Increased risk of breast cancer in individuals carrying the *TNRC9* rs3803662 C>T polymorphism: a meta-analysis of case-control studies

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ABSTRACT. Currently, the relationship between the trinucleotide repeat containing 9 (*TNRC9*) rs3803662 C>T polymorphism and risk of breast cancer (BC) is uncertain. Here, we attempted to obtain a more accurate assessment of this association by conducting a meta-analysis of all eligible case-control investigations, comprising 44,820 cases and 58,316 controls. A comprehensive search was performed to identify all suitable studies involving the *TNRC9* rs3803662 polymorphism and BC risk. Pooled odds ratios (ORs) and 95% confidence intervals (95%CIs)
were estimated using fixed- or random-effect models. Heterogeneity, publication bias, and sensitivity analyses were also carried out. We found that the variant T allele of rs3803662 C>T greatly increases BC risk (CT vs CC: OR = 1.14, 95%CI = 1.07-1.22, P < 0.001; TT vs CC: OR = 1.38, 95%CI = 1.25-1.53, P < 0.001; CT/TT vs CC: OR = 1.19, 95%CI = 1.11-1.28, P < 0.001; TT vs CT/CC: OR = 1.28, 95%CI = 1.19-1.38, P < 0.001). Stratified analysis based on ethnicity also revealed a markedly increased risk in Asian and Caucasian populations. Moreover, studies with hospital-based control groups showed elevated risk under the four genetic models employed, as did those using population-based controls, except under heterozygote comparison. The \textit{TNRC9} rs3803662 C>T polymorphism is greatly related to increased risk of BC, in both Asian and Caucasian populations.

**Key words:** Genetic polymorphism; \textit{TNRC9}; rs3803662 C>T; Breast cancer; Meta-analysis

**INTRODUCTION**

Breast cancer (BC) is the most common malignancy affecting women worldwide. Despite an overall 5-year survival rate of 83-92\% among women with stages I and II BC (Guo et al., 2012), the 10-year risk of recurrence is 20-40\% (Early Breast Cancer Trialists’ Collaborative Group, 2005). Aspects such as tumor stage, grade, and size, and lymph node metastasis status remain the most important prognostic factors for BC; however, we know little of the influence of inherited genetic variation on recurrence and overall survival rates.

It seems likely that a combination of environmental factors and susceptibility genes may contribute to the pathogenesis and progression of BC (Lichtenstein et al., 2000). In addition to the well-defined, highly penetrant modifications to genes such as \textit{BRCA1} and \textit{BRCA2}, which are only observed in a limited number of cases, certain common, low-penetrance variations have been identified in other genes potentially implicated in BC (Dong et al., 2008). Recently, several genome-wide association studies (GWAS; Antoniou et al., 2008; Garcia-Closas et al., 2008; Mcinerney et al., 2009; Latif et al., 2010) have revealed multiple BC-associated loci, of which the majority are single nucleotide polymorphisms (SNPs). However, these contribute only insignificant effects to BC risk.

One important locus is the trinucleotide repeat containing 9 (\textit{TNRC9}) gene, also termed \textit{TOX3}. This gene, on chromosome 16q12, contains a presumed high-mobility group (HMG), suggesting that it may act as a transcription factor. Moreover, it is related to bone metastasis in BC (Smid et al., 2006). Recently, Easton et al. (2007) provided strong evidence that rs3803662 C>T, which lies 8 kb upstream of \textit{TNRC9}/\textit{LOC643714}, confers even greater BC risk than mutations in both \textit{BRCA1} and \textit{BRCA2}. The rs3803662 polymorphism is either restricted to or more strongly associated with estrogen receptor (ER)-positive BC than ER-negative cases (Stacey et al., 2007; Garcia-Closas et al., 2008; Dittmer et al., 2011). Several GWAS have evaluated the relationship between this variant and BC risk, but their results
TNRC9 rs3803662 C>T polymorphism and breast cancer

remain to be verified in different ethnic groups (Li et al., 2009; Zheng et al., 2009; Ruiz-Narváez et al., 2010). Hence, we performed a meta-analysis of 18 eligible studies, involving 44,820 cases and 58,316 controls, to achieve an accurate estimation of this association and better understand BC risk factors.

