Genes other than *BRCA1* and *BRCA2* involved in breast cancer susceptibility

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This review focuses on genes other than the high penetrance genes *BRCA1* and *BRCA2* that are involved in breast cancer susceptibility. The goal of this review is the discovery of polymorphisms that are either associated with breast cancer or that are in strong linkage disequilibrium with breast cancer causing variants. An association with breast cancer at a 5% significance level was found for 13 polymorphisms in 10 genes described in more than one breast cancer study. Our data will help focus on the further analysis of genetic polymorphisms in populations of appropriate size, and especially on the combinations of such polymorphisms. This will facilitate determination of population attributable risks, understanding of gene-gene interactions, and improving estimates of genetic cancer risks.

Breast cancer is the most common cancer in women in the western world. In breast cancer development, genetic and environmental factors play a role with family history being the most important factor for determining breast cancer risk. This risk is a function of the number of relatives affected with breast and ovarian cancer, the degree of relationship to these relatives, and their age at diagnosis of the disease. Hereditary breast cancer accounts for 5-9% of all breast cancers. It was estimated that the combination of *BRCA1* and *BRCA2* gene mutations was responsible for approximately 80% of the families with hereditary breast cancer. These estimates, however, may be too high owing to the way patients are selected, namely on the basis of a pronounced family history of the disease. More recent estimates put this risk at about 30%. Mutations of the *BRCA1* and *BRCA2* genes do not explain the occurrence of breast cancer in every breast cancer prone family. At least one other major breast cancer susceptibility gene is proposed to exist. In addition, a number of rare genetic syndromes are associated with high breast cancer risk. Together, these rare syndromes account for less than 1% of all hereditary breast cancers.

Apart from these well defined, high penetrance genes, there may be other genes that also increase the susceptibility to breast cancer. Candidates are proto-oncogenes and genes involved in metabolic, oestrogen, and immunomodulatory pathways. Each of these genes probably confers only a small (odds ratio (OR) 1-1.5) to moderate (OR 1.5-2) increase in the lifetime breast cancer risk. Because mutations in these so-called low penetrance genes are expected to be present in a large number of people, the population attributable risk (PAR) for breast cancer explained by these genes (in combination with environmental exposures) may be substantial and (potentially) considerably higher than the PAR caused by rare mutations of high penetrance genes such as *BRCA1* and *BRCA2*. The published polymorphisms (a locus where two or more alleles are each present at a frequency >1% in the population) were, in general, studied because of their biological plausibility. Some polymorphisms may partly account for the difference in the sensitivity of women to environmental factors, such as the use of replacement oestrogens. In subjects carrying low penetrance gene mutations, environmental factors might especially affect the risk of developing breast cancer. One subject may be 10-200 times more sensitive than another and may therefore develop cancer, while others at the same level of exposure will not. With the identification of important low penetrance gene mutations along with their interaction with environmental factors, specific prevention may become possible. Research on low penetrance genes involved in breast cancer is still in its infancy. Whole genome screens for determining low penetrance genes are currently not yet financially feasible.

This review focuses on genes other than *BRCA1* and *BRCA2* that may be involved in breast cancer susceptibility. Although mutations or polymorphisms in many of the genes described can also play a role in other types of common cancer, such as colorectal, ovarian, or prostate cancer, this review addresses only breast cancer. The aim of the pooled analysis was to find polymorphisms that may either have a causative relation to breast cancer or that are in strong linkage disequilibrium (LD) with breast cancer causing variants (for example, situations where certain haplotype combinations of alleles at different loci occur more frequently than would be expected from random association). We studied only the relation of the polymorphisms to breast cancer risk, since we assumed that environmental factors will play...
followed by somatic mutations, loss of heterozygosity (LOH, in familial and sporadic breast cancer patients were addressed, breast cancer susceptibility, the possible germline mutations for the known genetic syndromes associated with increased susceptibility, the possible germline mutations and presumed function are presented in table 1.

Table 1  Breast cancer susceptibility genes with their localisation and presumed function

<table>
<thead>
<tr>
<th>Gene</th>
<th>Location and function</th>
</tr>
</thead>
</table>
| BRCAl              | 17q21, DNA repair pathway, 
|                    | TP53                  |
|                    | ATM                   |
|                    | PTEN                  |
|                    | LKB1                  |
|                    | HRAS1                 |
|                    | NAT1                  |
|                    | NAT2                  |
|                    | GSTM1                 |
|                    | GSTP1                 |
|                    | GSTT1                 |
|                    | CYP1A1                |
|                    | CYP1B1                |
|                    | CYP1D6                |
|                    | CYP1F7                |
|                    | CYP1F9                |
|                    | ER                    |
|                    | PR                    |
|                    | AR                    |
|                    | COMT                  |
|                    | UGT1A1                |
|                    | TNFa                  |
|                    | HSF70                 |
|                    | HFE                   |
|                    | TFR                   |
|                    | VDR                   |
|                    | APC                   |
|                    | APOE                  |
|                    | CYP2E1                |
|                    | EDH17B2               |
|                    | HER2                  |
|                    | Tjfl                  |

<table>
<thead>
<tr>
<th>Gene</th>
<th>Location and function</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>5q22, inhibits the progression of cells from G1 to S phase, apoptosis, cell-cell interactions</td>
</tr>
<tr>
<td>APOE</td>
<td>19p13.2, lipid metabolism</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>10q24.3, er, metabolism of acetone, ethyl glycol, and ethanol</td>
</tr>
<tr>
<td>EDH17B2</td>
<td>17q12.2, EP, catalyses the reaction between oestrone and oestriol</td>
</tr>
<tr>
<td>HER2</td>
<td>17q21, proto-oncogene, control of cell growth and proliferation</td>
</tr>
<tr>
<td>Tjfl</td>
<td>9q33-34, cell growth</td>
</tr>
</tbody>
</table>


an equal role in all studies. The external variables taken into account were, where possible, menopausal state and ethnicity. Pooled analyses were performed on all polymorphisms. In addition, the sample size required to detect an association with breast cancer susceptibility with sufficient power was addressed.

METHODS

Search of published studies

Published studies were identified using the PubMed databases from 1980 to 2000, using the search terms “breast”, “cancer”, “risk”, and “polymorphism(s)”. For each specific candidate gene, a separate search was performed. For the terms “HRAS1”, “breast”, “cancer”, and “risk” were used for HRAS1. In the addition, the references of studies identified by the electronic searches were also evaluated. Studies eligible for our pooled analysis were those that compared genotype or allele frequencies of candidate genes in breast cancer cases with non-breast cancer controls using genomic DNA. The polymorphisms reported in breast cancer patients and controls in more than one study are described separately. When more than one polymorphism in one gene (for example, CYP1A1) or polymorphisms in different genes in the same region (for example, the HLA region) were examined in only one single study, they are also included. The different genes along with their localisation and presumed function are presented in table 1.

Lay out per gene

For the known genetic syndromes associated with increased breast cancer susceptibility, the possible germline mutations in familial and sporadic breast cancer patients were addressed, followed by somatic mutations, loss of heterozygosity (LOH, the loss of one of the two alleles at a given locus in a tumour), and hypermethylation. According to Knudson’s “two hit theory”, the two hits required for tumour development are generally thought of as an intragenic mutation (germline or somatic) and LOH. Recently, it has been shown that hypermethylation of the promoter region of a gene can also be one of the hits required in cancer development because this can silence the gene.

