GSTM1, GSTT1, and GSTP1 Genotypes in Relation to Breast Cancer Risk and Frequency of Mutations in the p53 Gene

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Abstract

The glutathione S-transferase (GST) genes are involved in the metabolism of various carcinogens. Deletion polymorphisms in the GSTM1 and GSTT1 genes and an A-G polymorphism in the GSTP1 gene were investigated in relation to breast cancer risk in 500 breast cancer patients and 395 controls. The effects of the GST genotypes on the frequency and pattern of p53 mutations in 388 breast carcinomas were also studied. A suggestive trend of increasing risk of breast cancer with increasing number of G alleles of the GSTP1 was observed (P for trend, 0.11). The GSTM1 and GSTT1 polymorphisms did not show an association with breast cancer. No increase in risk was observed with a combination of genotypes. A statistically significant association was observed between the GSTT1 genotype and p53 mutation status of the tumors, with patients carrying the GSTT1 null genotype more frequently having mutations in the p53 gene compared with patients with a GSTT1 gene present (24.6% versus 12.4%; P = 0.019). There was also a suggestive trend for the GG genotype of the GSTP1 gene, but it was not statistically significant (P = 0.19). No association was observed with the type or location of mutations. We conclude that the GSTP1 and GSTT1 genes could play a role in carcinogenesis in the breast, possibly through increased frequency of mutations in tumor suppressor genes such as p53.

Introduction

Genetic factors participate to a varying degree in carcinogenesis. Germ-line mutations in the BRCA1 and BRCA2 genes confer a high risk to the individual, but they are rare in the population. One founder mutation in the BRCA2 gene has been detected in the Icelandic population. This is a 5-bp deletion in exon 9, 999del5 (1). The frequency of the 999del5 mutation is estimated to be 0.6% in the Icelandic population and accounts for 7–8% of breast cancer cases (2). The estimated risk of breast cancer for female carriers of this mutation is 37% by the age of 70 (3). At the other end of the spectrum are the genetic polymorphisms. It has been postulated that polymorphisms in enzymes involved in carcinogen metabolism increase the risk of cancer in some individuals. The risk to the individual carrying a variant in one of those genes is estimated to be low, but the high frequency in the population of some of the variants makes the potential population attributable risk high (4).

The GSTs (5) are a family of enzymes involved in detoxification of a wide range of chemicals, including possible carcinogens. In some of the GST genes there are known polymorphisms, which in some instances affect the activity of the enzyme product. The GSTM1 and GSTT1 genes both exhibit deletion polymorphisms. Homozygous deletions of those genes, called GSTM1 and GSTT1 null genotypes, result in a lack of enzyme activity (5, 6). An A-G polymorphism at nucleotide 313 in the GSTP1 gene results in an amino acid substitution (Ile105Val). This residue lies in the substrate binding site of the enzyme and the polymorphism has been shown to affect enzyme activity (7–10). A decrease in GST enzyme activity could result in inefficient detoxification of various carcinogens, which could lead to genetic damage and increased cancer risk.

It is not yet clear whether the GST polymorphisms affect breast cancer risk. Most of the studies published thus far have found no association, but most of them have also been based on relatively few cases, which could lead to false-negative findings. Dunning et al. (11) combined these results in a meta-analysis to obtain more precise risk estimates. They observed an association between the GSTM1 null genotype and postmenopausal breast cancer (OR = 1.33; 95% CI = 1.01–1.76). For the GSTP1 gene they observed an increased risk of breast cancer for Val (G) carriers (OR = 1.60; 95% CI = 1.08–2.39). No association was observed with the polymorphism in the GSTT1 gene. They concluded that the possible effects of these GST genotypes on breast cancer risk need to be studied further.

