Polymorphic Glutathione S-Transferases as Genetic Risk Factors for Senile Cortical Cataract in Estonians

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PURPOSE. To investigate the possible association between glutathione S-transferase GSTM1, GSTM3, GSTT1, and GSTP1 polymorphism and the occurrence of age-related cataracts in Estonian patients.

METHODS. Patients with cortical (155), nuclear (77), posterior subcapsular (120), mixed type (151) of senile cataract and control individuals (202) were genotyped for GSTM1 and GSTT1 by enzyme-linked immunosorbent assay and genotyped for GSTM3 and GSTP1 by polymerase chain reaction.

RESULTS. The frequency of the GSTM1-positive phenotype was significantly higher in the cortical cataract group (60.6%) than in the controls (45.0%) with odds ratio of 1.88 (95% CI, 1.23–2.94; P = 0.004). The cortical cataract risk associated with the GSTM1-positive phenotype was increased in carriers of the combined GSTM1-positive/GSTT1-positive phenotype (OR = 1.99; 95% CI, 1.30–3.11; P = 0.002) and the GSTM1-positive/GSTM3 AA genotype (OR = 2.28; 95% CI, 1.51–3.73; P < 0.001). The highest risk of cortical cataract was observed in patients having all three susceptible genotypes (OR = 2.56; 95% CI, 1.59–4.11; P < 0.001). Also, a significant interaction between the presence of the GSTP1*A allele and cortical cataract was found with prevalence of the GSTP1*A allele among the cortical cataract cases compared with the controls. Ninety-five percent of subjects with cortical cataract had the GSTP1 (AA, AB, or AC) genotype, whereas in controls 87% of persons had a genotype with GSTP1*A allele (OR = 3.1; 95% CI, 1.31–7.35; P = 0.007). In contrast to the GSTP1*A allele, the presence of the GSTP1*B allele in one or two copies leads to decreased cortical cataract risk (OR = 0.09 for GSTP1 BB genotype).

CONCLUSIONS. The GSTM1-positive phenotype as well as the presence of the GSTP1*A allele may be a genetic risk factor for development of cortical cataract.

Oxidation-reduction mechanisms have special importance in many tissues, including the lens. Oxidative damage can result in a number of molecular changes that contribute to the development of cataract.1–3 Crystallins and other proteins in lens fiber cells do not turn over and must serve the lens for the lifetime of the person. Thus, the lens and other proteins in lens fiber cells do not turn over and must have efficient reducing and detoxification systems. Enzymes catalyzing the conjugation of reduced glutathione (GSH) with a broad spectrum of electrophiles.5 This is important for detoxification of xenobiotics and endogenous toxic compounds and for protection of tissues from oxidative damage. The members of the GST family are placed into multigene classes of Alpha, Mu, Pi, Kappa, Theta, and Zeta according to the basis of sequence identity.6–8 Among these classes of GSTs, genetic polymorphism is described for the GSTM1, GSTM3, GSTT1, GSTP1, and GSTZ1 loci. There are three alleles at the GSTM1 locus: GSTM1*0, GSTM1*A, and GSTM1*B. GSTM1*0 is a deletion, and homozygotes express neither mRNA nor protein. Alleles GSTM1*A and GSTM1*B encode monomers that form homo- and heterodimeric enzymes: GSTM1a-1a, GSTM1b-1b, and GSTM1a-1b.6 As in GSTM1, the null allele is also described for the GSTT1 locus, and homozygotes for the GSTT1*0 allele express no enzyme.9 The human GSTM3 gene is polymorphic with two alleles: GSTM3*A and GSTM3*B. GSTM3*B has a 3-bp deletion in intron 6, which creates a recognition motif for the YY1 transcription factor.10 Recently, polymorphism in the glutathione S-transferase GSTP1 gene has been identified with six common phenotypes resulting from homo- and heterodimeric combinations of GSTP1*A, GSTP1*B, and GSTP1*C.11 The transitions changed codon 105 from ATC (Ile) in GSTP1*A to GTC (Val) in GSTP1*B and GSTP1*C and also codon 114 from GCG (Ala) to GTG (Val) in GSTP1*C. Both amino acid changes are in the electrophile-binding active site of the GST P1-1 enzyme, and GST P1-1 isoforms have shown to possess different enzymatic activities.12

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Several studies have revealed an association between specific alleles of GST genes and increased susceptibility to certain disorders. Although the role of GSTM1 and GSTT1 genes in modifying disease risk has been extensively investigated, the role of two other recently described polymorphic GSTs, GSTM3 and GSTP1, in determining genetic predisposition to diseases has been poorly studied. Recently, an association between homozygous deletion of GSTM1 and cataracts was found in a Japanese population, but no association was found in an Italian population. The role of GSTM3, GSTP1, and GSTT1 in cataract formation has not been investigated. In the present study we have examined the glutathione S-transferase GSTM1, GSTM3, GSTP1, and GSTT1 status in Estonian senile cataract patients to elucidate the putative connection between allelic variants of polymorphic GSTs and the incidence of cataract formation.

