Penetrance Analysis of the \textit{PALB2} c.1592\textit{del}T Founder Mutation

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\textbf{Abstract} Purpose: \textit{PALB2} is a recently identified breast cancer susceptibility gene. We have previously identified in the Finnish population a \textit{PALB2} c.1592\textit{del}T founder truncation mutation that is associated with an increased risk of breast cancer. In the present study, we wanted to assess in more detail the increased risk (hazard ratio, HR) and the age-specific cumulative risk (penetrance) of c.1592\textit{del}T with regard to susceptibility to breast and other forms of cancer.

\textbf{Experimental Design:} Modified segregation analyses fitted under maximum likelihood theory were used to estimate age-specific cumulative risks and HRs using the families of mutation carriers identified from a consecutive series of breast cancer cases unselected for age at onset or family history.

\textbf{Results:} We found a substantially increased risk of breast cancer [HR, 6.1; 95\% confidence interval (95\% CI), 2.2-17.2; \(P = 0.01\)] equivalent to a 40\% (95\% CI, 17-77) breast cancer risk by age 70\% years, comparable to that for carriers of mutations in \textit{BRCA2}. We found marginal evidence (\(P = 0.06\)) that the HR for breast cancer decreased with age by 4.2\% per year (95\% CI, 0.2-8.1), from 7.5-fold at age 30\% years to 2.0-fold at age 60\% years.

\textbf{Conclusions:} Our results suggest that it may be appropriate to offer \textit{PALB2} c.1592\textit{del}T mutation testing to Finnish women with breast cancer, especially those with an early age at onset or a family history of breast or related cancers, and to offer carriers the option of participation in extended disease surveillance programs.

\textbf{BRCA1} and \textbf{BRCA2} are clinically the most important genes associated with breast cancer (BC) susceptibility. The BC risks are estimated to be around 65\% and 45\% to age 70\% years for \textit{BRCA1} and \textit{BRCA2} mutation carriers, respectively (1–3). Mutations in these two genes are rare in most populations and account for, at most, 25\% of familial aggregation of the disease (1, 2). There are also other high-risk genes such as \textit{TP53} and \textit{PTEN}, but mutations in these are very rare. In addition, mutations in at least six other genes (\textit{CHEK2, ATM, NBS1, RAD50, BRIP, and PALB2}) have been found to be associated with, on average, a moderately increased BC risk (4). Recently, several genes with common variants associated with small increases in risk have been identified, but these variants are not necessarily causal and explain less than 4\% of familial aggregation (5). Therefore, much is yet to be learned about the familial, and especially genetic, causes of BC.

\textit{PALB2} (partner and localizer of \textit{BRCA2}) was originally identified as a \textit{BRCA2}-interacting protein. The \textit{PALB2}-\textit{BRCA2} interaction is essential for the double-stranded break repair functions of \textit{BRCA2} (6). \textit{PALB2} is also known as FANCN, the gene responsible for a novel subtype of Fanconi anemia (7, 8). Biallelic FANCN mutation carriers resemble Fanconi anemia patients carrying \textit{FANC1}/\textit{BRCA2} mutations in that they are prone to bone marrow failure and embryonal tumors such as...
Wilms tumor and medulloblastoma. Patients with novel subtype Fanconi anemia also display typical Fanconi anemia features including congenital malformations and cellular sensitivity to interstrand cross-linking agents (e.g., mitomycin C; refs. 7, 8).

Recent studies conducted in several populations have identified \( \text{PALB2} \) as a novel BC susceptibility gene (9–11). A number of protein-truncating mutations have been discovered that are substantially more common in familial and unselected cases than in controls (9, 10, 12). Potential increased risk for other cancer types such as prostate and male BC has also been suggested (9, 10). In our previous study, we identified a founder mutation in \( \text{PALB2} \), c.1592delT, associated with a 4-fold increased risk of BC for a randomly sampled heterozygous carrier, estimated by comparison of unselected cases and population controls. Should absolute BC risk for \( \text{PALB2} \)

