Mammary Tumor Development in Dogs Is Associated with 
BRCA1 and BRCA2

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Abstract
Breast cancer is a major contributor to overall morbidity and 
mortality in women. Several genes predisposing to breast cancer 
have been identified, but the majority of risk factors remain 
unknown. Even less is known about the inherited risk factors 
underlying canine mammary tumors (CMT). Clear breed 
predispositions exist, with 36% of English springer spaniels 
(ESS) in Sweden being affected. Here, we evaluate 10 hu-
man breast cancer genes (BRCA1, BRCA2, CHEK2, ERBB2, 
FGFR2, LSP1, MAP3K1, RCAS1, TOX3, and TP53) for association 
with CMTs. Sixty-three single-nucleotide polymorphisms 
(NSNs; four to nine SNPs per gene) were genotyped by iPLEX in 
canine ESS dogs, 212 CMT cases and 143 controls. Two genes, 
BRCA1 and BRCA2, were significantly associated with CMT 
(Bonferroni corrected P = 0.005 and P = 0.0001, respectively). 
Borderline association was seen for FGFR2. Benign and malignant 
cases were also analyzed separately. Those findings supported 
the association to BRCA1 and BRCA2 but with a stronger associ-
ation to BRCA1 in malignant cases. Both BRCA1 and BRCA2 
showed odds ratios of ~4. In conclusion, this study indicates 
that BRCA1 and BRCA2 contribute to the risk of CMT in ESS, 
suggesting that dogs may serve as a good model for human 
breast cancer. [Cancer Res 2009;69(22):8770–4]

Introduction
Mammary tumors are the most common neoplasia in intact fe-
male dogs (Canis familiaris; refs. 1–6). Mammary tumors constitute 
about half of all tumors in female dogs and approximately half of 
the canine mammary tumors (CMT) are malignant (7, 8). In both 
women and dogs, mammary tumors develop with age and they 
rarely occur before 25 and 5 years of age, respectively (9). The 
median age of occurrence is 10 to 11 years for dogs; however, some 
breeds develop CMT at a younger age. The English springer spaniel 
(ESS) has been shown to have a median age of onset at 6.9 years of 
age in the Swedish dog population (1). The development of both 
canine and human mammary tumors is hormone dependent (10, 
11). Canine mammary carcinoma have epidemiologic, clinical, 
and limited population size has also resulted in a high degree of 
large number of loci, each contributing with a small effect on 
breast cancer risk (25). Four genes, FGFR2, LSP1, MAP3K1, 
and TOX3, were recently found to be associated with a mild increase 
in risk of breast cancer in humans in a genome-wide association 
study (22). RCAS1 and TP53 have been reported to be associated 
with many types of cancer, including breast cancer (26). ERBB2 
has been shown to have altered expression in human breast can-
cancer, and a deletion in the CHEK2 gene has been reported as 
associated with a 2-fold to 3-fold increased risk of breast cancer (27, 
28). CMT is also considered a heterogeneous disease with a com-
plex background. It has been suggested that the origin of CMT is 
multifactorial and depends on an interaction between multiple 
major and minor genes and environmental factors.

Dogs have a history of inbreeding, which has resulted in low le-
vels of genetic variation within breeds. The recent breed formation 
and limited population size has also resulted in a high degree of 
linkage disequilibrium within breeds (29, 30), particularly compared 
with what is seen in humans (31). Certain breeds are predis-
posed to specific disorders, and CMT in ESS dogs in Sweden is one 
such clear example, with 36% of ESS in Sweden being affected by 
CMT (1). Due to the small genetic variation, CMT should have a 
more homogenous origin within a single breed compared with 
breast cancer in the larger human population. This should allow 
for an easier identification of risk factors within a breed. As part 
of the dog genome sequencing project, a power calculation for 
case-control association within dog breeds was performed, sug-
 suggesting that with 15,000 single-nucleotide polymorphisms (SNPs) 
the power to detect a locus with a sample of 100 affected and 
100 unaffected dogs is 97% for λ = 5 and 50% for λ = 2 (30) if
the frequency of the associated allele is <20%. This supports the notion that, in a genetically isolated population, it is relatively easy to identify a specific founder haplotype, which is significantly more frequent in cases than in controls.

Here we selected 10 genes (BRCA1, BRCA2, CHEK2, ERBB2, FGFR2, LSP1, MAP3K1, RCAS1, TOX3, and TP53; Table 1) as candidate genes for CMT and performed association using an initial sample set of 89 unrelated cases and 85 unrelated controls and a similar replication set. We found that at least two genes, BRCA1 and BRCA2, were associated with CMT in ESS.