Pooled analysis

For a better insight into the possible effects of the various genes on breast cancer susceptibility, a pooled analysis for each polymorphism was performed. The results of this analysis are shown in table 2. The raw numbers of cases and controls from comparable studies were analysed together. The genotype specific ORs and the 95% confidence intervals (CIs) were calculated for all studies combined, without adjustment for external variables. This can result in values that differ from those in the original article. Whenever possible, a distinction was made between women heterozygous and homozygous for the variant allele. Where metabolic polymorphisms are assumed to be associated with a specific phenotype, a distinction was made between phenotype and genotype based studies (for example, CYP2D6, NAT2). For the genotype studies, the genotypes were combined according to phenotypic classes. Also, where possible, separate analyses were performed for the three major ethnic subgroups, white, African-American, and Asian.

The ORs and the 95% CIs of the studies used in the pooled analysis of polymorphisms in three genes are shown in figs 1 (the HRAS1 polymorphism), 2 (the PROGINS polymorphism of the PR gene), and 3 (the polymorphisms in the vitamin D receptor (VDR) gene).
<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Allele frequency</th>
<th>Cases*</th>
<th>Controls*</th>
<th>Risk G†</th>
<th>OR</th>
<th>95% CI</th>
<th>PAR</th>
<th>Sample size‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tp53 Intron 3</td>
<td>0.17</td>
<td>667</td>
<td>1981</td>
<td>W/V/V</td>
<td>0.97</td>
<td>0.79-1.18</td>
<td>2900</td>
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<tr>
<td>Tp53 Exon 4</td>
<td>0.36</td>
<td>552</td>
<td>2219</td>
<td>W/V/W</td>
<td>1.08</td>
<td>0.88-1.33</td>
<td>2200</td>
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<tr>
<td>Tp53 Intron 6</td>
<td>0.17</td>
<td>733</td>
<td>2160</td>
<td>W/V/V</td>
<td>0.70</td>
<td>0.51-0.95</td>
<td>1500</td>
<td></td>
</tr>
<tr>
<td>Hras1</td>
<td>0.08</td>
<td>2029</td>
<td>3252</td>
<td>V/V/V</td>
<td>2.04</td>
<td>1.73-2.41</td>
<td>430</td>
<td></td>
</tr>
<tr>
<td>L-myc</td>
<td>0.50</td>
<td>593</td>
<td>214</td>
<td>W/V</td>
<td>1.12</td>
<td>0.77-1.63</td>
<td>4000</td>
<td></td>
</tr>
<tr>
<td>Nat1</td>
<td>0.24</td>
<td>646</td>
<td>796</td>
<td>V carriers</td>
<td>1.13</td>
<td>0.91-1.39</td>
<td>5900</td>
<td></td>
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<tr>
<td>Nat2</td>
<td>0.62</td>
<td>476</td>
<td>785</td>
<td>W/V</td>
<td>0.87</td>
<td>0.75-1.00</td>
<td>7400</td>
<td></td>
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<tr>
<td>Gstm1</td>
<td>0.72</td>
<td>3394</td>
<td>3514</td>
<td>V carriers</td>
<td>1.59</td>
<td>1.01-2.48</td>
<td>1%</td>
<td></td>
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<tr>
<td>Gstm2</td>
<td>0.36</td>
<td>593</td>
<td>214</td>
<td>W/V</td>
<td>1.12</td>
<td>0.77-1.63</td>
<td>4000</td>
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<td>Gstm3</td>
<td>0.09</td>
<td>85</td>
<td>178</td>
<td>V carriers</td>
<td>1.04</td>
<td>0.54-2.03</td>
<td>1500</td>
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<tr>
<td>Cyp1a1</td>
<td>0.14</td>
<td>339</td>
<td>361</td>
<td>W/V</td>
<td>1.62</td>
<td>1.15-2.29</td>
<td>870</td>
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<tr>
<td>Cyp1b1</td>
<td>0.11</td>
<td>791</td>
<td>1468</td>
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<td>1.18</td>
<td>0.94-1.48</td>
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<tr>
<td>Cyp1f1</td>
<td>0.13</td>
<td>223</td>
<td>221</td>
<td>V carriers</td>
<td>0.99</td>
<td>0.43-2.24</td>
<td>5900</td>
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<td>Cyp1f2</td>
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<td>599</td>
<td>433</td>
<td>W/V</td>
<td>0.79</td>
<td>0.61-1.03</td>
<td>4500</td>
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<tr>
<td>Cyp1g1</td>
<td>0.13</td>
<td>655</td>
<td>808</td>
<td>W/V</td>
<td>0.99</td>
<td>0.80-1.11</td>
<td>8200</td>
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<tr>
<td>Cyp1g2</td>
<td>0.04</td>
<td>143</td>
<td>300</td>
<td>W/V</td>
<td>1.59</td>
<td>1.04-2.42</td>
<td>42%</td>
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<tr>
<td>Tfr</td>
<td>0.08</td>
<td>260</td>
<td>332</td>
<td>E2 allele</td>
<td>0.99</td>
<td>0.62-1.59</td>
<td>2800</td>
<td></td>
</tr>
<tr>
<td>Edf1/2r</td>
<td>0.54</td>
<td>190</td>
<td>190</td>
<td>W/V</td>
<td>0.59</td>
<td>0.37-0.94</td>
<td>1500</td>
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<td>Her2</td>
<td>0.13</td>
<td>339</td>
<td>359</td>
<td>W/V</td>
<td>1.26</td>
<td>0.88-1.79</td>
<td>20%</td>
<td></td>
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<tr>
<td>Tbr 1</td>
<td>0.06</td>
<td>152</td>
<td>733</td>
<td>W/V</td>
<td>1.58</td>
<td>0.96-2.99</td>
<td>6%</td>
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<tr>
<td>Tfr</td>
<td>0.40</td>
<td>165</td>
<td>294</td>
<td>W/V</td>
<td>1.01</td>
<td>0.66-1.55</td>
<td>4%</td>
<td></td>
</tr>
</tbody>
</table>

*Cases and controls: all studies combined. †Risk G: risk genotype. ‡Sample size: the number of patients and controls required to detect an association, with power of 90% and a significance level of 0.0026 (correction for multiple testing). W: wild type. V: variant allele.
The pooled analysis also shows the PAR and sample size required to detect the association with breast cancer for each polymorphism, with a power of 90% and a significance level of 0.0026 corrected for multiple testing (also shown in table 2). A description of the sample size calculation is given in Appendix 1.

