or sporadic breast cancers, the 1592delT mutation was strongly associated with negative estrogen receptor and progesterone receptor status, and although not statistically significant, all but one of the PALB2 carrier tumors with HER2 data available had negative HER2 status. PALB2 mutation carriers were at significantly higher risk for developing triple-negative (estrogen receptor/progesterone receptor/HER2 negative) tumors than noncarriers ($P < 0.0001$). The tumors of PALB2 mutation carriers were more often of higher grade. The cytokeratin 5/6 and 17 expression of tumors was not different between PALB2 mutation carriers and other breast cancer patients. However, the tumors of PALB2 mutation carriers expressed cytokeratin 14 more often than sporadic tumors ($P = 0.0205$). Altogether, the basal-like subtype was significantly more common among patients with a PALB2 mutation than among either sporadic or other familial patients ($P = <0.0001$). The tumors of PALB2 carriers also presented higher expression of Ki67, and they showed more often low cyclin D1 expression than tumors of other familial or sporadic breast cancer patients. BRCA1 carrier tumors were even more often estrogen receptor- and progesterone receptor-negative than PALB2 carrier tumors (Supplementary Table 1) but had less frequently positive lymph node status than PALB2 carriers. BRCA1 (97.6%; $P = 0.0223$) and BRCA2 (100%; $P = 0.0017$) carrier tumors were significantly more often negative for HER2 than familial and sporadic tumors (84.9%). Of the tumors of BRCA1

### Table 1. Frequencies of PALB2 1592delT in different patient groups

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Total</th>
<th>1592delT</th>
<th>OR*</th>
<th>95% CI</th>
<th>$P$</th>
<th>OR†</th>
<th>95% CI</th>
<th>$P$</th>
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<tbody>
<tr>
<td>Population controls</td>
<td>1,079</td>
<td>1,077</td>
<td>2</td>
<td>(0.2%)</td>
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<tr>
<td>Familial, three or more affected</td>
<td>426</td>
<td>415</td>
<td>11</td>
<td>(2.6%)</td>
<td>14.27</td>
<td>3.09-132.82</td>
<td>&lt;0.0001</td>
<td>4.19</td>
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<tr>
<td>Familial, two affected</td>
<td>521</td>
<td>513</td>
<td>8</td>
<td>(1.5%)</td>
<td>8.40</td>
<td>1.67-81.34</td>
<td>0.0028</td>
<td>2.47</td>
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<tr>
<td>Sporadic breast cancer patients</td>
<td>1,274</td>
<td>1,266</td>
<td>8</td>
<td>(0.6%)</td>
<td>3.40</td>
<td>0.68-32.95</td>
<td>1</td>
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<tr>
<td>All familial breast cancer patients</td>
<td>947</td>
<td>928</td>
<td>19</td>
<td>(2.0%)</td>
<td>11.03</td>
<td>2.65-97.78</td>
<td>&lt;0.0001</td>
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<tr>
<td>Unselected breast cancer patients</td>
<td>1,706</td>
<td>1,694</td>
<td>12</td>
<td>(0.7%)</td>
<td>3.81</td>
<td>0.85-35.15</td>
<td>0.0952</td>
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</tbody>
</table>

Abbreviation: OR, odds ratio.

*Odds ratio as compared with population controls.

†Odds ratio as compared with sporadic breast cancer patients.
**Table 2. Tumor characteristics of PALB2 1592delT mutation carriers compared with the tumors of familial and sporadic patients**