Materials and Methods

Sample collection and DNA isolation. All dogs used in this study were privately owned and registered in the Swedish Kennel Club’s database (SKK) with complete pedigrees. The dogs were selected from the databases of Agria pet insurances and SKK, and information was collected regarding possible risk factors for the development of mammary tumors (signalment, age of onset, sex, spaying, lactation, use of contraceptives, diet, pregnancy, disease status, and family cancer history) pathology reports and/or other clinical diagnostic information. All dogs included in the study were female ESS dogs. All the control dogs were older than 8 y, with a confirmed absence of CMT based on palpation of the mammary gland performed by a veterinarian. The dogs were subdivided into two study populations. In the first population (data set 1, n = 192), 100 ESS cases diagnosed with CMT and 92 control dogs were selected. All cases and controls were selected to be unrelated at the parental level. There were 28 cases with malignant tumors, 57 cases with benign tumors, 15 unclassified cases; 28 with malignant tumors, 57 with benign tumors, 15 unclassified cases.

In the replication population (data set 2, n = 182), the diagnostic criteria were less stringent and fewer dogs with diagnosed malignant disease were available. One hundred twenty-one ESS cases were selected based on pathology reports if available and, otherwise, based only on physical examination data (the presence of a single or multiple nodules within the mammary gland). Most of these mammary tumors were not surgically excised, or excised and not histopathologically evaluated. Of the 121 cases, 4 were confirmed as malignant and 39 as benign by histopathology. Sixty-one control dogs were available. Siblings were allowed in this population.

Blood samples were collected by veterinarians in different veterinary animal hospitals and veterinary clinics throughout Sweden between the years 2005 and 2009. All sampling of dogs were approved by the owners and conform to the decision of the Swedish Animal Ethical Committee (no. C9/5) and the Swedish Animal Welfare Agency (no. 30-83/95). DNA was extracted from EDTA blood samples using the QIAamp DNA Blood Mini Kit according to the manufacturer’s protocol (Qiagen).

Candidate gene selection and genotyping. Ten genes (BRCA1, BRCA2, CHEK2, ERBB2, FGFR2, LSP1, MAP3K1, RCAS1, TOX3, and TP53) were selected in the present study as candidate genes for CMT. The samples were genotyped for 63 SNPs using the iPLEX Gold Mass ARRAY according to the manufacturer’s protocol (Sequenom). Due to the difference in population structure in humans and dogs, a different SNP selection approach was used here rather than the tagSNP approach, which would have been used in humans based on the human HapMap (32). Within dog breeds there are long haplotypes (~1 Mb in size) resulting from the recent breed creation. This means that most genes reside within a block of complete linkage disequilibrium, where no recombination has occurred since breed creation. Because haplotype maps for individual dog breeds do not exist at this point, it is not possible to pick tagSNPs, but instead one can guess that most of the three to five haplotypes expected to cover a gene would be tagged with at least five SNPs. Thus, the SNPs were chosen from the 2.5 million SNP map described in Lindblad-Toh and colleagues (30). This map has roughly one SNP per thousand bases of sequence and is not exhaustive enough to thoroughly describe coding SNPs. No difference was therefore made between coding and noncoding SNPs. We choose evenly spaced, nonrepetitive SNPs from the start to the end of each gene; we aimed for at least five SNPs. Thus, four to nine SNPs per candidate gene (63 SNPs in total) were selected from the available dog genome sequences in the UCSC Genome Browser, Dog May 2005 (CanFam2) assembly (Table 1 and Supplementary Table S1).

The primers for amplification and extension were designed using Mass ARRAY Assay Design v3.1 software. DNA was amplified using PCR, and the remaining nucleotide triphosphates were deactivated by phosphatase treatment (SAP). A single base primer extension step was performed, and the allele specific extension products of different masses were quantitatively analyzed using MALDI TOF Mass Specs.

Data analysis. The primary genotype data was analyzed using the Typer 4.0 Analyzer User Interface software (Sequenom) for cluster analysis. SNPs with a call rate of >75% and a minor allele frequency (MAF) of at least 5% were included in each analysis. Samples with a call rate of ≤75% were excluded from further analysis. After filtering, the number of informative SNPs ranged from 32 to 39 SNPs in the different analyses (Supplementary Table S2).