RESULTS

The unknown “BRCA3” gene

Based on several studies, a region on chromosome 8p11-21 is considered to be involved in hereditary and sporadic breast cancer. Linkage analysis in eight French breast cancer families showed a multipoint lod of 2.51 (a lod score >3.0 is the accepted statistical significance level for linkage of a genetic locus with a disease) with two markers on chromosome 8p.27 Linkage analysis in two large German breast cancer families, with negative lod scores for the BRCA1 and BRCA2 locus, showed a multipoint lod score of 3.30 at two other markers, localised between the two markers in the French study, on chromosome 8p.28 In studies focusing on chromosome 8p, LOH...
was observed in 46-74% of unselected human breast tumours,22, 23, 27 in 86% of the familial tumours,28 in 78% of tumours in women with a specific BRCAl2 mutations (that is, the 999del5 mutation),29 and in 83% of male breast tumours.30 In ductal breast carcinoma in situ, LOH on 8p was observed in 0-37% of cases,30-34 suggesting LOH on 8p is associated with invasive behaviour of the tumour. Based on these LOH studies, there are at least two different regions of minimal overlap of LOH on chromosome 8p. The region on 8p discussed earlier,24 is localised in one of these regions. Mapped genes in this region include hEXTIL,53 WRN,53 and LHRH.54 No somatic or germline mutations have, as yet, been detected in these genes in breast cancer cases.

Rare genetic syndromes with increased breast cancer risk

The Tp53 gene and Li-Fraumeni syndrome

Inactivating mutations in the Tp53 gene have been found in many tumour types including breast cancer.4 Li-Fraumeni syndrome is an autosomal dominant disorder, caused by germline mutations in the Tp53 gene. This syndrome is characterised by an increased risk of soft tissue and osteosarcomas, leukaemias, brain tumours, adrenocortical carcinomas, and breast cancers.5 The risk of developing breast cancer before the age of 45 is 18-fold higher for affected females as compared to the general population.6 The excess is greatest below the age of 20 and declines with increasing age (relative risk (RR) for breast cancer after the age of 45 = 1.8).7 Germ-line mutations in the Tp53 gene have been estimated to account for less than 1% of breast cancer cases.4, 52-54 However, somatic mutations in the Tp53 gene are reported in 19-57% of human breast cancers,52-57 and LOH is found in 30-42%.52-54 There is no association between somatic Tp53 mutations and LOH at the Tp53 locus,24-25 suggesting that one inactivated allele may be sufficient for breast cancer development.26 Hypermethylation of the promoter region of the Tp53 gene does not play a role in breast cancers.58

Three different Tp53 polymorphisms (in intron 3, exon 4, and intron 6) have been studied in breast cancer patients. All three polymorphisms exhibit strong linkage disequilibrium with each other.55 In five studies examining the intron 3 polymorphism, none found an association with increased breast cancer risk.54-56 Surprisingly, the breast cancer risk for homozygous carriers of the variant allele was decreased. When all studies were combined, the OR for heterozygous carriers of the variant allele was 0.97 (95% CI 0.79-1.18) and was 0.46 (95% CI 0.25-0.84) for homozygous carriers. The exon 4+ and the intron 6- polymorphisms showed similar results. Thus, a decreased breast cancer risk for homozygous variant allele carriers was found for all three polymorphisms in the Tp53 gene. These homozygous variant allele carriers comprise 3% (intron 3), 13% (exon 4), and 3% (intron 6) of the women and, thus, up to 13% of women have decreased breast cancer risks.

Four studies examined all three polymorphisms in the Tp53 gene in relation to breast cancer risk. In one of the two studies examining haplotypes, an association was observed between the haplotype composed of the three variant alleles and the risk of breast cancer among white populations (OR=2.57, 95% CI 1.37-4.07). This association was not found in Hispanic (OR=0.24, 95% CI 0.05-1.11) or African-American patients (OR=1.13, 95% CI 0.46-2.81)56 nor in patients from Pakistan (OR=0.77, 95% CI 0.38-1.56). The two other studies did not construct haplotypes, but compared genotypic combinations. The first study found a marginally significant association between breast cancer and the genotypic combination that is heterozygous for all three polymorphisms (OR=1.68, 95% CI 0.99-2.86).57 This genotype combination did not exclude the haplotype composed of three variant alleles from being at risk. The second study found associations between breast cancer and two genotype combinations.58 With the first genotype combination (OR=2.94, 95% CI 1.37-6.27), heterozygous for the intron 3 and 6 polymorphisms and homozygous for the exon 4 variant allele, the haplotype of three variant alleles is still supported. With the second genotype combination (OR=1.61, 95% CI 1.13-2.30), homozygous for the intron 3 and 6 wild type allele and heterozygous for the exon 4 polymorphism, the variant allele haplotype is not possible. The fact that the four studies used different methods to examine the polymorphisms hampers the comparison of results. However, the analysis showed that the haplotype composed of the three variant alleles is associated with an increased breast cancer risk, particularly in white breast cancer patients.59

The ATM gene and ataxia telangiectasia

Most A-T patients do not survive to an age at which breast cancer generally occurs.60 A-T carriers (heterozygous for ATM mutations) are sensitive to late onset apoptosis after x ray irradiation owing to accumulation of cell cycle checkpoint abnormalities.61 In several studies, A-T carriers appear to have an increased breast cancer risk (OR 3.3-876 and PAR 3.8-5.857-73). However, in all studies the OR was determined with the observed/expected method and all groups were small. One study found no increased breast cancer risk among A-T carriers.62 The risk of A-T carriers to develop breast cancer is estimated to be 11% by the age of 50 and 30% by the age of 70.63

Germline mutations in the ATM gene are rare in breast cancer families without features of A-T.64-65 In sporadic breast cancers, germline and somatic mutations in the ATM gene are also rare,66 even in young patients67 and patients with bilateral breast cancer.68 In 88 breast cancer patients with a family history of breast cancer and leukaemia or lymphoma, three germline mutations in the ATM gene have been found.69 Chen et al.70 examined these three mutations and none appeared to be causal. In 82 Dutch breast cancer patients (diagnosed before the age of 45, > 5 years survival) including 33 bilateral cases, seven germline mutations were found, one out of frame splice site mutation (detected three times), three truncating mutations, and one in frame deletion.66 It was hypothesised that the existence of two distinct classes of A-T mutations (truncating and missense) might explain some of the seemingly contradictory data on cancer risk associated with the ATM gene.67-68 The truncating mutations act as null mutations because they produce low cellular levels of an unstable ATM protein. Because truncating mutation carriers have 50% of wild type ATM activity, they will have an almost normal phenotype. Some missense mutations encode stable, but functionally abnormal proteins that are present at normal intracellular levels. These proteins could compete in complex formation with the normal ATM protein, resulting in a dominant negative cellular phenotype. The functional loss in ATM missense mutation carriers might be more severe than in ATM truncating mutation carriers and, thus, only ATM missense mutations might be associated with an increased cancer risk.68 In most studies, A-T carrier detection in breast cancer cases was based on the protein truncation test and could only detect truncating mutations. Support for the existence of two functionally distinct classes of mutations can be derived from a study describing an increased breast cancer risk in two A-T families with a specific ATM missense mutation (T7271G) in both homozygotes and heterozygotes, with an age specific incidence rate based OR of 12.7 (95% CI=3.53-45.9).79 This mutation results in a aberrant full length ATM protein level comparable with unaffected subjects.72 Another explanation for the seemingly contradictory data on breast cancer risk is that the carrier frequency of A-T mutations could be much lower than the described 1% of the general population causing a low PAR. If this is the case, the OR for breast cancer in A-T

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carriers can be high (as found in the A-T families), while mutations in the ATM gene are rarely detected in sporadic breast cancer patients.