MATERIAL AND METHODS

Identification of relevant studies

We searched PubMed and Embase (last update: June 30, 2014) using the following terms: “rs3803662”, “TNRC9”, “TOX3”, “16q12”, “genetic susceptibility”, “SNP”, “polymorphism” or “variation”, and “breast cancer”. The search was conducted in English. A manual search of references included in retrieved papers was performed to identify additional studies. Articles in our meta-analysis conformed to the following criteria: i) they evaluated the TNRC9 rs3803662 polymorphism and BC risk, ii) they used a case-control design, and iii) they contained genotype data.

Data extraction

Two investigators independently extracted data and reached a consensus in cases of disagreement. The following data were recorded: first authors’ names, year of publication, nationality, control source, and genotype frequencies. Ethnicity was categorized as Caucasian or Asian (one study of an African population was excluded). Data were extracted separately.

Statistical analysis

All analyses were carried out with the Stata software (version 8.2; StataCorp. LP, College Station, TX, USA), using two-sided P values. The relationship between the TNRC9 rs3803662 polymorphism and BC risk was assessed by odds ratios (ORs) and 95% confidence intervals (95% CIs). The significance of pooled ORs was assessed by the Z-test. Pooled ORs were generated from the respective studies using heterozygote (CT vs CC) and homozygote comparisons (TT vs CC), and dominant (CT/TT vs CC) and recessive models (TT vs CT/CC). Bonferroni correction was adopted for multiple comparisons. Since such comparisons were carried out four times, P values below 0.05/4 (0.0125) were considered statistically significant. Both the Cochran Q-test of heterogeneity and the I² statistic, which quantifies the proportion of overall variation due to heterogeneity, were calculated (Cochran, 1950; Higgins et al., 2003). Where the Q-test P value of at least 0.05 indicated a lack of heterogeneity, the OR of each dataset was calculated using a fixed-effect model (the Mantel-Haenszel method; Mantel and Haenszel, 1959). Otherwise, a random-effect model (the DerSimonian-Laird method; DerSimonian and Laird, 1986) was applied. Stratified analyses were also carried out based on ethnicity. A funnel plot and the Egger linear regression test were used to assess potential publication bias (Egger et al., 1997). In addition, sensitivity analysis was undertaken to verify the reliability of our results, by sequentially removing single studies from the meta-analysis to determine their individual effects on the pooled OR.
RESULTS

Study characteristics

In total, 18 studies involving 44,820 cases and 58,316 controls met the inclusion criteria and were used in pooled analyses (Antoniou et al., 2008; Garcia-Closas et al., 2008; Li et al., 2009; Mcinerney et al., 2009; Gorodnova et al., 2010; Latif et al., 2010; Liang et al., 2010; Tamimi et al., 2010; Campa et al., 2011; Han et al., 2011; Slattery et al., 2011; Butt et al., 2012; Harlid et al., 2012; Shan et al., 2012; Mizoo et al., 2013; Ottini et al., 2013; Chen et al., 2014; Elematore et al., 2014). The primary features of these investigations are shown in Table 1. All were case-control studies concerning BC. Five involved Asian subjects, 12 focused on Caucasians, while one comprised African participants. Twelve and six studies were population- and hospital-based, respectively.