![Fig. 1. Pedigrees of \( \text{PALB2} \) c.1592delT carrier families. Some of the shown pedigrees represent updated versions of those reported previously in Erkko et al. (9). Black circles, breast cancer cases; age at diagnosis is mentioned. Arrows, probands. Slashed symbols, deceased individuals. Gray, other cancer types (Ca, colorectal; Leu, leukemia; Lu, lung; Me, melanoma; Pan, pancreatic; Sp, spinal; Sto, stomach); cancer type and age at diagnosis are presented when known. Individuals genotyped for c.1592delT are indicated either as carrier (+) or noncarrier (-). Age at monitoring is shown for unaffected individuals genotyped for c.1592delT.](image)

![Table 1. All models considered and their estimated hazard ratios and confidence intervals](table)

<table>
<thead>
<tr>
<th>Model</th>
<th>No. parameters</th>
<th>Log likelihood</th>
<th>( \text{HR}_{\text{BC}} ) (95% CI)</th>
<th>( \text{HR}_{\text{NBC}} ) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No effect of ( \text{PALB2} ) mutation</td>
<td>0</td>
<td>-256.29</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mutation only affects BC risk</td>
<td>1</td>
<td>-253.31</td>
<td>6.1 (2.2-17.2)</td>
<td>1</td>
</tr>
<tr>
<td>Mutation only affects NBC risk</td>
<td>1</td>
<td>-256.28</td>
<td>1</td>
<td>1.0 (0.3-3.0)</td>
</tr>
<tr>
<td>Mutation affects BC and NBC risks</td>
<td>2</td>
<td>-253.09</td>
<td>6.4 (2.4-17.2)</td>
<td>1.4 (0.6-3.2)</td>
</tr>
<tr>
<td>Mutation only affects BC risk, ( \text{HR}_{\text{BC}}(t) = \exp(a + b \times t) )</td>
<td>3</td>
<td>-251.52</td>
<td>a: 3.30 (1.39-3.2)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b: -0.043 (-0.084-0.002)</td>
<td></td>
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</tbody>
</table>
mutation carriers be greater for women with other genetic risks, as was implicitly assumed by the fitted model applied to data from UK familial cases and controls (10), the BC risks associated with c.1592delT could be somewhat higher (9), especially for women with a family history of breast cancer, which is likely to be the setting in which testing for this mutation might take place.

The penetrance (i.e., age-specific cumulative cancer risk) for PALB2 mutation carriers has not been extensively studied before. Detailed penetrance estimates are needed to properly predict the cancer risks associated with PALB2 c.1592delT. This information is highly relevant for genetic counselling purposes and for assessing needs for extended disease surveillance of mutation carriers. In the current investigation, we have used all available data on the family history of cancer and ages of disease onset for PALB2 c.1592delT carriers identified in a previously described series of BC cases unselected for family history (9). We estimated the penetrance of this germ-line mutation for BC alone, as well as for cancer of any site other than the breast (NBC).

**Materials and Methods**

**Subjects.** As previously reported (9), 1,918 women with BC (proband) were recruited at three university hospitals in Finland (Oulu, Tampere, and Kuopio) without selection for or against family history of cancer or age at disease onset. All samples were obtained according to protocols approved by each of the participating hospitals and by the informed consent of the patients. Further, the patients had agreed that any medical record information considered relevant for carrying out the indicated study on cancer genetics should be readily available for the authorized researchers. A total of 17 probands carrying PALB2 c.1592delT were included in the current penetrance study, with a mean age at disease onset of 54.3 years (range, 41-73 years). For seven carriers there was no report of cancer family history; their mean age at onset was 59.3 years [95% confidence interval (95% CI), 51.5-67.1], 8.5 years (P = 0.04) later than for the other case carriers (50.8 years; 95% CI, 46.7-54.9). All available DNA samples from their family members were obtained and genotyped for c.1592delT by direct sequencing. Carrier pedigrees were constructed using all available information.

**Statistical methods.** The hazard ratio (HR; i.e., the ratio of sex- and age-specific cancer incidence for carriers to that for noncarriers) was estimated for BC and for NBC. These HRs were estimated by modified segregation analyses fitted under maximum likelihood theory. The likelihood function was based on the Elston-Stewart formulation (13), to phenotypes were assumed to be conditionally independent given genotypes. Hardy-Weinberg equilibrium was also assumed. To adjust for ascertainment, the likelihood for each pedigree was conditioned on the ascertainment criteria, namely that the proband was both a carrier and a BC case. Under the assumption that all pedigrees were independent, the sum of the conditional log likelihoods was maximized over the parameter space using the statistical package MENDEL version 3.2 (14).