We analyzed all cases versus all controls for data sets 1 and 2 separately and together to investigate whether a single SNP or haplotype was present at a significantly higher or lower frequency in cases compared with controls and thus associated with CMT. Haplotypes were created from all SNPs remaining after filtering in each gene. Haplotype analysis could not be performed for the TP53 and LSP1 genes, because only one SNP remained after filtering for these genes. Malignant versus controls, benign versus controls, and malignant versus benign were analyzed only for affected dogs in data set 1 with diagnosis confirmed by histopathology. Cases in data set 2 were not included due to the low number of cases with confirmed diagnosis by histopathology and to the relatedness of the dogs in this data set. Association analyses for all comparisons were performed with the PLINK software (33) for single χ² SNP association, haplotype association, odds ratios, and MAF. Nominal (raw) χ² and Bonferroni corrected χ² P values were calculated to adjust for the multiple testing that arises from evaluating several SNPs or haplotypes (34, 35). A Bonferroni corrected P < 0.05, with correction for total SNP number remaining after filtering in each analysis, was considered statistically significant, although this likely overcorrects due to the fact that most SNPs within a gene are likely linked to each other and are therefore not unrelated observations.

Table 1. Genes evaluated for association to CMT risk

<table>
<thead>
<tr>
<th>Gene</th>
<th>Human chromosome</th>
<th>Canine chromosome</th>
<th>No. SNPs</th>
<th>Span covered</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA2</td>
<td>13</td>
<td>25</td>
<td>4</td>
<td>10.729–10.786 Mb</td>
</tr>
<tr>
<td>BRCA2</td>
<td>17</td>
<td>2</td>
<td>8</td>
<td>23.278–23.399 Mb</td>
</tr>
<tr>
<td>FGFR2</td>
<td>10</td>
<td>2</td>
<td>29</td>
<td>34.303–34.406 Mb</td>
</tr>
<tr>
<td>TOX3</td>
<td>16</td>
<td>2</td>
<td>7</td>
<td>65.949–65.964 Mb</td>
</tr>
<tr>
<td>CHEK2</td>
<td>22</td>
<td>2</td>
<td>6</td>
<td>25.089–25.133 Mb</td>
</tr>
<tr>
<td>MAP3K1</td>
<td>5</td>
<td>2</td>
<td>6</td>
<td>46.823–46.858 Mb</td>
</tr>
<tr>
<td>LSP1</td>
<td>11</td>
<td>18</td>
<td>5</td>
<td>49.138–49.143 Mb</td>
</tr>
<tr>
<td>RCAS1</td>
<td>8</td>
<td>13</td>
<td>5</td>
<td>13.125–13.138 Mb</td>
</tr>
<tr>
<td>TP53</td>
<td>17</td>
<td>5</td>
<td>5</td>
<td>35.617–35.686 Mb</td>
</tr>
<tr>
<td>ERBB2</td>
<td>17</td>
<td>9</td>
<td>5</td>
<td>26.098–26.110 Mb</td>
</tr>
</tbody>
</table>
was found for six genes: BRCA1, BRCA2, FGFR2, MAPK1, LSP1, and TP53 (Table 2 and Supplementary Table S1). When data set 2 (4 malignant, 39 benign, and 78 unclassified cases and 61 controls) was analyzed, BRCA2 replicated and CHEK1 and TOX3 also reached nominal significance. The TP53 and LSP1 genes only had one SNP with a MAF of >5% and could therefore not be conclusively studied. When both data sets were combined, BRCA2 reached the strongest significance ($P_{raw} = 3.9 \times 10^{-6}$ and $P_{Bonf} = 1.4 \times 10^{-4}$) together with BRCA1 ($P_{raw} = 1.3 \times 10^{-4}$ and $P_{Bonf} = 0.0049$; Table 2). For BRCA2, the most significant association was seen for the SNP BICF2G630470214 located in intron 24 of the BRCA2 gene. The SNP is in a region showing limited levels of conservation and is thus likely not the causative variant. Two SNPs in BRCA1, BICF2G630829454 and BICF2G630829457, reached statistical significance. BICF2G630829454 is located within a conserved element in intron 10 of the BRCA1 gene, whereas BICF2G630829457 is located 3’ of the BRCA1 gene.