<table>
<thead>
<tr>
<th>Category</th>
<th>PALB2</th>
<th>Familial*</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
<th>Sporadic</th>
<th>P</th>
<th>OR</th>
<th>95% CI</th>
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<tr>
<td>Pos</td>
<td>15 (45.5%)</td>
<td>541 (58.1%)</td>
<td>0.1482</td>
<td>0.60</td>
<td>0.30-1.21</td>
<td>707 (54.4%)</td>
<td>0.3093</td>
<td>0.70</td>
<td>0.35-1.40</td>
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<tr>
<td>Neg</td>
<td>32 (97.0%)</td>
<td>918 (97.5%)</td>
<td>0.5818</td>
<td>0.84</td>
<td>0.11-6.38</td>
<td>1,262 (96.0%)</td>
<td>0.9681</td>
<td>0.99</td>
<td>0.49-2.00</td>
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<td>Pos</td>
<td>1 (3.0%)</td>
<td>24 (2.5%)</td>
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<tr>
<td>Neg</td>
<td>14 (46.7%)</td>
<td>181 (20.9%)</td>
<td>0.0008</td>
<td>3.31</td>
<td>1.58-6.90</td>
<td>217 (16.8%)</td>
<td>&lt;0.0001</td>
<td>4.33</td>
<td>2.08-9.00</td>
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<tr>
<td>Pos</td>
<td>16 (53.3%)</td>
<td>390 (41.9%)</td>
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<td>Pos</td>
<td>32 (97.0%)</td>
<td>918 (97.5%)</td>
<td>0.1482</td>
<td>0.60</td>
<td>0.30-1.21</td>
<td>707 (54.4%)</td>
<td>0.3093</td>
<td>0.70</td>
<td>0.35-1.40</td>
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<td>p53</td>
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<tr>
<td>Neg</td>
<td>18 (78.3%)</td>
<td>517 (80.0%)</td>
<td>0.8349</td>
<td>0.90</td>
<td>0.33-2.46</td>
<td>459 (79.3%)</td>
<td>0.9064</td>
<td>0.94</td>
<td>0.34-2.59</td>
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<tr>
<td>Pos</td>
<td>5 (21.7%)</td>
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<td>3 (9.4%)</td>
<td>223 (25.4%)</td>
<td>0.0027</td>
<td>0.35</td>
<td>0.17-0.72</td>
<td>351 (27.6%)</td>
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<td>404 (46.1%)</td>
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<td>3</td>
<td>17 (53.1%)</td>
<td>250 (28.5%)</td>
<td>0.3803</td>
<td>1.43</td>
<td>0.64-3.21</td>
<td>874 (66.4%)</td>
<td>0.2608</td>
<td>1.58</td>
<td>0.71-3.53</td>
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<td>Ductal</td>
<td>25 (75.8%)</td>
<td>652 (68.6%)</td>
<td>0.3803</td>
<td>1.43</td>
<td>0.64-3.21</td>
<td>874 (66.4%)</td>
<td>0.2608</td>
<td>1.58</td>
<td>0.71-3.53</td>
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<tr>
<td>Lobular</td>
<td>3 (9.1%)</td>
<td>197 (20.7%)</td>
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<td>Medullary</td>
<td>0 (0.0%)</td>
<td>18 (1.9%)</td>
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<tr>
<td>Other</td>
<td>5 (15.2%)</td>
<td>84 (8.8%)</td>
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<td>LumA</td>
<td>9 (40.9%)</td>
<td>453 (72.6%)</td>
<td>&lt;0.0001</td>
<td>8.65</td>
<td>3.61-20.71</td>
<td>447 (75.1%)</td>
<td>&lt;0.0001</td>
<td>11.55</td>
<td>4.78-27.93</td>
</tr>
<tr>
<td>LumB</td>
<td>6 (14.6%)</td>
<td>150 (25.9%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Her2 pos</td>
<td>3 (4.5%)</td>
<td>16 (2.5%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triple-neg</td>
<td>5 (22.7%)</td>
<td>26 (4.2%)</td>
<td>&lt;0.0001</td>
<td>6.31</td>
<td>2.44-16.29</td>
<td>19 (3.2%)</td>
<td>&lt;0.0001</td>
<td>12.15</td>
<td>4.50-32.81</td>
</tr>
<tr>
<td>CK-5/6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neg</td>
<td>17 (77.3%)</td>
<td>518 (84.8%)</td>
<td>0.3389</td>
<td>0.61</td>
<td>0.22-1.70</td>
<td>331 (86.9%)</td>
<td>0.2020</td>
<td>0.51</td>
<td>0.18-1.45</td>
</tr>
<tr>
<td>Pos</td>
<td>5 (22.7%)</td>
<td>93 (15.2%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK-14</td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Neg</td>
<td>15 (71.4%)</td>
<td>487 (83.4%)</td>
<td>0.1519</td>
<td>0.50</td>
<td>0.19-1.32</td>
<td>332 (88.5%)</td>
<td>0.0205</td>
<td>0.32</td>
<td>0.12-0.88</td>
</tr>
<tr>
<td>Pos</td>
<td>6 (28.6%)</td>
<td>97 (16.6%)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>CK-17</td>
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<td></td>
</tr>
<tr>
<td>Neg</td>
<td>19 (90.5%)</td>
<td>513 (94.3%)</td>
<td>0.3505</td>
<td>0.57</td>
<td>0.13-2.58</td>
<td>360 (96.8%)</td>
<td>0.1680</td>
<td>0.32</td>
<td>0.07-1.52</td>
</tr>
<tr>
<td>Pos</td>
<td>2 (9.5%)</td>
<td>31 (5.7%)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK combined</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neg</td>
<td>14 (63.6%)</td>
<td>368 (69.0%)</td>
<td>0.5916</td>
<td>0.78</td>
<td>0.32-1.91</td>
<td>285 (79.8%)</td>
<td>0.0708</td>
<td>0.44</td>
<td>0.18-1.09</td>
</tr>
<tr>
<td>Pos</td>
<td>8 (36.4%)</td>
<td>165 (31.0%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ki67</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (&lt;5%)</td>
<td>3 (10.3%)</td>
<td>205 (25.9%)</td>
<td>0.0004</td>
<td>0.27</td>
<td>0.12-0.59</td>
<td>225 (18.6%)</td>
<td>0.0490</td>
<td>0.36</td>
<td>0.16-0.77</td>
</tr>
<tr>
<td>1 (5-19%)</td>
<td>7 (24.1%)</td>
<td>319 (40.3%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (20-29%)</td>
<td>8 (27.6%)</td>
<td>138 (17.4%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (≥30%)</td>
<td>11 (37.9%)</td>
<td>130 (16.4%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclin D1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>7 (31.8%)</td>
<td>308 (53.6%)</td>
<td>0.0449</td>
<td>0.40</td>
<td>0.16-1.01</td>
<td>203 (51.8%)</td>
<td>0.0683</td>
<td>0.43</td>
<td>0.17-1.09</td>
</tr>
<tr>
<td>Low</td>
<td>15 (68.2%)</td>
<td>267 (46.4%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: T, tumor size; N, nodal status; M, primary metastasis; ER, estrogen receptor; PR, progesterone receptor; LumA, luminalA; LumB, luminalB; Neg, negative; CK, cytokeratin; Pos, positive.