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Country/Region</th>
<th>Ethnicity</th>
<th>Source</th>
<th>Cases (44,820)</th>
<th>Controls (58,316)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CC CT TT</td>
<td>CC CT TT</td>
</tr>
<tr>
<td>Antoniou</td>
<td>2008</td>
<td>Europe</td>
<td>Caucasian</td>
<td>Hospital</td>
<td>2422 2173 497</td>
<td>2244 1831 382</td>
</tr>
<tr>
<td>Garcia-Closas</td>
<td>2008</td>
<td>Europe</td>
<td>Caucasian</td>
<td>Hospital</td>
<td>7359 7132 1848</td>
<td>13,295 9,005 2026</td>
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<tr>
<td>Mcinerney</td>
<td>2009</td>
<td>UK</td>
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<td>Population</td>
<td>486 382 82</td>
<td>532 396 48</td>
</tr>
<tr>
<td>Latif</td>
<td>2010</td>
<td>UK</td>
<td>Caucasian</td>
<td>Hospital</td>
<td>106 103 18</td>
<td>217 137 19</td>
</tr>
<tr>
<td>Li</td>
<td>2009</td>
<td>China</td>
<td>Asian</td>
<td>Hospital</td>
<td>32 141 118</td>
<td>40 128 123</td>
</tr>
<tr>
<td>Tamimi</td>
<td>2010</td>
<td>Sweden</td>
<td>Caucasian</td>
<td>Population</td>
<td>333 300 54</td>
<td>415 273 50</td>
</tr>
<tr>
<td>Gorodnova</td>
<td>2010</td>
<td>Russia</td>
<td>Caucasian</td>
<td>Population</td>
<td>74 50 16</td>
<td>77 82 15</td>
</tr>
<tr>
<td>Liang</td>
<td>2010</td>
<td>China</td>
<td>Asian</td>
<td>Population</td>
<td>126 413 486</td>
<td>127 464 455</td>
</tr>
<tr>
<td>Han</td>
<td>2011</td>
<td>Korea</td>
<td>Asian</td>
<td>Population</td>
<td>369 1435 1481</td>
<td>516 1617 1361</td>
</tr>
<tr>
<td>Campa</td>
<td>2011</td>
<td>Europe</td>
<td>Caucasian</td>
<td>Population</td>
<td>3706 3528 1074</td>
<td>5721 4724 1150</td>
</tr>
<tr>
<td>Slattery</td>
<td>2011</td>
<td>America</td>
<td>Caucasian</td>
<td>Population</td>
<td>569 495 109</td>
<td>708 530 90</td>
</tr>
<tr>
<td>Harlid</td>
<td>2012</td>
<td>Europe</td>
<td>Caucasian</td>
<td>Population</td>
<td>1394 1420 330</td>
<td>2768 1898 352</td>
</tr>
<tr>
<td>Butt</td>
<td>2012</td>
<td>Sweden</td>
<td>Caucasian</td>
<td>Population</td>
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<td>780 512 95</td>
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<tr>
<td>Shan</td>
<td>2012</td>
<td>Tunisia</td>
<td>African</td>
<td>Population</td>
<td>190 271 141</td>
<td>126 165 78</td>
</tr>
<tr>
<td>Chen</td>
<td>2014</td>
<td>China</td>
<td>Asian</td>
<td>Population</td>
<td>159 178 51</td>
<td>217 227 58</td>
</tr>
<tr>
<td>Ottini</td>
<td>2013</td>
<td>Italy</td>
<td>Caucasian</td>
<td>Hospital</td>
<td>143 195 74</td>
<td>352 323 70</td>
</tr>
<tr>
<td>Mizoo</td>
<td>2013</td>
<td>Japan</td>
<td>Asian</td>
<td>Hospital</td>
<td>74 230 160</td>
<td>91 227 142</td>
</tr>
<tr>
<td>Elematore</td>
<td>2014</td>
<td>Chile</td>
<td>Caucasian</td>
<td>Population</td>
<td>330 371 100</td>
<td>100 185 62</td>
</tr>
</tbody>
</table>

Quantitative synthesis

The variant T allele of rs3803662 C>T markedly increased BC risk under heterozygote (CT vs CC: OR = 1.14, 95%CI = 1.07-1.22, P < 0.001, $I^2 = 68.3\%$) and homozygote comparisons (TT vs CC: OR = 1.38, 95%CI = 1.25-1.53, P < 0.001, $I^2 = 71.2\%$), as well as under dominant (CT/TT vs CC: OR = 1.19, 95%CI = 1.11-1.28, P < 0.001, $I^2 = 74.9\%$) and recessive models (TT vs CT/CC: OR = 1.28, 95%CI = 1.19-1.38, P < 0.001, $I^2 = 58.8\%$; Figure 1 and Table 2).