Both BRCA1 and BRCA2 had odds ratios of ~4, suggesting the presence of a common predisposing allele with a relative risk of ~4 (Table 2). The BRCA1 risk allele showed a frequency of 97% in cases and 91% in controls, and the BRCA2 risk allele a frequency of 97% of cases compared with 88% of the controls, supporting the notion that the risk alleles are indeed very common in the ESS dog breed. The results for all SNPs included in the single SNP association analysis are presented in Supplementary Table S3. Haplotype analysis revealed similar frequencies and $P$ values (Table 3, BRCA1 $P_{raw} = 1.5 \times 10^{-4}$, BRCA2 $P_{raw} = 4.8 \times 10^{-4}$). No other genes besides BRCA1 and BRCA2 reached significance after Bonferroni correction for multiple testing, although FGFR2 had a significant nominal $P$ value ($P < 0.005$). The SNP with the strongest association for FGFR2 is positioned within intron 1 and is not conserved.

To examine if there was a stronger association to any particular gene in the malignant tumors, we used data set 1 where samples were clearly unrelated and had a pathologically validated diagnosis (28 malignant, 57 benign, and 92 controls). When malignant cases and controls were compared, the strongest tentative association was seen to BRCA1 (Table 4, $P_{raw} = 0.007$ and $P_{Bonf} = 0.27$). A similar association was also seen when malignant cases were compared with the benign cases ($P_{raw} = 0.03$ and $P_{Bonf} = 0.81$).

**Discussion**

We identified two genes associated with CMT in ESS, BRCA1 and BRCA2. Germ line mutations in BRCA1 and BRCA2 are thought to account for 5% to 10% of all breast cancer in women (36, 37), and our results suggest that they also predispose to CMTs based on candidate gene association. In this study, we were able to detect association to risk factors conferring a ~4-fold increased risk for both BRCA1 and BRCA2 to CMT using data from only 212 cases and 143 controls (Table 2). This is in concordance with previous power calculations stating that canine complex traits can be mapped with a few hundred dogs (30) and shows the advantages of mapping genetic risk factors in dogs compared with humans. However, expanding the cohort in our study could possibly generate significant associations for additional genes, because the disease frequency is as high as ~36% in the ESS breed. This increased disease frequency could either

### Table 2. Association of the best single SNP in each gene to CMT risk

<table>
<thead>
<tr>
<th>Gene</th>
<th>Best $P_{raw}$ data set 1</th>
<th>$6.7 \times 10^{-4}$</th>
<th>Best $P_{raw}$ data set 2</th>
<th>$1.3 \times 10^{-4}$</th>
<th>Best $P_{raw}$ total</th>
<th>$3.9 \times 10^{-6}$</th>
<th>Best $P_{Bonf}$ total</th>
<th>$1.4 \times 10^{-4}$</th>
<th>Odds ratio total</th>
<th>$F_{cases}/F_{controls}$ total</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA2</td>
<td>0.0032</td>
<td>2.00E-03</td>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
<td>0.00E+00</td>
<td>4.24</td>
<td>0.9750 ± 0.88</td>
<td></td>
</tr>
<tr>
<td>BRCA1</td>
<td>0.012</td>
<td>1.00E-02</td>
<td></td>
<td></td>
<td></td>
<td>1.30E-04</td>
<td>0.00049</td>
<td>3.74</td>
<td>0.9750 ± 0.91</td>
<td></td>
</tr>
<tr>
<td>FGFR2</td>
<td>0.018</td>
<td>1.00E-02</td>
<td></td>
<td></td>
<td></td>
<td>1.00E-02</td>
<td>0.00047</td>
<td>1.88</td>
<td>0.9600 ± 0.83</td>
<td></td>
</tr>
<tr>
<td>TOX3</td>
<td>0.29</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
<td>0.0041</td>
<td>1.00</td>
<td>0.9200 ± 0.86</td>
<td></td>
</tr>
<tr>
<td>CHEK2</td>
<td>0.37</td>
<td>0.0015</td>
<td></td>
<td></td>
<td></td>
<td>0.004</td>
<td>0.014</td>
<td>1.00</td>
<td>0.9200 ± 0.86</td>
<td></td>
</tr>
<tr>
<td>MAPK1</td>
<td>0.025</td>
<td>0.0042</td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
<td>0.023</td>
<td>1.00</td>
<td>0.9750 ± 0.91</td>
<td></td>
</tr>
<tr>
<td>LSP1</td>
<td>0.036</td>
<td>0.0014</td>
<td></td>
<td></td>
<td></td>
<td>0.004</td>
<td>0.014</td>
<td>1.00</td>
<td>0.9200 ± 0.86</td>
<td></td>
</tr>
<tr>
<td>RCAS1</td>
<td>0.025</td>
<td>0.015</td>
<td></td>
<td></td>
<td></td>
<td>0.034</td>
<td>1.00</td>
<td>1.40</td>
<td>0.5750 ± 0.49</td>
<td></td>
</tr>
<tr>
<td>TP53</td>
<td>0.010</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
<td>0.014</td>
<td>1.00</td>
<td>0.9750 ± 0.91</td>
<td></td>
</tr>
<tr>
<td>EBB2</td>
<td>0.30</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
<td>0.004</td>
<td>0.009</td>
<td>1.00</td>
<td>0.9750 ± 0.91</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** $P_{raw}$, the best $\chi^2$ single SNP $P$ value obtained for each gene [nominal association ($P_{raw} < 0.05$) is indicated in bold]; $P_{Bonf}$, Bonferroni corrected $P$ values [significant association ($P_{Bonf} < 0.05$) is indicated in bold]; $F_{cases}/F_{controls}$, the risk allele frequency in cases and controls.