It has been postulated that a decrease in GST enzyme activity could result in higher frequency and a specific pattern of mutations in cancers. The most frequently mutated gene in human cancer is the p53 gene. It is well known that certain cancers have a specific mutation pattern associated with specific exposures, e.g., lung cancer and smoking (12). This is not so evident for p53 mutations in breast cancer, but the pattern has been shown to vary between different populations (13). This suggests that the risk factors are not the same from one country to another. One study has investigated possible associations between GST genotypes and the status of the p53 gene in breast tumors (14). They found that breast cancer patients carrying the G allele of the GSTP1 gene had mutations in the

1 The abbreviations used are: GST, glutathione S-transferase; OR, odds ratio; CI, confidence interval.
Materials and Methods

Study Population. The study population, both cases and controls, are of the same ethnic background. The breast cancer group consists of 500 Icelandic females, unselected with respect to family history, who were born in the years 1894 to 1964. They were diagnosed with breast cancer in the years 1989–1995, with a mean age at diagnosis of 58.5 years (58.5 ± 14; range, 28–94 years), which is similar to the mean age at diagnosis in Iceland (60.4 years). Samples were collected at the time of diagnosis. DNA was extracted from 455 blood samples and 45 tumor samples using a standard phenol/chloroform method.

A total of 388 breast tumor samples had previously been investigated for mutations in the *p53* gene. Of those, 185 overlap with the breast cancer group described above, and matching blood samples were available for 140. DNA was extracted from tumor samples for the rest of the cases.

The control group consists of 395 females without breast cancer, born in the years 1909 to 1966. Blood samples were collected during the same time period as the cases (1987–1996) and were selected as being disease-free by the end of the year 2000. Their mean age at time of sample collection was 49 years (49 ± 12; range, 25–82 years). DNA was extracted from blood, either using a standard phenol/chloroform method or from frozen lymphocytes using a direct proteinase K digestion.

All blood samples were from the Icelandic Cancer Society’s Biological Specimen Bank, Reykjavik, Iceland. Tumor samples were from the Department of Pathology, National University Hospital of Iceland, Reykjavik, Iceland. All samples were precoded and stripped of personal identifiers in accordance with the requirements of the Icelandic Data Commission.

Genotyping Analysis. All three *GSTs* were genotyped in the same tube, as described previously, with certain modifications (14). DNA (25 ng) was mixed with 5 pmol of each primer (high-pressure liquid chromatography-purified), 0.2 mM of each dNTP, 1.5 mM MgCl₂, and 0.36 units of Dynazyme polymerase from Finnzymes in a final volume of 15 µl. The PCR reaction has been described previously (14). The PCR product was digested with 2 units of BsmAI restriction enzyme (from New England Biolabs), as described by the manufacturer, in a final volume of 25 µl. The fragments were analyzed on a 4% agarose gel (NuSieve 3:1; FMC BioProducts) or on an 8% acrylamide, 5% bis, gel.

On more difficult samples, in which case DNA had been extracted from paraffin-embedded tissue or in some instances from frozen lymphocytes, another PCR method was used for the *GSTT1* gene. A 112-bp fragment of the *GSTT1* gene was amplified along with a 175-bp fragment from the *GSTM2* gene (same fragment as in the multiplex PCR). The sequence for a new set of primers was obtained from the laboratory of Dr. Douglas A. Bell, NIEHS, Research Triangle Park, NC (15).

DNA (0.5–1 µl) was mixed with 10 pmol of each primer, 0.2 mM of each dNTP, 1.5 mM MgCl₂, and 0.36 units of Dynazyme polymerase in a final volume of 15 µl. Initial denaturation was carried out at 95°C for 3 min and then 35 cycles of 94°C for 30 s, 59°C for 30 s, 72°C for 1 min and a final polymerization step of 72°C for 5 min. The PCR products were electrophoresed on a 3% agarose gel (NuSieve; 3:1).

*p53* mutation analysis of exons 5–8 was carried out by constant denaturant gel electrophoresis, and mutants were confirmed by sequencing. The conditions were as described previously (16–18).

Statistical Analysis. Genotype proportions were compared between groups using the χ² test, with Yates’ correction, or Fishers’ exact test. ORs and 95% CIs were calculated, and *Ps* <0.05 were considered significant. Tests for Hardy-Weinberg equilibrium were conducted by comparing observed and expected genotype frequencies using a χ² test. All analyses were performed using the statistical software package STATA 6.0 (STATA Corporation, College Station, TX).