A recent study of 138 Austrian hereditary breast and ovarian cancer (HBOC) patients without BRCA1 and BRCA2 mutations showed functionally significant ATM germline mutations in at least 8.7% of the HBOC patients. The penetrance for one of the mutations (L1420F) was estimated to be 85% at age 60.

In conclusion, although the exact association remains unclear, a role for the ATM gene in breast cancer susceptibility is plausible.

**The PTEN gene and Cowden syndrome**

Cowden syndrome is an autosomal dominant disorder, characterised by the development of hamartomas and benign tumours. Mutations in the PTEN gene are present in 80% of Cowden syndrome families. Truncating PTEN mutations in Cowden syndrome families are associated with cancer and cause a 25-50% lifetime breast cancer risk in women. No mutations in the PTEN gene have been detected in breast cancer families and families with breast and brain cancer without features of Cowden syndrome. In sporadic breast cancer patients, germline and somatic mutations in the PTEN gene are rare even in young patients. LOH at the PTEN locus is found in 11-41% of sporadic breast cancers, but no somatic mutations have been observed in the remaining allele. It is, however, still possible that an epigenetic phenomenon such as hypermethylation of the promoter region inactivates the remaining allele. In one study (in 177 breast cancer patients with a positive family history for breast cancer and without BRCA1 and BRCA2 mutations), an association was found between a polymorphism in intron 4 of the PTEN gene and a lower age of diagnosis of breast cancer (42.7 versus 48.1 years). No comparison, however, was made with healthy controls. In conclusion, the PTEN gene is not likely to play a role in classical hereditary breast cancer. In sporadic breast cancers, LOH at the PTEN locus is detected, but since no alterations have been found in the remaining allele, it is not currently known whether PTEN plays a role in sporadic breast cancer susceptibility.

**The LKB1 gene and Peutz-Jeghers syndrome**

Peutz-Jeghers syndrome is an autosomal dominant disorder characterised by hamartomatous polyps in the small bowel and pigmented macules of the buccal mucosa, lips, fingers, and toes. This syndrome is caused by truncating germline mutations in the LKB1 gene. Patients with Peutz-Jeghers syndrome have an increased breast cancer risk. No germline mutations were detected in 22 patients from 14 breast cancer families with LOH on chromosome 19p. In 62 primary breast cancers in women without Peutz-Jeghers syndrome, no somatic mutations were found in the LKB1 gene and LOH was observed in only 8%. In conclusion, the LKB1 gene seems to play a role in breast cancer susceptibility, but only in patients with Peutz-Jeghers syndrome.

**Low penetrant breast cancer susceptibility genes**

There are several classes of potential low penetrance breast cancer susceptibility genes, such as proto-oncogenes, metabolic pathway genes, oestrogen pathway genes, and immunomodulatory pathway genes.

**Proto-oncogenes**

Proto-oncogenes are involved in the regulation of normal cell growth and differentiation. Mutations in proto-oncogenes lead to disturbances in the cell cycle and can result in abnormal growth or proliferation. Well known proto-oncogenes are the RAS genes, the HER2 gene, and the myc genes.

**HRAS1**

The HRAS1 gene encompasses four exons flanked by a variable tandem region repeat at the 3’ end. This minisatellite locus is composed of four common alleles (94% of the white population) and dozens of variants, the so-called intermediate and rare alleles. Each variant allele is derived from the common allele nearest in size to it. The HRAS1 polymorphism was examined in 13 studies. Positive ORs were detected in all studies (fig 1), five of which reached significance with ORs of 2-7. Combining the studies showed an association between rare HRAS1 alleles and breast cancer (OR=2.03, 95% CI 1.72-2.40), with a PAR of 14%. There are, however, several methodological problems in performing this pooled analysis, since the choice of the cut-off point between rare, intermediate, and common alleles is difficult to make and the distribution of the alleles in subgroups of the population varies between studies. The choice of rare alleles (frequency <4%) is not the same for all studies and does not correspond to any previous biological interpretation. In most studies there are four common alleles and the rest of the alleles are listed as rare. With this as the criterion, our pooled analysis indicated that the rare HRAS1 alleles are associated with a moderately increased risk of breast cancer.

**L-myc**

Three studies focused on L-myc and breast cancer risk. In one study, no controls were examined. Another study found an association with breast cancer for women heterozygous (OR=2.25, 95% CI 1.12-4.51) and homozygous (OR=2.63, 95% CI 1.22-5.68) for the variant allele. When all three studies were combined for our pooled analysis, no association with breast cancer was found.

**Metabolic pathway genes**

Enzymes involved in metabolic pathways are of interest because of their possible role in (de)toxification of chemical compounds. A number of metabolic pathway genes, including the cytochrome p450 family, the GST family, and the NAT1 and NAT2 genes, are thought to have evolved as an adaptive response to environmental exposure to toxins, including some carcinogens. The prediction is therefore that any alteration in the activity of these enzymes would result in an altered susceptibility to potentially toxic (mutagenic) compounds. This may determine the rate at which somatic mutations occur in genes in response to environmental exposures, resulting in an altered cancer susceptibility. The cytochrome p450 family proteins are known as phase I enzymes. In general, these enzymes metabolically activate carcinogens. A genotype associated with an increased phase I enzyme activity might therefore increase breast cancer risk. The NAT and GST family proteins are known as phase II enzymes. These enzymes metabolically inactivate carcinogens. The substrates for phase II enzymes include carcinogenic compounds activated by the phase I enzymes. A genotype associated with decreased phase II activity might therefore increase breast cancer risk.

**N-acetyl transferase (NAT)**

Both NAT1 and NAT2 are polymorphic with so-called fast and slow phenotypes. Slow acetylators produce proteins that are either poorly expressed, are unstable, or have partially reduced catalytic activities. In theory, having the slow acetylator phenotype could mean that aromatic amines are metabolised more slowly and that slow acetylators might, therefore, be at increased breast cancer risk.
ancestry. Two studies found no association with breast cancer risk either separately or combined. The NAT1 polymorphism does not appear to play a major role in breast cancer susceptibility, although a small increase in breast cancer risk cannot be excluded.

**NAT2**
The acetylation capacity in NAT2*4* homozygotes in vivo is higher than in NAT2*4* heterozygotes and all variant alleles have lower acetylation capacities. The population frequency of the fast acetylator genotype of the NAT2 gene is 22-78%. None of seven studies found an increased breast cancer risk for the slow acetylator NAT2 phenotype, while two studies found a decreased breast cancer risk. No association with breast cancer risk was found when all studies were combined. The results for the NAT2 genotype were similar. No effect on breast cancer risk was found when the studies were combined in our pooled analysis. In conclusion, the NAT2 polymorphism does not play a role in breast cancer susceptibility.

**Combination of NAT1 and NAT2**
One study examined both polymorphisms. No association between these polymorphisms and breast cancer was observed for either the NAT1 or NAT2 genes separately or combined.