*Non-BRCA1/2 and non-PALB2.*
carriers, 27.5% were of the basal-like subtype compared with 6.9% of the tumors of mutation-negative familial patients ($P < 0.0001$; odds ratio, 5.31; 95% CI, 2.40-10.96). BRCA2 carrier tumors were less frequently of basal-like type ($P = 0.0076$) and more frequently of luminal A type ($P = 0.0058$) than PALB2 carrier tumors. PALB2 and BRCA1 carrier tumors differed significantly for cyclin D1 expression, wherein BRCA1 tumors expressed even more frequently low levels of cyclin D1 ($P = 0.0039$).

Survival analyses. The Kaplan-Meier survival analyses of PALB2, BRCA1, and BRCA2 mutation carriers are presented in Fig. 1. Among all cases, BRCA1 and especially BRCA2 mutation carriers had significantly reduced 10-year breast cancer–specific survival (76.0% and $P = 0.047$, and 63.7% and $P < 0.001$, respectively). PALB2 mutation carriers had a similar estimated cumulative survival to BRCA1 carriers, but the difference from nonmutation carriers was not statistically significant ($P = 0.2$; Fig. 1A). Because all three mutations were associated with HER2-negative tumors, we further explored their survival effect, especially among HER2-negative breast cancer patients. Among HER2-negative patients, the 10-year breast cancer–specific survival was significantly worse for BRCA2 carriers (55.3%; $P < 0.001$) and PALB2 1592delT mutation carriers (63.5%; $P = 0.007$) than for nonmutation carrier patients (85.7%; Fig. 1B). Because PALB2 mutations were strongly
associated with the risk for familial breast cancer and because also poor prognosis of breast cancer has been found to cluster in families (38), we explored whether the PALB2 mutation might influence also survival of the affected cases in breast cancer families. Among familial cases, the mutation carriers had reduced survival (P = 0.041; Fig. 1C), especially so among familial patients with HER2-negative tumors (P < 0.001; Fig. 1D).

Univariate hazard ratios calculated with Cox’s regression analysis for breast cancer death within 10 years from diagnosis for patients with PALB2 1592delT, BRCA1, and BRCA2 mutation are presented in Table 3. BRCA2 carriers had significantly increased risk for breast cancer–specific death (hazard ratio, 2.34; P = 0.0002), particularly among HER2-negative patients (hazard ratio, 3.75; P < 0.0001), whereas the result for BRCA1 mutation carriers was not statistically significant. The risk for death for PALB2 mutation carriers was significantly higher among all HER2-negative breast cancer patients (hazard ratio, 2.94; P = 0.010) and among familial non-BRCA1/2 patients (hazard ratio, 2.94; P = 0.047), especially so among HER2-negative familial patients (hazard ratio, 4.57; P < 0.001).