Results

The frequency of the *GSTM1*, *GSTT1*, and *GSTP1* genotypes was compared between 500 breast cancer cases and 395 controls (Table 1). The *GG* genotype of the *GSTP1* gene was found to be more frequent in cases (14.6%) than in controls (11.6%), although the difference did not reach statistical significance (*P* = 0.15). There was also a suggestive trend of increasing risk with increasing number of *G* alleles (*P* for trend, 0.11). We did not observe an association between breast cancer and polymorphism in the *GSTM1* or the *GSTT1* gene. No significant departures from Hardy-Weinberg equilibrium were observed for *GSTP1* genotypes among controls (*P* = 0.95) or cases (*P* = 0.86).

A difference in genotype frequencies between controls and cases diagnosed before the age of 45 or after the age of 55 was observed (Tables 2 and 3), although the difference never reached statistical significance. The *GSTM1* null genotype was observed to be more frequent among controls (54.2%) than among cases diagnosed before the age of 45 (45.5%). The opposite was true for the *GSTT1* null genotype (20.5% versus 25.0%). No difference in genotype frequencies of *GSTM1* and *GSTT1* was observed between controls and cases diagnosed after the age of 55. The *GG* genotype of the *GSTP1* gene was more frequent among cases than controls, and the difference was more marked in the older age group of cases.

We investigated whether breast cancer risk was affected by *GST* genotype combinations (Table 4). The reference group

<p>| Table 1 Association between <em>GSTM1</em>, <em>GSTT1</em>, and <em>GSTP1</em> genotypes and breast cancer |
|---------------------------------|-----------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (n = 500)</th>
<th>Controls (n = 395)</th>
<th>OR (95% CI)</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>GSTM1</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>227 (45.4%)</td>
<td>181 (45.8%)</td>
<td>1.0 (reference)</td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>273 (54.6%)</td>
<td>214 (54.2%)</td>
<td>1.02 (0.78–1.33)</td>
<td>0.95</td>
</tr>
<tr>
<td><em>GSTT1</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>401 (80.2%)</td>
<td>314 (79.5%)</td>
<td>1.0 (reference)</td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>99 (19.8%)</td>
<td>81 (20.5%)</td>
<td>0.96 (0.69–1.33)</td>
<td>0.86</td>
</tr>
<tr>
<td><em>GSTP1</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>202 (40.4%)</td>
<td>177 (44.8%)</td>
<td>1.0 (reference)</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>225 (45.0%)</td>
<td>172 (43.6%)</td>
<td>1.15 (0.86–1.52)</td>
<td>0.38</td>
</tr>
<tr>
<td>GG</td>
<td>73 (14.6%)</td>
<td>46 (11.6%)</td>
<td>1.39 (0.91–2.12)</td>
<td>0.15</td>
</tr>
</tbody>
</table>
consisted of individuals with a putative low-risk genotype combination, i.e., GSTM1 present, GSTT1 present, and GSTP1 AA. We did not observe a trend of increasing breast cancer risk with increasing number of putative high-risk genotypes. An increase in risk seemed mainly associated with the G allele of the GSTP1 gene, which is in accordance with our findings in Table 1. The 388 breast cancer patients with a known p53 status in their tumors were genotyped for GSTM1, GSTT1, and GSTP1 (Table 5). The patients carrying the GSTT1 null genotype had more frequent mutations in the p53 gene compared with patients with a GSTT1 gene present (24.6% versus 12.4%; \( P = 0.019 \)). No association was observed with the GSTM1 genotype, but p53 mutations were more common in patients carrying the GG genotype of the GSTP1 gene compared with the AA genotype (20.4% versus 12.8%), although the difference was not statistically significant (\( P = 0.19 \)). No association was observed with the type or location of the mutations (results not shown). Four of 14 transversions and none of the six deletions in the p53 gene were in patients carrying the GSTT1 null genotype. Eight transversions and three deletions were in patients carrying the G allele of the GSTP1 gene.