### Table 3. Association of the best haplotypes in each gene to CMT risk

<table>
<thead>
<tr>
<th>Gene</th>
<th>$N_{SNPs}$</th>
<th>Best $P_{raw}$</th>
<th>Best $P_{Bonf}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA2</td>
<td>2</td>
<td>4.80E-06</td>
<td>2.60E-04</td>
</tr>
<tr>
<td>BRCA1</td>
<td>5</td>
<td>1.50E-03</td>
<td>0.0082</td>
</tr>
<tr>
<td>FGFR2</td>
<td>7</td>
<td>0.019</td>
<td>1.0</td>
</tr>
<tr>
<td>TOX3</td>
<td>6</td>
<td>0.025</td>
<td>1.0</td>
</tr>
<tr>
<td>CHEK2</td>
<td>5</td>
<td>0.070</td>
<td>1.0</td>
</tr>
<tr>
<td>MAPK1</td>
<td>2</td>
<td>0.065</td>
<td>1.0</td>
</tr>
<tr>
<td>LSP1</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>RCAS1</td>
<td>5</td>
<td>0.19</td>
<td>1.0</td>
</tr>
<tr>
<td>TP53</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>EBB2</td>
<td>3</td>
<td>0.20</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**NOTE:** $N_{SNPs}$, number of SNPs included in the haplotypes; $P_{raw}$, the best $\chi^2$ haplotype $P$ value obtained for each gene [nominal association ($P_{raw} < 0.05$) is indicated in bold]; $P_{Bonf}$, Bonferroni corrected $P$ values [significant association ($P_{Bonf} < 0.05$) is indicated in bold]; $N_{cases}$, N controls 143.
be caused by many different risk factors accumulated within the breed or by a few risk alleles of very high frequency. The latter notion is supported by the high frequency (≈90%) of both the \textit{BRCA1} and \textit{BRCA2} risk alleles in the healthy ESS dogs. Despite this high allele frequency both risk factors confer a ≈4-fold increased risk, suggesting a complex etiology of multiple strong risk factors for this disease.

The polymorphisms showing association in our study are not within coding regions and have unknown function. The most likely scenario is that they tag association signals present over entire \textit{BRCA1} and \textit{BRCA2} or parts of the genes due to the long linkage disequilibrium, and the causative variants are thus to be discovered. Still, the associated SNP present within a noncoding conserved element of intron 10 of the \textit{BRCA1} gene could potentially affect gene regulation and should be evaluated for effects on expression levels of the \textit{BRCA1} transcript. One can also not completely exclude that these associations stem from neighboring genes.

One additional gene, \textit{FGFR2}, showed nominal, but not Bonferroni corrected, association and a 2-fold increased risk together with a high allele frequency (90% in affected, 83% in controls). The \textit{FGFR2} (fibroblastic growth factor receptor 2) has been associated to human breast cancer in several studies, but no disease-causing variants have been detected thus far (22, 38, 39). However, a recent study indicates that an intronic SNP in \textit{FGFR2} might alter the function of \textit{FGFR2} and cause the association in several ethnic groups (40). It is possible that also the intronic SNP detected in this study is of functional character, although the fact that it is not conserved makes this less likely. More importantly, additional dogs would possibly yield a significant association also for this gene. Adding further SNP markers in the study could also give a similar effect because several markers were removed from the analysis due to a MAF of <5%. In particular, the \textit{TP53} and \textit{LSP1} gene results would probably benefit from more SNPs being included because only one SNP remained for analysis after MAF filtering. This observation could be caused by a random sampling of uninformative SNPs or could be caused by a low level of diversity within the ESS breed in this region.