The results from multivariate Cox’s regression analysis by mutation status, adjusting for established prognostic factors, are summarized in Table 4. Carrying a BRCA2 mutation was clearly an independent factor on breast cancer survival, with ~2-fold increased risk for breast cancer–specific death among all patients (hazard ratio, 2.06; P = 0.04) and among patients stratified by negative HER2 status (hazard ratio, 2.04; P = 0.05). Carrying a PALB2 mutation was an independent prognostic factor among all HER2-negative breast cancer cases (hazard ratio, 2.45; P = 0.04), along with the conventional prognostic factors as well as among familial breast cancer patients (hazard ratio, 2.38; P = 0.05), especially the HER2-negative cases (hazard ratio, 3.49; P = 0.008).

Discussion

The 1592delT mutation was found in 2.0% of familial breast cancer patients in Southern Finland, with a higher prevalence among cases with a stronger family history (2.6%) than among patients with only one affected first-degree relative (1.5%) or among family history–negative cases (0.6%). The observed increase in frequency of the variant with increasing family history is consistent with it having multiplicative effects with other and thus far unknown susceptibility alleles in the PALB2 mutation carrier families, as has previously been suggested also for carriers of another moderate penetrance variant CHEK2 1100delC (39). The high odds ratio (odds ratio, 14.27; 95% CI, 3.09-132.87) for the 1592delT mutation among the breast cancer cases with strongest family history suggests a significantly elevated familial breast cancer risk, which may warrant consideration of genetic testing for the 1592delT mutation and more careful presymptomatic screening of carriers in Finnish breast cancer families (this study; ref. 16). However, identification of putative other risk alleles with synergistic effects with the PALB2 allele will be highly important for more accurate risk estimation for the carriers. We observed no increased risk for contralateral breast cancer for PALB2 mutation carriers.

Tumors of PALB2 carriers exhibited a phenotype of ductal histology and high grade, as well as negative estrogen receptor, progesterone receptor, and HER2 status, and high proliferation rate as compared with tumors of other familial or sporadic patients. Interestingly, although PALB2 is an interaction partner of BRCA2 and it could be presumed that tumors of PALB2 mutation carriers would be more similar to those of BRCA2 carriers, tumors of PALB2 carriers shared similar features with tumors of BRCA1 carriers. Furthermore, tumors of PALB2 carriers, similar to BRCA1, associate very strongly with the triple-negative (estrogen receptor/progesterone receptor/HER2 negative) and basal-like phenotype. This is not shared by tumors of BRCA2 carriers that correlate strongly with the luminalA subtype, as has also previously been suggested in a gene expression study (40), although also luminalB subtype has been found among BRCA2 tumors (41). In previous studies, only few PALB2 mutation carrier tumors have been studied, mainly for the estrogen receptor, progesterone receptor, or HER2 status, with inconsistent results (11–15). However, because PALB2 mutations are moderate penetrance mutations, presumably predisposing to familial breast cancer in concert with other, thus far unknown risk alleles, the tumor phenotype, and behavior might also vary depending on the genetic background of other such alleles.

In this study, BRCA2 mutation was found to be an independent predictor of poor survival among all breast cancer cases, and our exploratory analysis further suggests that BRCA2 and PALB2 mutations affect survival especially among

### Table 3. Univariate Cox’s regression analyses for 10-y breast cancer–specific survival

<table>
<thead>
<tr>
<th>Category</th>
<th>WT, n (deaths)</th>
<th>Mutant, n (deaths)</th>
<th>P</th>
<th>HR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1 patients*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>2,135 (241)</td>
<td>67 (15)</td>
<td>0.0546</td>
<td>1.67</td>
<td>0.99-2.82</td>
</tr>
<tr>
<td>HER2-neg patients</td>
<td>1,011 (102)</td>
<td>35 (6)</td>
<td>0.3592</td>
<td>1.47</td>
<td>0.64-3.35</td>
</tr>
<tr>
<td>BRCA2 patients*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>2,135 (241)</td>
<td>68 (21)</td>
<td>0.0002</td>
<td>2.34</td>
<td>1.50-3.66</td>
</tr>
<tr>
<td>HER2-neg patients</td>
<td>1,011 (102)</td>
<td>38 (15)</td>
<td>&lt;0.0001</td>
<td>3.75</td>
<td>2.18-6.45</td>
</tr>
<tr>
<td>PALB2 patients*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>2,135 (241)</td>
<td>29 (6)</td>
<td>0.2524</td>
<td>1.61</td>
<td>0.71-3.61</td>
</tr>
<tr>
<td>HER2-neg patients</td>
<td>1,011 (102)</td>
<td>18 (6)</td>
<td>0.0104</td>
<td>2.94</td>
<td>1.29-6.69</td>
</tr>
<tr>
<td>Familial patients</td>
<td>888 (109)</td>
<td>21 (6)</td>
<td>0.0466</td>
<td>2.30</td>
<td>1.01-5.24</td>
</tr>
<tr>
<td>Familial HER2-neg patients</td>
<td>531 (52)</td>
<td>14 (6)</td>
<td>0.0004</td>
<td>4.57</td>
<td>1.96-10.64</td>
</tr>
</tbody>
</table>