A competing risks model, similar to that in ref. 15, was used. In this model, individuals are censored at death, last interview, or first cancer, whichever is earliest. Until the onset of an individual’s first cancer, cancer occurrences in different organs are assumed to be conditionally independent given genotype. Therefore, the probability density of an individual’s observed phenotype given his or her genotype, g, is

\[ S_{g,BC}(t) × S_{g,NBC}(t), \]

or

\[ h_{g,BC}(t) × S_{g,BC}(t) × S_{g,NBC}(t), \]

depending on whether the individual was unaffected, a BC case, or an NBC case, respectively, where \( t \) is the age at censoring, \( S_{g,BC} \) is the BC survival function for genotype \( g \), \( h_{g,BC} \) is the BC hazard function for genotype \( g \), and \( S_{g,NBC} \) and \( h_{g,NBC} \) are the analogous quantities for NBC. As in refs. 9, 10, the PALB2 mutation was assumed to act dominantly for cancer risks.

In the above notation, the breast cancer hazard ratio \( HR_{BC} \) is by definition \( h_{1,BC}/h_{0,BC} \), where \( g = 0 \) for noncarriers and \( g = 1 \) for carriers. The hazards \( h_{0,BC} \) and \( h_{1,BC} \) were determined by the equality \( HR_{BC} = \frac{h_{1,BC}}{h_{0,BC}} \) and the requirement that the average of the genotype-specific BC hazards, weighted by the corresponding genotype frequencies, should equal the Finnish population BC incidences. Because the PALB2 mutation is rare, this is approximately the same as taking the noncarrier hazard \( h_{0,BC} \) to be the population BC incidence. Similar comments apply to NBC. Survival functions were calculated as the exponential of minus the corresponding cumulative hazards. Age-specific cumulative risks were calculated from the survival functions.

Finnish population cancer incidence rates were obtained from the 2001-2005 Finnish Cancer Registry age-standardized and age-specific rates.\(^{11}\) NBC population incidences were calculated as the difference of the BC and all cancer incidences. The population allele frequency of the c.1592delT mutation was assumed to be 0.001, as estimated in ref. 9 for controls; sensitivity analyses were conducted for allele frequencies in the range of 0.0001 to 0.01. Only probands and their first- and second-degree relatives were included in the main analysis; another sensitivity analysis was conducted to assess the effect of excluding data on more distant relatives.

Our analytic methods require a censoring age for each of the 213 individuals in the 17 carrier families, but this was missing for 118 unaffected individuals and 5 NBC cases. For NBC cases, we imputed an age at diagnosis of 60 years. For unaffected individuals, we imputed an age from the mean ages of their parents, spouses, and siblings, in order of increasing preference; otherwise we imputed an age of 50 years.

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The likelihood ratio test was used to compare the goodness-of-fit of nested models. Forward model selection was used to choose the most parsimonious model. Student’s t-tests were used to test the equality of the means of ages of probands with and without a recorded family history. These and other calculations were done with R version 2.5.0.12 All hypothesis tests assumed two-sided alternative hypotheses and the nominal level of statistical significance was taken to be 0.05, following convention.

For the seven case carriers without reported cancer family histories, no family structures were recorded, however it is likely that none of their first- or second-degree relatives had cancer. If this was the case, then excluding the families of case carriers with no family histories would result in an overestimate of penetrance. Therefore, we conducted analyses first including only these case carriers but no relatives, and second by duplicating seven of the other families (Fig. 1C-D and F-I), changing all nonproband cancers to noncancers, and making these the family histories for the cases for whom no pedigree had been recorded. The latter estimates from the augmented data set are preferred.

### Results

Based on the 10 families with a reported family history and the seven singletons for whom no family history was recorded, the PALB2 c.1592delT mutation was estimated to be associated with a 14.3-fold (95% CI, 6.6-31.2) increased risk of BC (P = 0.0001) and a 2.6-fold (95% CI, 1.3-5.1) increased risk of NBC, as a group (P = 0.03), but this may be an overestimate (see “Statistical methods”).