Both the \textit{BRCA1} and \textit{BRCA2} genes are part of the granin gene family, mostly functioning as tumor suppressor genes. Both genes are frequently seen together with somatic \textit{p53} mutations (41–45). Whereas the \textit{BRCA1} and \textit{BRCA2} genes belong to the same gene family, the histology of breast cancers in women predisposed by \textit{BRCA1} and \textit{BRCA2} mutations differ in several ways, including the presence on \textit{BRCA2} mutations in male breast cancer and the frequent association of \textit{BRCA1} with ovarian cancer. In addition, \textit{BRCA1} tumors more frequently show a higher grade and are more likely to lack estrogen and progesterone receptors. They are also associated to worse prognosis compared with sporadic cases (46). Less is known about \textit{BRCA2} tumors, but they seem to resemble tumors in sporadic cases to a higher degree (46). To test if a similar coupling of malignancy could be seen in CMT, we compared the malignant and benign cases. No significant association could be detected (Table 4), but a stronger tentative association for \textit{BRCA1} was found in the malignant cases, whereas \textit{BRCA2} seemed to be equally strongly associated with malignant and benign disease. Despite the limited sample numbers in our study, these results are only preliminary and need further confirmation, but the finding agrees with a report that \textit{BRCA1} nuclear expression is particularly reduced in malignant CMTs (47). Additional samples would help determine if a germ line mutation in \textit{BRCA1} truly is predictive of malignancy in CMT in ESS. However, the presence of \textit{BRCA2} in both groups supports the theory that hyperplastic and benign mammary gland proliferation precedes malignant transformation (48) and have a similar inherited predisposition. This is in concordance with breast cancer development being a molecular continuum from benign disease to actual breast cancer, as has been proposed (49).

In conclusion, the association data obtained in this study indicates that the candidate genes \textit{BRCA1} and \textit{BRCA2} are involved in the development of CMTs. Further studies are necessary to find the actual mutation and to understand the functional mechanism, whereby these genes influence the development and malignancy of this disease in the ESS breed. The study suggests that CMT is an excellent model for human breast cancer, indicating that both humans and dogs can benefit from further comparative studies. A genome-wide association study for CMT is currently in progress and is expected to identify additional strong risk factors.

### Table 4. Association of the best single SNP in each gene to malignant and benign tumor risk (data set 1)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Best $P_{raw}$ malignant versus controls</th>
<th>Best $P_{raw}$ benign versus controls</th>
<th>Best $P_{raw}$ malignant versus benign</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA2</td>
<td>0.020</td>
<td>0.032</td>
<td>0.97</td>
</tr>
<tr>
<td>BRCA1</td>
<td>0.0068</td>
<td>0.086</td>
<td>0.027</td>
</tr>
<tr>
<td>FGFR2</td>
<td>0.027</td>
<td>0.068</td>
<td>0.067</td>
</tr>
<tr>
<td>TOX3</td>
<td>0.34</td>
<td>0.37</td>
<td>0.41</td>
</tr>
<tr>
<td>CHEK2</td>
<td>0.60</td>
<td>0.17</td>
<td>0.32</td>
</tr>
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<td>MAP3K1</td>
<td>0.044</td>
<td>0.028</td>
<td>0.12</td>
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<td>LSP1</td>
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<td>0.17</td>
<td>0.32</td>
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<td>RCAS1</td>
<td>0.038</td>
<td>0.25</td>
<td>0.18</td>
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<tr>
<td>TP53</td>
<td>0.82</td>
<td>0.036</td>
<td>0.14</td>
</tr>
<tr>
<td>ERBB2</td>
<td>0.75</td>
<td>0.28</td>
<td>0.44</td>
</tr>
<tr>
<td>$N_{cases}$</td>
<td>28</td>
<td>53</td>
<td>26</td>
</tr>
<tr>
<td>$N_{controls}$</td>
<td>84</td>
<td>84</td>
<td>50</td>
</tr>
</tbody>
</table>

Abbreviation: $P_{raw}$, the best $\chi^2$ single SNP $P$ value obtained for each gene. Nominal association ($P_{raw} < 0.05$) is indicated in bold. None of the SNPs gives significant $P$ values after Bonferroni correction.
Disclosure of Potential Conflicts of Interest

The authors have full access to the data and take responsibility for their integrity. The authors declare no commercial associations or conflict of interest and have nothing to disclose.

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