Abbreviation: HR, hazard ratio.
*Compared with non-BRCA1/2 and non-PALB2 patients.
HER2-negative breast cancer patients; PALB2 1592delT mutation as well as BRCA2 mutation was an independent prognostic factor when adjusted also for conventional prognostic factors. Carrying a PALB2 mutation also seems to affect survival specifically in breast cancer families, which is consistent with epidemiologic studies suggesting that breast cancer prognosis may be inherited (38). Although the basic histopathologic tumor phenotype seems different for PALB2 and BRCA2 mutation carrier tumors, the former representing triple-negative, basal-like tumors and the latter luminal subtype, these results suggest that perturbation of the BRCA2 function either through inactivating BRCA2 or PALB2 mutations may result in specific progression pathway leading to aggressive behavior of the tumor and/or reduced response to therapy. Indeed, comparative genomic hybridization analysis has shown major similarities in the genomic profile of PALB2 and BRCA2 mutation carrier tumors (13). At the same time, the triple-negative phenotype of PALB2 mutation carrier tumors also supports a poor prognosis among PALB2 mutation carriers.

Our results suggest a more significant impact of BRCA2 mutation on long-term breast cancer survival than previously found (42–45), especially when stratified by tumor HER2 status, which has not previously been studied. The reduced survival among BRCA2 mutation carriers was not affected by other second cancers (data not shown). The prognostic effect of BRCA2 mutation as well as PALB2 mutation becomes

Table 4. Multivariate Cox’s regression models (final step) in different patient groups