In our stratified analysis based on ethnicity, each genetic model also revealed obviously enhanced risk in the Asian group (CT vs CC: OR = 1.16, 95%CI = 1.04-1.29, P = 0.010, $I^2 = 65.4\%$).
TNRC9 rs3803662 C>T polymorphism and breast cancer

= 18.0%; TT vs CC: OR = 1.42, 95%CI = 1.26-1.59, P < 0.001, F = 35.0%; CT/TT vs CC: OR = 1.26, 95%CI = 1.13-1.40, P < 0.001, F = 18.5%; TT vs CT/CC: OR = 1.24, 95%CI = 1.15-1.34, P < 0.001, F = 38.0%; Figure 2). Moreover, similar results were obtained using the Caucasian dataset (CT vs CC: OR = 1.15, 95%CI = 1.06-1.24, P = 0.001, F = 77.2%; TT vs CC: OR = 1.40, 95%CI = 1.22-1.59, P < 0.001, F = 78.6%; CT/TT vs CC: OR = 1.19, 95%CI = 1.09-1.29, P < 0.001, F = 82.3%; TT vs CT/CC: OR = 1.32, 95%CI = 1.19-1.46, P < 0.001, F = 64.1%; Figure 3). Likewise, we noted that data from studies with hospital-based control groups showed elevated risk under these same four models (CT vs CC: OR = 1.25, 95%CI = 1.13-1.38, P < 0.001, F = 57.3%; TT vs CC: OR = 1.53, 95%CI = 1.25-1.87, P < 0.001, F = 74.4%; CT/TT vs CC: OR = 1.32, 95%CI = 1.17-1.49, P < 0.001, F = 73.2%; TT vs CT/CC: OR = 1.31, 95%CI = 1.10-1.55, P = 0.002, F = 73.2%). Data from population-based investigations demonstrated an interesting and clear association under homozygote comparison (TT vs CC: OR = 1.31, 95%CI = 1.14-1.51, P < 0.001, F = 69.7%) and dominant (CT/TT vs CC: OR = 1.13, 95%CI = 1.03-1.24, P = 0.007, F = 73.1%) and recessive models (TT vs CT/CC: OR = 1.27, 95%CI = 1.16-1.39, P < 0.001, F = 48.3%), but not using heterozygote comparison (CT vs CC: OR = 1.09, 95%CI = 1.00-1.19, P = 0.058, F = 66.4%; Table 2).

Figure 1. Forest plot concerning our meta-analysis of the relationship between the TNRC9 rs3803662 C>T polymorphism and breast cancer risk under the dominant genetic model. OR = odds ratio, CI = confidence interval.
Table 2. Stratified analysis of the relationship between the TNRC9 rs3803662 C>T polymorphism and breast cancer risk.