**Glutathione S-transferase (GST) family**
Deletion variants that are associated with a lack of enzyme function exist at GSTM1 and GSTT1. Homozygotes for null deletions in the GSTM1 and/or GSTT1 genes may have an impaired ability to eliminate carcinogens metabolically and may therefore be at increased cancer risk.

**GSTM1**
*GSTM1* is polymorphically expressed as *GSTM1-0* or null (homozygous deletion) and *GSTM1a* and *GSTM1b*. Between 20 and 60% of the general population are homozygous null for the *GSTM1* gene. The null variant has been well examined in breast cancer studies with varying results. Our pooled analysis showed an association between this polymorphism and breast cancer risk, although this increase is very small and only marginally significant (OR=1.13, 95% CI 1.00-1.26). The combined sample size is large enough to exclude a moderate increase in breast cancer risk for *GSTM1* null homozygous carriers.

**GSTT1**
In 31% (24/77) of the breast cancer cases, hypermethylation of the GSTT1 promoter region was detected. A polymorphism, the isoleucine to valine substitution at codon 105, has been combined. The isoleucine to valine substitution at codon 105, has been combined. The results were similar for premenopausal and postmenopausal women. Based on the combined sample size, a moderate increase in breast cancer risk can be excluded for women homozygous null for the GSTT1 polymorphism.

**Combination of GSTM1, GSTT1, and GSTP1 polymorphisms**
Six studies examined both *GSTM1* and *GSTT1* polymorphisms and breast cancer risk and four of them also analysed the GSTP1 polymorphism. In one study, no controls were examined and it was therefore not included in our pooled analysis.

Of the five studies that examined the combination of two of the GST genes, one found an association between the two risk genotype and breast cancer for all three combinations of GST genes. Another detected an association between the two risk genotype for the *GSTM1* and *GSTT1* gene and breast cancer. Pooled analysis showed an increased breast cancer risk for the one and the two risk genotype of all three gene combinations, although only the two risk genotype of the *GSTM1* and *GSTP1* combination reached significance (OR=1.65, 95% CI 1.00-2.71).

For the two studies where all three polymorphisms were examined, the results were similar. The study that observed associations with the two risk genotypes also showed an association between the three high risk alleles and breast cancer. When both studies were combined, increased breast cancer risks were observed for carriers of the one, two, or three risk genotype, although only the two risk genotype reached significance (OR=1.90, 95% CI 1.13-3.22).

**CYP1A1**
This gene codes for aryl hydrocarbon hydroxylase (AHH). AHH is strongly inducible; differences in xenobiotic metabolic activity between subjects even within a family can be over 200-fold. Changes in AHH activity, resulting in different oestrogen levels, could affect breast cancer risk. Four polymorphisms have been described in the *CYP1A1* gene, namely the m1 polymorphism, the m2 polymorphism (associated with increased enzyme activity in vitro, both for homozygotes and heterozygotes for the variant allele), the m3 polymorphism (only present in African-Americans), and the m4 polymorphism.

**GSTM1**
*GSTM1* is polymorphically expressed as *GSTM1-0* or null (homozygous deletion) and *GSTM1a* and *GSTM1b*. Between 20 and 60% of the general population are homozygous null for the *GSTM1* gene. The null variant has been well examined in breast cancer studies with varying results. Our pooled analysis showed an association between this polymorphism and breast cancer risk, although this increase is very small and only marginally significant (OR=1.13, 95% CI 1.00-1.26). The combined sample size is large enough to exclude a moderate increase in breast cancer risk for *GSTM1* null homozygous carriers.

**GSTT1**
The *GSTT1* gene has two functionally different genotypes, *GSTT1-0* or null (homozygous deletion) and *GSTT1+* (one or two undeleted alleles). The *GSTT1* null genotype has been linked to increased DNA damage from experimental carcinogens. In different populations, 9-64% are homozygous null for the *GSTT1* gene. Six studies examining this polymorphism showed no association with breast cancer. The results were similar for premenopausal and postmenopausal women. Based on the combined sample
combined. However, by combining the two studies which ana-
lysed postmenopausal women only,193 194 an association with breast cancer was found for both heterozygous carriers of the m2 variant allele (OR=1.59, 95% CI 1.07-2.37) and women homozygous for the variant allele, although the latter associ-
ation is not significant (OR=2.53, 95% CI 0.92-6.96). This is 
probably because of lack of power. The African-American spe-
cific m3 variant allele does not seem to play a role in breast 
cancer susceptibility.195 196 No association with breast cancer was 
observed in the one study197 that examined the m4 poly-
morphism.

In conclusion, a small increased breast cancer risk was 
found in the white population for the m1 polymorphism and 
a moderately increased breast cancer risk in postmenopausal 
women was detected for the m2 polymorphism. A moderate 
increase in breast cancer risk for variant allele carriers can-
not be excluded for all four polymorphisms owing to lack of 
power. Additional data are required to define the precise 
association between this gene and breast cancer, particularly 
in the white population and in postmenopausal women.

CYP1B1

The CYP1B1 enzyme exceeds other p450 enzymes in both oes-
trogen hydroxylation activity and expression in breast 
tissue.198 Four polymorphisms have been described in this gene 
and all variants have higher hydroxylation activity.199 These 
variant alleles may be associated with changes in oestrogen 
metabolism and therefore breast cancer risk. Three of these 
polymorphisms were examined in breast cancer patients and 
controls. The codon 432 polymorphism was examined in three 
studies.196 198 199 Large differences in variant allele frequencies 
were found between different populations with the variant 
allele frequency ranging from 0.15 to 0.68, with even large 
differences between two Asian studies.195 196 When all studies 
were combined, our pooled analysis found no association 
between the codon 432 polymorphism and breast cancer. The 
study examining the codon 119 polymorphism detected an 
association with an increased breast cancer risk in women 
heterozygous for the variant allele (OR=1.62, 95% CI 1.15- 
2.29).196 However, in women homozygous for the variant allele, 
a non-significant decrease in risk was found (OR=0.6, 95% CI 
0.11-3.31). No association with breast cancer was observed for 
the codon 453 polymorphism.196

One study examined both the codon 119 and codon 432 
polymorphisms.195 The two polymorphisms were genetically 
independent and no association with an increased breast can-
cer risk was found for any combination of them.

CYP2D6

The CYP2D6 variant allele is the result of a deletion of a 17.5 kb 
region including the entire CYP2D6 gene.200 In white popula-
tions, 5% are homozygous for this polymorphism.194 195 These 
poor metabolisers are unable to metabolise agents such as 
dehydroepiandrosterone and codeine.17 Seven studies, with varying 
results, examined this polymorphism, three 
phenotypically196 199 200 and four genotypically.196 201 202 When 
the phenotype studies were combined, a moderately increased 
breast cancer risk was found for poor metabolisers (OR=2.22, 
95% CI 1.39-3.55) with a PAR of 8%. When the genotype 
studies were combined, an association was detected for carriers 
(homozygous and heterozygous combined) of the variant 
allele (OR=1.49, 95% CI 1.26-1.77), with a PAR of 6%. In con-
clusion, this polymorphism may play a role in increased breast 
cancer susceptibility.