When the augmented data set was used (see “Statistical methods”), the estimated HR for BC became 6.1 (95% CI, 2.2-17.2; P = 0.01) and the estimated HR for NBC became 1.4 (95% CI, 0.6-3.2; P = 0.5); see Table 1. The corresponding age-specific cumulative risk is shown in Fig. 2, indicating an estimated BC risk to age 70 years of 40% (95% CI, 17-77). The HR for BC decreased with age by 4.2% per year (95% CI 0.2-8.1), decreasing from 7.5-fold at age 30 years to 2.0-fold at age 60 years (P = 0.06). These estimates were fairly insensitive both to small changes in the c.1592delT allele frequency and to restrictions of the families to relatives of various degrees of relatedness to the proband (results not shown).

### Discussion

Using the families of all mutation carriers identified in a series of unselected BC cases, we have found an ~6-fold increased incidence of BC for PALB2 c.1592delT carriers. The estimates of BC risks is robust in the sense that it is fairly insensitive to the exact allele frequency and degree of family histories used. The estimate is also conservative because we have assumed that the seven case carriers without a recorded family history had no relatives with cancer. Our analyses suggest that the risk of BC for mutation carrying relatives of unselected case carriers is ~40% to age 70 years (Fig. 2). There was no evidence for an increased risk of NBC for carriers, but we had little power to address this issue.

Our results should be interpreted with caution considering that the estimates are based on a relatively small number of carriers and their families when compared with published population-based estimates for the penetrance of mutations in BRCA1 and BRCA2. We have also had to make some assumptions about missing data on family members, most particularly those for which family structure and cancer history had not been recorded. It would have been advantageous to know the mutation status for more relatives of the case carriers; however, this information is not essential. The statistical approach we have taken provides valid estimates no matter how many, if any, relatives are genotyped. Furthermore, having missing ages for some unaffected relatives is not optimal, but most information on penetrance comes from the affected women for whom ages at diagnosis were known.

If there are familial factors, such as other genes, which modify risks, then estimates of penetrance using families selected for increased cancer occurrence will, on average, be higher than found in this setting. Similarly, estimates based on the family histories of mutation carrying controls (unselected for family history) will, on average, be lower. At this stage, there is no evidence for there being familial modifiers of penetrance for PALB2 mutation carriers, as was assumed by Rahman and colleagues (10). This scenario, however, is consistent with the marginally significant observations that the HR decreases with age and that the age at onset of the seven case carriers with no known family history was 8.5 years later compared with that of the probands of the 10 families with family histories of cancer.

The fact that we have observed about half of the 17 PALB2 mutation carriers to have a family history of breast cancer is consistent with the estimated 40% lifetime risk for carriers. For example, about half of BRCA1 and BRCA2 mutation–carrying breast cancer families identified through population-based sampling of cases, such as in the present study, also do not have a family history. Although this observation has been found repeatedly in several investigations, it is perhaps not well recognized (16).

One of the strengths of this study was its focus on one particular mutation. Larger studies in which the relative risks of different PALB2 mutations can be compared are needed to determine if the risks caused by other PALB2 mutations are the same as those attributed here to c.1592delT. High penetrance was suggested for c.229delT, the most 5′ mutation in PALB2 currently reported (11). In contrast, a much lower penetrance estimate was estimated for mutations located in the 3′ terminus of the gene (10). Therefore, it is possible that mutations closer to the 5′ terminus could confer higher relative risks than those in the 3′ terminus. The observed penetrance could also reflect differences in protein stability. The c.1592delT mutation produces a stable but functionally defective product (9) and could therefore confer a higher disease risk compared with a mutation acting merely in a haploinsufficient manner.

The estimated increased BC risk for PALB2 c.1592delT carriers is high, considering that it has been estimated that for BRCA2 mutation carriers the BC risk to age 70 years is 45% (95% CI, 31-56; ref. 3). Given this substantially enhanced cancer risk observed for heterozygous carriers and the roughly 1% occurrence rate of PALB2 c.1592delT in unselected breast cancer cases (9), genetic testing for this germ-line mutation could be considered in Finland for women diagnosed with breast cancer, particularly with early-onset disease, and in the context of women with a family history of breast and other related cancers not explained by high-risk mutations in BRCA1.
and BRCA2, as explained by Byrnes et al. (17). Both affected and disease-free PALB2 c.1592delT carriers are likely to benefit from inclusion in extended cancer surveillance programs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

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