<table>
<thead>
<tr>
<th>Variable</th>
<th>N°</th>
<th>Case/controls</th>
<th>CT or CC</th>
<th>TT or CC</th>
<th>CT/TT or CC</th>
<th>TT or CT/CC</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>OR (95%CI)</td>
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<td>OR (95%CI)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>P (Q)</td>
<td>P (Q)</td>
<td>F (%)</td>
<td>P (Q)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>I² (%)</td>
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<td></td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>Total</td>
<td>18</td>
<td>44,820/58,316</td>
<td>1.14 (1.07-1.22)</td>
<td>&lt;0.001</td>
<td>1.13 (1.25-1.51)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ethnicity</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>12</td>
<td>38,765/52,174</td>
<td>1.15 (1.06-1.24)</td>
<td>0.001</td>
<td>1.40 (1.22-1.59)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Asian</td>
<td>5</td>
<td>5453/5773</td>
<td>1.06 (1.04-1.20)</td>
<td>0.001</td>
<td>1.42 (1.26-1.59)</td>
<td>&lt;0.001</td>
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<tr>
<td>Control source</td>
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<td></td>
</tr>
<tr>
<td>Population</td>
<td>12</td>
<td>21,595/26,964</td>
<td>1.09 (1.00-1.19)</td>
<td>0.058</td>
<td>1.43 (1.14-1.81)</td>
<td>&lt;0.001</td>
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<tr>
<td>Hospital</td>
<td>6</td>
<td>23,225/31,352</td>
<td>1.25 (1.13-1.38)</td>
<td>0.001</td>
<td>1.53 (1.25-1.87)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Number of datasets. The single African study was excluded in the stratified analysis based on ethnicity. †A random-effect model was used when the heterogeneity test P value was <0.05, otherwise, a fixed-effect model was employed. P (Q): P value for the Q-test of heterogeneity. P (Z): P value for the Z-test of overall odds ratio. P < 0.0125 was defined as the significance threshold after Bonferroni correction. OR = odds ratio, CI = confidence interval.
Figure 2. Forest plot concerning our meta-analysis of the relationship between the TNRC9 rs3803662 C>T polymorphism and breast cancer risk among Asians, under the dominant genetic model. OR = odds ratio, CI = confidence interval.

Figure 3. Forest plot concerning our meta-analysis of the relationship between the TNRC9 rs3803662 C>T polymorphism and breast cancer risk among Caucasians, under the dominant genetic model. OR = odds ratio, CI = confidence interval.

Heterogeneity tests

We found obvious heterogeneity in data regarding the relationship between the TNRC9 rs3803662 polymorphism and susceptibility to BC under heterozygote (CT vs CC: $P_{\text{heterogeneity}} < 0.001, F^2 = 68.3\%$) and homozygote comparisons (TT vs CC: $P_{\text{heterogeneity}} < 0.001, F^2 = 71.2\%$), and dominant (CT/TT vs CC: $P_{\text{heterogeneity}} < 0.001, F^2 = 74.9\%$) and recessive models (TT vs CT/CC: $P_{\text{heterogeneity}} < 0.001, F^2 = 58.8\%$). We assessed the source of this heterogeneity...
by subgroup analysis, which showed no significant level in the Asian dataset, but revealed a
significant presence in the Caucasian group, under all genetic models. One possible reason
for this is that the Caucasian subjects derived from various countries associated with different
genetic backgrounds.

**Sensitivity analysis**

We attempted to test the influence of single datasets on the pooled ORs by excluding
each study included in our meta-analysis in turn. The corresponding results were not materially
altered (data not shown), suggesting that our findings were statistically robust.

**Publication bias**

Begg’s funnel plot and the Egger test were used to assess publication bias. The
former revealed no evidence of obvious asymmetry (the funnel plot of the overall CT/TT vs
CC comparison is shown in Figure 4). Furthermore, application of the Egger test to provide
statistical evidence of funnel plot symmetry also suggested the absence of publication bias
(CT/TT vs CC: P = 0.218).

![Begg's funnel plot with pseudo 95% confidence limits](image)

**Figure 4.** Begg’s funnel plot testing publication bias under the dominant genetic model. Each point represents
a separate study for the indicated association. The horizontal line represents mean effect size. LogOR = natural
logarithm of the odds ratio, SE = standard error.

**DISCUSSION**

We believe, based on the present meta-analysis, that the *TNRC9* rs3803662 C>T
polymorphism is clearly connected with increased BC risk. To the best of our knowledge,
this is the first report to explore the connection between this polymorphism and risk of BC in
different ethnicities.