Oestrogen pathway genes

Experimental, clinical, and epidemiological studies show that 
oestrogen and progesterone play a major role in growth and 
differentiation of normal breast tissue.114 203 A prolonged or 
increased exposure to oestrogen is associated with increased 
breast cancer risk.204 205 Endogenous and exogenous hormones 
stimulate cell proliferation, and thus enhance the chance of 
accumulating random genetic errors. The most widely 
accepted risk factors for breast cancer such as age at 
menarche, age at first pregnancy, number of pregnancies, 
breast feeding, age at menopause, and obesity 12 207 can be 
considered measures of the cumulative dose of oestrogen 
that breast epithelium is exposed to over time.206 207 Several oestro-
gen metabolites can directly or indirectly cause oxidative DNA 
damage.208 In conclusion, genes involved in the metabolism 
of sex hormones (that is, oestrogens) are interesting 
candidates for breast cancer susceptibility genes.207 209

Cytochrome p450 family

CYP17

A polymorphism in the CYP17 gene was detected in the 5’ 
untranslated region. The variant allele of this polymorphism 
has an additional Sp1 type promoter site. Since it is thought 
that the number of 5’ promoter elements correlates with pro-
moter activity,19 women with this allele might have higher 
oestradiol levels.211 An association between the presence of at 
least one variant allele and an increased serum oestrogen and 
progesterone level at day 11 and day 22 of the menstrual cycle 
is found in young, nulliparous women.212 One male breast 
cancer study (64 cases and 58 controls) observed an increased 
risk for variant allele carriers (OR=2.10, 95% CI 1.04-4.27).213 Ten 
studies on female breast cancer examined this.214 216 219 219 221 222 One 
found an association (OR= 1.99, 1.5-3.45) between variant 
allele carriers (homozygous and heterozygous) and breast 
cancer in young women (< 37 years of age),212 but the number 
of cases (n=109) and controls (n=117) were small. Six other studies 
in premenopausal women194 216 218 219 221 222 showed no 
association between this polymorphism and breast cancer. 
When all studies were combined in our pooled analysis, no 
association with breast cancer was found. In conclusion, based 
on the combined sample size, even a small increase in breast 
cancer risk overall can be excluded. However, because the 
studies did not further discriminate for age, an increased risk 
for breast cancer in young women carrying the variant allele 
cannot be excluded.

CYP19

Several polymorphisms have been described in the CYP19 
gene. A tetranucleotide repeat polymorphism, (TTTA)n, is 
located in intron 4, about 80 nucleotides downstream from 
exon 4.224 Our pooled analysis of the five studies examining the 
(TTTA)10 allele polymorphism215-220 showed an OR of 1.59 
(95% CI 1.01-2.48), with a PAR of 1%. There is, however, a 
problem in performing a pooled analysis on this polymor-
phism, because the studies use different methods to detect the 
alleles. Two studies found eight different alleles while the 
three others found seven, six, and five respectively. Two other 
polymorphisms in intron 4 and 6 described in one study were 
in strong linkage disequilibrium with the tetranucleotide 
polymorphism.220 No association with breast cancer was 
detected for either polymorphism. Another polymorphism 
(codon 264) also showed no association with breast cancer.220 
In conclusion, this gene might play a (minor) role in breast 
cancer susceptibility.

Oestrogen receptor (ER) gene

ER is a critical determinant of cellular responsiveness to oes-
trogen and is thought to play an important role in breast can-
cer promotion.107 Germline and somatic mutations in the ER 
gene in breast cancer cases are rare.232 Five somatic mutations 
in the ER gene have been found in only four out of 300 human 
breast tumours.232-234 Methylation of the promoter region of the 
ER gene was detected in 25% of ER negative breast cancers, 
while no methylation was found in ER positive tumours and 
normal breast specimens.235 Several polymorphisms in the ER
gene have been described. The PvuII polymorphism in intron 1 was examined in three studies,
235 236 237 one of which did not use controls. 238 The two other studies found no association with breast cancer, although the genotypes of cases (and not of controls) were only given in one. 237 When the three studies were combined, with control genotypes from one study, 238 no association was found. The total number of controls (n = 53), however, is very small. The XbaI polymorphism 237 showed a decreased breast cancer risk (OR = 0.50, 95% CI 0.25-0.99) for homozygous carriers of the variant allele (10.5 kb allele). In this study, both the number of cases (n = 191) and controls (n = 204) was small. A third polymorphism, in codon 325, is this study, both the number of cases (n = 191) and controls (n = 53), were only given in one. 237 No association with breast cancer was found when the three studies were combined.

Surprisingly, only five relatively small studies examined polymorphisms in the ER gene. Owing to the small sample sizes, an association with breast cancer risk can neither be confirmed nor excluded.

**Progesteron receptor (PR) gene**

Methylation of the CpG islands in the 5’ region of the PR gene was found in 40% of PR negative breast cancers cases (6/15) and not found in 15 PR positive tumours or normal breast stroma. A polymorphism in intron 7 of the PR gene has been described. The variant PROGINS allele consists of a 306 bp insertion of the Alu subfamily. 239 Four studies studied this polymorphism in relation to breast cancer risk. 239-242 Although the studies observed different ORs for heterozygous carriers, the ORs for women homozygous for the PROGINS allele were similar, ranging from 0.27-0.63 (fig 2). Pooled analysis of these studies showed that the OR for women homozygous for the variant allele was 0.32 (95% CI 0.16-0.65). Thus, instead of an increased risk, four studies separately and combined found a decreased breast cancer risk for homozygous carriers of the PROGINS allele.

**Androgen receptor (AR) gene**

A mutation in the AR gene was detected in three male breast cancer patients with (partial) androgen resistance, two brothers 243 and one sporadic patient. 244 An increased risk of breast cancer was found in women with BRCA1 mutations (165 women with breast cancer, 139 without) if they inherited at least one AR allele with >27 CAG repeats. 245 Two other studies in sporadic breast cancer patients found no association between the number of CAG repeats and breast cancer risk (in total, 876 cases and 810 controls). 246, 247 This polymorphism therefore does not appear to play a major role in breast cancer susceptibility.

**Catechol-O-methyltransferase (COMT)**

A polymorphism was identified in the COMT gene at codon 158 248; the normal allele was designated COMT-H and the variant allele COMT-L. The variant allele encodes a thermolabile form of the enzyme with reduced activity. Four studies examined this polymorphism. 249-251 No increased breast cancer risk (overall, for premenopausal or for postmenopausal women) was found when all studies were combined.

**Uridine diphospho-glucuronosyltransferase 1A1 (UGT1A1) gene**

A TA repeat polymorphism has been reported in the promoter region of the UGT1A1 gene. Increasing the number of repeats in this polymorphism leads to a decrease in enzyme activity. 252, 253 The wild type allele (UGT1A1*1) contains six TA repeats and the most common variant allele (UGT1A1*28) seven. Two other variant alleles (UGT1A1*33, five repeats and UGT1A1*34, eight repeats) have been found almost exclusively in the African-American population. 252-254 Among premenopausal women, an association was found in an African-American population. 255 An increased breast cancer risk (OR = 1.8, 95% CI 1.0-3.1) was detected for heterozygous and homozygous carriers of the variant alleles with a decreased enzyme activity (UGT1A1*28 and UGT1A1*34). In a white population in another study, no association was found. 256 No association between breast cancer risk and the UGT1A1*28 allele was detected when the studies were combined.