<table>
<thead>
<tr>
<th>Category</th>
<th>Variable</th>
<th>P</th>
<th>HR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BRCA1 patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>Grade</td>
<td>0.0002</td>
<td>1.74</td>
<td>1.30-2.33</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>&lt;0.0001</td>
<td>1.65</td>
<td>1.34-2.03</td>
</tr>
<tr>
<td></td>
<td>PR</td>
<td>0.0001</td>
<td>2.09</td>
<td>1.45-3.03</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>&lt;0.0001</td>
<td>3.30</td>
<td>2.19-5.00</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>&lt;0.0001</td>
<td>5.70</td>
<td>3.34-9.73</td>
</tr>
<tr>
<td></td>
<td>HER2</td>
<td>0.0293</td>
<td>1.55</td>
<td>1.04-2.29</td>
</tr>
<tr>
<td><strong>HER2-neg patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>Grade</td>
<td>0.0120</td>
<td>1.50</td>
<td>1.09-2.07</td>
</tr>
<tr>
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<td>T</td>
<td>0.0000</td>
<td>2.11</td>
<td>1.66-2.68</td>
</tr>
<tr>
<td></td>
<td>PR</td>
<td>0.0019</td>
<td>2.01</td>
<td>1.29-3.12</td>
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<td>N</td>
<td>0.0001</td>
<td>2.66</td>
<td>1.65-4.27</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>&lt;0.0001</td>
<td>5.70</td>
<td>3.03-10.73</td>
</tr>
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<td><strong>BRCA2 patients</strong></td>
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<td></td>
</tr>
<tr>
<td>All patients</td>
<td>Grade</td>
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<td>1.56</td>
<td>1.18-2.07</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>&lt;0.0001</td>
<td>1.65</td>
<td>1.35-2.02</td>
</tr>
<tr>
<td></td>
<td>PR</td>
<td>0.0001</td>
<td>2.10</td>
<td>1.46-3.02</td>
</tr>
<tr>
<td></td>
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<td>&lt;0.0001</td>
<td>3.34</td>
<td>2.20-5.07</td>
</tr>
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<td></td>
<td>M</td>
<td>&lt;0.0001</td>
<td>5.85</td>
<td>3.43-9.99</td>
</tr>
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<td>HER2</td>
<td>0.0263</td>
<td>1.58</td>
<td>1.06-2.36</td>
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<td>BRCA2</td>
<td>0.0418</td>
<td>2.06</td>
<td>1.03-4.15</td>
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</tr>
<tr>
<td>All patients</td>
<td>Grade</td>
<td>&lt;0.0001</td>
<td>1.83</td>
<td>1.37-2.44</td>
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<td>1.72</td>
<td>1.41-2.11</td>
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<tr>
<td></td>
<td>PR</td>
<td>&lt;0.0001</td>
<td>2.19</td>
<td>1.52-3.15</td>
</tr>
<tr>
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<td>N</td>
<td>&lt;0.0001</td>
<td>3.49</td>
<td>2.29-5.32</td>
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<tr>
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<td>M</td>
<td>&lt;0.0001</td>
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<td>3.30-9.60</td>
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<tr>
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<td>p53</td>
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<tr>
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<td>BRCA2</td>
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<td>1.01-4.13</td>
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<tr>
<td><strong>PALB2 patients</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>Grade</td>
<td>&lt;0.0001</td>
<td>1.83</td>
<td>1.37-2.44</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>&lt;0.0001</td>
<td>1.72</td>
<td>1.41-2.11</td>
</tr>
<tr>
<td></td>
<td>PR</td>
<td>&lt;0.0001</td>
<td>2.19</td>
<td>1.52-3.15</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>&lt;0.0001</td>
<td>3.49</td>
<td>2.29-5.32</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>&lt;0.0001</td>
<td>5.63</td>
<td>3.30-9.60</td>
</tr>
<tr>
<td><strong>HER2-neg patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>T</td>
<td>&lt;0.0001</td>
<td>2.27</td>
<td>1.80-2.85</td>
</tr>
<tr>
<td></td>
<td>PR</td>
<td>0.0040</td>
<td>1.91</td>
<td>1.23-2.96</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>&lt;0.0001</td>
<td>2.83</td>
<td>1.75-4.58</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>&lt;0.0001</td>
<td>5.85</td>
<td>3.10-11.02</td>
</tr>
<tr>
<td></td>
<td>p53</td>
<td>0.0041</td>
<td>2.01</td>
<td>1.25-3.23</td>
</tr>
<tr>
<td></td>
<td>PALB2</td>
<td>0.0399</td>
<td>2.45</td>
<td>1.04-5.77</td>
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*p-Compared with non-BRCA1/2 and non-PALB2 patients.*
noticeable mainly after 5 years of follow-up, whereas BRCA1 mutation carriers seem to have significantly higher survival difference compared with noncarriers at 60 months (Fig. 1A). Most of the studies published previously have only had short-term follow-up data available or the number of the mutation carriers has been too small for significant analysis of breast cancer survival, as reviewed in Bonadona et al. (42). In addition, the survival rate of sporadic or familial breast cancer patients in general have, in some studies (43, 45), been far worse than among patients in our study, which may also have played down the specific role of BRCA2 mutation on survival. In general, HER2-negative patients have better prognosis than do patients with HER2 amplification/overexpression (35). Interestingly, the survival effect by PALB2 or BRCA2 mutation was especially pronounced among patients with negative HER2 status, and this effect was independent of the estrogen receptor or progesterone receptor status of the tumors. Currently, no direct functional interaction between BRCA2 and HER2 pathways is known, and the association of BRCA2 and PALB2 mutations, and their survival effect with negative HER2 status may suggest mutual exclusion of the HER2 and BRCA2/PALB2 pathways in progression of the carrier tumors.

In conclusion, we have shown here that the PALB2 1592delT has a strong effect on familial breast cancer risk, with presumed multiplicative effects with other alleles on familial breast cancer risk. The tumors rising in the patients carrying this mutation manifest a phenotype associated with a more aggressive disease. Our exploratory analysis suggests that the mutation associates also with reduced survival especially in breast cancer families and among patients with HER2-negative tumors, and larger studies are warranted to confirm these results. Our results also suggest a significant impact of BRCA2 mutation on long-term breast cancer survival.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References
The Breast Cancer Susceptibility Mutation PALB2 1592delT Is Associated with an Aggressive Tumor Phenotype

Tuomas Heikkinen, Hanni Kärkkäinen, Kirsimari Aaltonen, et al.