### Discussion

In this study, an increased risk of breast cancer associated with the G allele of the GSTP1 gene was suggested, although not reaching statistical significance. The difference was more prominent among cases diagnosed after the age of 55 than before the age of 45. Our results support the findings of Helzlsoeur et al. (19) who found a trend of increasing risk with an increasing number of G alleles that was confined to postmenopausal women. In a meta-analysis published by Dunning et al. (11) they also found an increased risk for breast cancer in association with the G allele of the GSTP1 gene (OR = 1.6; 95% CI = 1.08–2.39), but this analysis was only based on the results of two studies with a total of 172 cases (19, 20). However, in two recent reports (21, 22) a trend of decreased risk associated with the G allele was suggested, and no risk was observed in another study by Curran et al. (23). The discrepancy in results across studies could be attributed in part to chance and because of small sample size in many of them (19, 20, 23). Our study had 90% power to detect a relative risk of 1.8 for the carriers of the GG genotype, but additional studies are needed that can detect a relative risk of 1.5 for more reliable results.

We did not observe an association between the GSTM1 null or the GSTT1 null genotypes and an increased risk for breast cancer despite having 90% power to detect a relative risk of 1.2 associated with the GSTM1 null genotype and of 1.5 associated with the GSTT1 null genotype. This supports the findings of Milikan et al. (22), Curran et al. (23), and Garcia-Closas et al. (24), but not that of other studies that have found an increased risk of breast cancer among postmenopausal women carrying the GSTM1 null genotype (19, 21, 25).

Increased breast cancer risk was not found to be associated with an increasing number of putative high-risk GST genotypes (Table 4). Our results support the findings of Milikan et al. (22) and Curran et al. (23) and also those of Garcia-Closas et al. (24), who looked at combinations of GSTM1 and GSTT1 genotypes. On the other hand, both Helzlsoeur et al. (19) and Mitruten et al. (21) have observed an increased risk of breast cancer associated with combined effects of GST genotypes, but the genotype combinations were not the same for both papers. We found a significantly higher frequency of mutations in the p53 gene in patients carrying the GSTT1 null genotype (24.6%) as compared with patients with a GSTT1 gene present (12.4%; \( P = 0.019 \)). These results indicate that the lack of the GSTT1 gene, possibly leading to inefficient detoxification of certain mutagens, could result in increased DNA damage and frequency of mutations and a greater risk for the individual of developing cancer. Our results differ from the results of the only published study on this matter by Nedelcheva Kristensen et al. (14), which was based on fewer tumor samples (131) than the present study (388). They did not find an association between p53 mutations and the GSTT1 null genotype; instead they observed a higher frequency of mutations in the p53 gene in patients carrying the GG genotype of the GSTP1 gene compared with carriers of the AA genotype. Our results do suggest a trend in that direction, but it is not statistically significant (\( P = 0.19 \)). Nedelcheva Kristensen et al. also found an increased frequency of transversions and deletions in relation to the G allele of the GSTP1 gene. We did not observe this in our study for any of the genotypes.

The discrepancies between p53 study results are difficult to explain. Possible explanations include different sample sizes, but also variations in the distribution of risk factors between countries, although this seems unlikely when comparing similar countries like Norway and Iceland. The p53 mutation spectra have, however, been found to be different between different populations, which could indicate that risk factors are not the same from one country to another (13). This could affect the importance of each GST gene product in detoxifying carcinogens and thus could have an effect on study results.

In summary, our results indicate that the G allele of the GSTP1 gene could be associated with increased risk of breast
cancer, although the mechanism behind that is still not clear. Polymorphisms in the GSTT1 gene and possibly the GSTP1 gene may have an effect on the frequency of mutations in the p53 gene, and thus breast cancer risk, through inefficient detoxification. More studies are needed to determine the significance of these findings. The possible effect of the GST polymorphisms on DNA damage and the frequency of mutations in cancer-related genes, such as p53, is of importance in relation to other factors, most notably, the possible modifying effects on the risk associated with germ-line mutations in the two BRCA genes.

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