**HLA region**

The principal function of the highly polymorphic HLA antigens is to bind peptide fragments, so that they can be optimally presented to cytotoxic T lymphocytes and natural killer cells. 257 The HLA antigens play a major role in immunity, self-recognition, and cell and tissue differentiation. Several studies observed no association with breast cancer. 258-260 Other studies have indicated that different HLA antigens may either be risk factors for or protective against breast cancer. 261-263 No strong associations with specific alleles were found and (some of) the results were contradictory. In one study, a family was examined in which more than 40% of the members of two generations had cancer (mostly breast, endometrial, and gastrointestinal). 264 Positive lod scores to markers within or near the HLA region were found. None of the lod scores, however, reached significance. 265 In conclusion, several reports have indicated that different HLA alleles may be risk factors for or protective factors against cancer. No clear associations with specific alleles have been detected.

**Tumour necrosis factor α (TNFα) gene**

The TNFα gene is a central mediator in the inflammatory response and immunological activities towards tumour cells. 266, 267 One polymorphism in the TNFα gene occurs in a series of repeating conserved motifs and is not randomly distributed. It therefore most likely has some functional and selective effect. 268 The rare TNF2 allele of this polymorphism lies on the extended haplotype A1-B8-DR3-DQ2, 269 which is associated with autoimmunity and high TNFα production. 266, 270 A comparison of the data suggests that there may be a small effect of the ~308 polymorphism, with the TNF2 allele being associated with slightly higher levels of TNFα production. An association between TNF2 allele carriers (heterozygous and homozygous) and breast cancer risk was shown in one study which included 40 breast cancer patients and 106 controls (OR = 3.33, 95% CI 1.65-7.54). 270

**Heat shock protein 70 (HSP70) gene**

In the HLA region, three intronless genes encoding members of HSP70 are located centromerically to the TNF genes. The genes have been identified as HSP70-1, HSP70-2, and HSP70-3. 271 HSP70 is a determining factor in immunological mechanisms against tumour cells and HSPs can serve as a target for anti-tumour immune recognition by antibodies and T cells. 272, 273 Conversely, HSP70 expression on tumour cells is correlated with the inhibition of monocytotoxic activity, which can protect tumour cells against host immunological reactions. 274 Whether HSP70 acts as an anti-tumour immune response enhancer or as a tumour promoter may depend on HSP70 genotypes. 275 One study showed that the variant allele carriership of the HSP70-hom gene was associated with breast cancer (OR = 3.56, 95% CI 1.26-10.01), whereas carriership of the HSP70-2 gene was not (OR = 2.36, 95% CI 0.75-7.33). 275

**Iron metabolism**

Experimental, clinical, and epidemiological investigations have shown that iron can influence carcinogenesis. 276 Increased body iron stores have been associated with cancer risk. A number of genes are involved in iron metabolism, including the haemochromatosis gene (HFE) and the transferrin receptor (TFR) gene.
The HFE gene and hereditary haemochromatosis (HH)
So far, two point mutations (Cys282Tyr and His63Asp) have been detected in the HFE gene of HH patients. Over 80% of haemochromatosis patients are homozygous for the Cys282Tyr mutation.277 Heterozygous carriers, comprising 15% of the American population, have, on average, increased iron stores as compared to non-carriers.276–277 In a study of 1950 HH heterozygotes and 1656 controls, no increased breast cancer risk was detected (OR=0.98, 95% CI 0.81-1.19).277 In another study with 165 cases and 294 controls, no association was found between breast cancer and the HFE and TFR genotypes when the genotypes were tested both separately and together.278 In conclusion, the HFE and TFR genes do not play a major role in breast cancer susceptibility.

Other genes
Vitamin D receptor (VDR) gene
Five polymorphisms of the VDR gene have been studied in breast cancer patients and controls. Four of these, the TaqI, Apal, BsmI, and the poly-A polymorphism, are located in the 3’ region of the gene and are in linkage disequilibrium with each other. One polymorphism, FokI, is located in the 5’ region of the gene and is not in linkage disequilibrium with the other polymorphisms. The TaqI polymorphism, associated with increased serum vitamin D3 levels,279 was examined in three studies.276–278 No association with breast cancer was found in our pooled analysis (fig 3). The BsmI polymorphism was investigated in two studies and, when they were combined, again no association with breast cancer was found (fig 3).277–280 The two other polymorphisms in the 3’ region of the gene, the Apal and the poly-A polymorphism, were each addressed in one small study. For both polymorphisms, an increased breast cancer risk was found for carriers (homozygous and homozygous) of the variant allele (Apal, OR=1.56, 95% CI 1.09-2.24276; poly-A, OR=1.73, 95% CI 1.16-2.59278; fig 3). Pooled analysis of the two studies on the FokI polymorphism277–280 detected no association with breast cancer (fig 3). In one study, haplotypes of the Apal (variant allele a) and the TaqI (variant allele T) polymorphisms were tested. Women with the genotype aaTT (homozygous for the haplotype of both variant alleles) had an increased breast cancer risk (OR=2.5, 95% CI 1.02-6.35278) as compared to the genotype Aatt.276 The results of the different polymorphisms in this gene are contradictory, and it remains unclear whether the VDR gene plays a role in breast cancer susceptibility.

The APC gene
Breast tumours (n=227) were screened for truncating mutations in exon 15 of the APC gene (77% of the coding sequence) and only one somatic mutation was found.281 Somatic mutations in the APC gene were detected in 13 of 70 breast cancer cases in another study.282 Most of these mutations were outside the mutation cluster region that has been noted for colorectal cancer.281 One of the polymorphisms in the APC gene, the 11307K polymorphism, is specific to Ashkenazi Jews. In Ashkenazi Jewish women with breast cancer without a BRCA1 or BRCA2 mutation, no association between breast cancer and the 11307K polymorphism was detected.282–284 In another study, the presence of the 11307K allele among BRCA1/2 carriers was not associated with a further increase of cancer risk.282 This polymorphism probably does not play a role in breast cancer susceptibility.

Combinations of polymorphisms in different genes
One study examined the four polymorphisms in the CYP1A1 gene and the GSTM1 and GSTT1 polymorphisms.285 None of these polymorphisms, either separately or combined, was associated with increased breast cancer risks. Others analysed a combination of the m2 polymorphism in the CYP1A1 gene and the GSTM1 polymorphism and again no associations were found.286 One study287 examined the m1 polymorphisms in the CYP1A1 gene and the polymorphisms in the CYP17 and COMT genes. The presence of at least two putative high risk genotypes was associated with an increased risk of breast cancer (OR=3.47, 95% CI 1.21-9.99).

In conclusion, in a few studies with small sample sizes, combinations of polymorphisms were examined.