The *TNRC9* gene, also known as *TOX3* and *CAGF9*, contains a trinucleotide
repeat motif (Margolis et al., 1997) and an HMG-box (O’Flaherty and Kaye, 2003). It is
involved in the regulation of calcium-dependent transcription and interacts with cAMP
response element-binding protein (Yuan et al., 2009). In addition, *TNRC9* can interact
with CITED1 to increase transcription (Yuan et al., 2009; Dittmer et al., 2011). CITED1 is a transcription coregulator that enhances the activity of transcription factors such as ERs (Yahata et al., 2001). A correlation between the rs3803662 genotype and decreased TNRC9 mRNA has been observed in breast tumors, in which transcription of this gene decreases in an allele-dependent manner (Riaz et al., 2012). To date, one study has shown an effect of TNRC9 expression on BC, showing that increased levels of TNRC9 mRNA predict bone metastasis in this disease (Smid et al., 2006).

Recently, Easton et al. (2007) found that the TNRC9 polymorphisms rs3803662 C>T, rs8051542 C>T, and rs12443621 A>G demonstrate a convincing connection with heightened BC risk. Moreover, another GWAS of 1600 Icelandic BC patients and 11,563 controls, followed by a replication study of 4554 affected individuals and 17,577 control subjects, showed the rs3803662 C>T TNRC9 variant to be associated with increased ER-positive BC risk (Stacey et al., 2007). However, in a Japanese study, the rs3803662 T allele exhibited no connection with risk of BC (Mizoo et al., 2013). As conflicting results have been obtained, the clinical importance of this polymorphic gene is uncertain. Thus, this meta-analysis was conducted to establish the possible risk of BC conferred by the TNRC9 rs3803662 C>T polymorphism. Based on 44,820 cases and 58,316 controls, the present study did reveal a connection between the TNRC9 polymorphism and BC, in that the rs3803662 variant was found to be strongly associated with increased risk of this disease. The stratified analysis according to ethnicity also returned a similar result, using both Caucasian and Asian datasets. Our meta-analysis demonstrates that the T allele of the TNRC9 rs3803662 C>T variant constitutes a minor risk factor for the development of BC.

The interpretation of our results may be limited by certain factors. First, the published studies were not sufficiently numerous to enable a comprehensive analysis, and the lack of data in some articles restricted further investigation of potential interactions with, for example, ER/ progesterone receptor status, menopause, and pathological subtype. Second, although every effort was made to identify studies for use in our meta-analysis, we believe that some eligible investigations were not included. Third, our results were based on unadjusted data, whereas a more precise analysis could be conducted if detailed individual information were available, such as age, histological type, and BRCA1/2 status.

Nevertheless, the present meta-analysis has several advantages. First, we extensively explored the connection between the TNRC9 rs3803662 C>T polymorphism and BC risk, and our research further established an obvious association in Asian and Caucasian groups. This study attempted to reveal the role of genetic ancestry in BC etiology. Second, we pooled several datasets comprising a substantial number of cases and controls from different studies, which greatly aided our statistical analysis.

In conclusion, our meta-analysis suggests that the TNRC9 rs3803662 C>T polymorphism is associated with elevated BC risk among both Asians and Caucasians. This polymorphism is an independent factor in the development of BC, and represents a potential therapeutic target for new drugs. However, well-designed, multicenter studies of other potential BC risk factors, such as gene-gene and gene-environment interactions, should be conducted to validate our findings.

Conflicts of interest

The authors declare no conflict of interest.
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TNRC9 rs3803662 C>T polymorphism and breast cancer


Stacey SN, Manolescu A, Sulem P, Rafnar T, et al. (2007). Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat. Genet.* 39: 865-869. [http://dx.doi.org/10.1038/ng2064](http://dx.doi.org/10.1038/ng2064)