DISCUSSION
This review, which examined 34 polymorphisms in 18 different genes, described in more than one breast cancer study, whenever possible with pooled analysis, showed an association with breast cancer for 13 polymorphisms in 10 genes. Increased breast cancer risks were found for the polymorphisms in HRA51, GSTM1, CYP1B1 (codon 119), CYP2D6, CYP19, and VDR (Apal and poly-A), with PARs ranging from 1-41%. Interestingly, decreased breast cancer risks were found for women homozygous for the variant allele for the intron 3, exon 4, and intron 6 polymorphisms in the Tp53 gene, the XbaI polymorphism in the ER gene, and the PROGINS polymorphism in the PR gene. Women with these genotypes may represent a subpopulation where prevention strategies can be less intensive than in the general population. The pooled analysis was performed on large (>2000 cases) sample sizes for the HRA51, GSTM1, and CYP19 polymorphisms. There is, therefore, strong evidence for increased breast cancer risks associated with these three polymorphisms, although the increase in breast cancer risk for the GSTM1 polymorphism is very small. More research on these three genes will probably only narrow the confidence intervals and not change either the ORs or the allele frequencies. The sample sizes studied for other polymorphisms, such as Tp53 (intron 3, exon 4, and intron 6), GSTPI, CYP1B1 (codon 119), ER (XbaI), and VDR (Apal and poly-A), were quite small (<1000 cases). Because of this, the association with increased breast cancer risk is not confirmed. The sample sizes for the polymorphisms of CYP2D6 and PR were intermediate (between 1000 and 2000 cases). Our pooled analysis indicated that there is an association with breast cancer, although more research (larger sample size) could slightly change both the OR and the allele frequencies. The pooled analysis for 12 other polymorphisms in nine genes, namely L-myc, NAT1, NAT2, GSTT1, CYP1A1, CYP17, AR, COMT, and UGT1A1, showed no association with breast cancer. For NAT2, GSTT1, and CYP17, the polymorphisms with large sample sizes, an association with breast cancer can be excluded. For polymorphisms with small (L-myc, NAT1, CYP1A1 (m2, m3 and m4), AR, and UGT1A1) or intermediate (CYP1A1 (m1) and COMT) sample sizes, an association with breast cancer cannot be excluded.

Somewhat different rules are applicable for polymorphisms described in only one study. To conclude from a negative result that the original effect is likely to be an artefact, a sample size of roughly four times the initial study is needed when replicating these studies (see Appendix 1). Eight polymorphisms (TNF-α,287 HSP70-2,287 HSP70-hom,287 APOE,288 EDH17B2,289 HER2,289 TBR-1,289 and TFR289) are each described in only one study, all of which have (very) small sample sizes. A weak association with breast cancer was found for five of these (TNF-α, HSP70-2, HSP70-hom, EDH17B2, and HER2). Replication of this is needed either to confirm or reject the tentative findings.

Strikingly little research has been performed on combinations of polymorphisms which are addressed in only a few studies in breast cancer patients. An association with increased breast cancer risk was found for combinations of polymorphisms in the different GST genes, although the total number of cases (n=238) and controls (n=240) was small and the association was only marginally significant. No
Appendix 1 Sample size calculation

More than one study. The statistical power for actual investigations into disease genes can be tackled by postulating a simple biallelic genetic model and assuming that the disease gene itself (or a 100% linked and associated marker) is observed. Relative risks of the disease can then be specified for homozygotes for the wild type allele (aa), heterozygotes (Aa), and homozygotes for the variant allele (AA). This allows for variations in penetrance and dominant, additive, or recessive models. Other input parameters that the model needs are the disease frequency, the frequency of the variant allele, the required power, and the significance level. The basis of the calculation is the determination of the a posteriori genotype distribution of the frequencies of alleles transmitted from unaffected parents to their affected child. The genotypes constructed by the alleles that are not transmitted serve then as (pseudo) controls as in the transmission/disequilibrium test. Power calculations for the difference in the frequencies of binomial entities are performed using a normal approximation. Sample sizes required are then calculated for detecting differences between cases and controls in allele frequencies, in variant allele carriers and in homozygotes for the variant allele.

Although the assumption of 100% linked and associated markers is not entirely realistic, it does indicate an order of magnitude for the power of an actual investigation. This will probably be lower as a function of genetic distance and allelic frequency match between marker and disease alleles. The actual calculations are done on a Microsoft Excel® spreadsheet, available from the first author on request.

Only one study. When only one association study is available on a polymorphism, it is likely to be a positive one. It is therefore unlikely that a reliable disease model can be obtained from it. Because of this, the sample size cannot be computed with the formula presented in the previous section. A second study will need a sample size at least four times as large as the original one in order either to replicate or refute the association. The reason for the factor 4 is as follows. Assume that the power for the first study to find a significant difference between cases and controls is equal to 0.50. For example, for a disease model with a variant allele frequency of 0.20, a disease frequency of 10%, and genotypic relative risks for aA and AA both equal to 1.5, the power to detect a difference between cases and controls at a significance level of 5% is 0.502 in a study with 164 trios. To detect the same difference with a power of 0.95, 656 trios are required, which is exactly a factor of 4 larger. Even for a weak genetic disease model with a variant allele frequency of 0.50, a disease frequency of 10% and genotypic relative risks both equal to 1.1, this factor is 3.9996 (5001 trios in first study versus 20 002 in the replication study). In order to replicate the first study with a power of 90% (instead of 95%), the required increase in sample size is a factor of 3.165.

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1p32-p21.

11q22-q23.

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15q15-q21.

15q15-q22.

17q11-q12.

11q23.

12q13-q21.

1p36-p31.

12q21-q24.

1p21-p22.

11q23-q24.

13q32-q21.

17q21-q23.

17q21-q23.


17q21-q23.

17q21-q23.

17q21-q23.


17q21-q23.

17q21-q23.
Another risk factor for MI?

Platelet receptor gene Ibα-5T/C Kozak polymorphism may influence the risk of MI, concludes a preliminary UK study. In the prelude to MI platelets attach, through a glycoprotein complex GPIb/IX/V, to von Willebrand factor on plaques in the arterial walls. Four subunits (GPIbα, GPIbβ, GPIX, GPV) comprise the receptor complex, each specified by a single gene; the GPIbα subunit has the binding site for von Willebrand factor. Douglas et al speculated that the GPIbα-5T/C sequence Kozak polymorphism—affecting GPIb/IX/V expression—and GPIbα variable number tandem repeats (VNTRs)—affecting its structure and lengthening the receptor—might alter the risk of MI. GPIbα Kozak polymorphism is a thymine (T) or cytosine (C) at position −5 around the ATG initiator codon; GPIbα VNTR has four variants (A–D) in descending molecular size.

Douglas et al analysed how common these genotypes were in patients aged 33–80 years (180 white, 76 Indian Asian), recruited at cardiac catheterisation, for those with confirmed MI (one group) and without (another group) but otherwise matched for known risk factors. GPIbα Kozak homozygous TT genotype was strongly linked with MI (infarct group TT 85.2%, TC 12.5%, CC 2.3%; non-infarct group TT 67.3%, TC 32.7%, CC 32.7; p=0.001) as was TT versus TC and CC genotypes. GPIbα VNTR genotypes showed no link, except marginally, between CC and all other genotypes. Neither polymorphism was linked with severity of arterial disease.

TT Kozak genotype may be important in MI, the authors conclude, but confirmation awaits a larger prospective study.

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Genes other than BRCA1 and BRCA2 involved in breast cancer susceptibility

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