**Materials and Methods**

**Subjects**

Unrelated patients with senile cataract and control individuals of Estonian nationality (all the four grandparents were ethnically Estonians) were recruited from two ophthalmic clinics that provide outpatient care in the town of Tartu and the South-Estonian area. Cataract status was determined by the lens examination in transient and side illumination using a biomicroscope and ophtalmoscope, and opacities were classified into nuclear, cortical, posterior subcapsular, and mixed type. Any patients with secondary cataracts were excluded, for example, those due to trauma, diabetes, and other known causes. All persons were interviewed to obtain data on smoking habits, and participants were thereafter classified into nonsmokers and smokers. Both current and former smokers had smoked at least five cigarettes per day for at least 5 years. The case group consisted of 503 patients with senile cataract. We succeeded in identifying 155 patients with cortical, 77 with nuclear, 120 with posterior subcapsular, and 151 with mixed type of cataract. The mean age of the case group was 72.0 ± 8.7 years (range, 47–93 years); 349 (69.4%) were women and 127 (25.2%) were smokers, with average smoking duration 31.3 ± 14.0 years. The control group comprised 202 unrelated volunteers without cataract, glaucoma, or uveitis. The mean age of the control group was 65.7 ± 6.9 years (range, 43–90 years); 146 (72.3%) were women and 42 (20.8%) smokers, with average smoking duration 24.5 ± 11.1 years. EDTA-blood samples were collected from every individual participating in the study and stored at −20°C until used. The tenets of the Declaration of Helsinki were followed. Informed consent was obtained, and the protocols for human experimentation were approved by the Ethical Commission of the University of Tartu.

**Identification of GSTM1 and GSTT1 Phenotypes by ELISA**

Phenotyping of GSTM1 and GSTT1 enzymes using frozen and lyed whole blood was performed with the monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) as described earlier. Genotyping of GSTM3 locus was performed essentially as described by Inskip et al., except that electrophoresis was carried out under the denaturing conditions. Before being subjected to electrophoresis in 15% polyacrylamide gel (C = 4.8%) containing 6.4 M urea, the digested PCR products were incubated with equal volume of formamide at 56°C.

Two polymorphisms at the GSTP1 gene were genotyped by the RFLP analysis of PCR-amplified DNA. The fifth exon of the GSTP1 gene containing A-G polymorphism at codon 105 was amplified using primers P1E5A (5′-TGT GTG GCA GTC TCT CAT CCT T-3′) and P1E5B (5′-TAC TTG GCT GGT TGA TGT CCC A-3′). Using restriction endonuclease AluI (Fermentas, Vilnius, Lithuania), the 463-bp DNA sequence amplified from the GSTP1*B and GSTP1*C alleles was digested into 17-, 221-, and 225-bp fragments, whereas the PCR product derived from GSTP1*A gave only 17- and 446-bp segments. Primers complementary to intron five (P1E6A: 5′-TGG CAG CTG AAG TGG ACA GGA TT-3′ and intron six (P1E6B: 5′-ATG GCT CAC ACC TGT GTG CAT-3′) of the GSTP1 gene were used to amplify the DNA fragment containing the sixth exon of the gene. PCR products were digested with either CaeII or AcI (both from New England Biolabs GmbH, Schwalbach, Germany). Amplons from the GSTP1*A and GSTP1*B were cut into three fragments by CaeII (49, 110, and 173 bp) and two fragments by AcI (158 and 174 bp). The amplified DNA sequence from the sixth exon of the GSTP1*C was cut into 49- and 283-bp fragments by CaeII and left undigested by AcI.

**Statistical Analysis**

To compare the cataract cases with the controls, relative risks of the polymorphic GST pheno- and genotypes were evaluated by computing odds ratios (ORs), 95% confidence intervals (CIs), and CIs-based P values according to Mantel and Haenszel. The limit of statistical significance was set at P = 0.05. The etiologic fraction (EF), i.e., proportion of all cases that may be attributable to a certain genotype was calculated, as described previously, from ORs under the assumption that the genotype can be approximately treated like exposure to a risk factor.

**Results**

Distribution of the GSTM1, GSTT1, GSTM3, and GSTP1 pheno- and genotypes in patients with cataracts and the control individuals are shown in Tables 1 and 2. Significant association with polymorphic GSTs was found only in cortical cataract cases and in the whole group of cataract patients. Differences in frequency distribution of GSTs in patients with nuclear, posterior subcapsular, and mixed type of cataract compared with the control group were similar to those in the cortical cataract group, but were less expressed and did not meet the criterion of significance. The distribution of the GSTM1 A, GSTM1 B, and GSTM1 A/B phenotypes was also studied, but no effect of GSTM1*A and GSTM1*B alleles on any type of cataract was detected.

The strongest allelic association between GSTs and cataract was found in the subgroup of patients with cortical opacity, where gene frequencies in the GSTM1 and GSTP1 loci were significantly different than the control individuals. Proportion of the GSTM1-positive individuals in the cortical cataract group was significantly higher (60.6%) than in the controls (45.0%),
with odds ratio of 1.88 (95% CI, 1.23–2.88; P = 0.004) (Table 2). The frequency of two alleles of the three present in the GSTP1 locus was different in cortical cataract patients, with the prevalence of GSTP1*A allele (73.2% vs. 64.9%; P = 0.017) and decreased incidence of GSTP1*B allele (15.8% vs. 24.3%; P = 0.006) compared with the controls. Considering all the genotypes having either the GSTP1*A or GSTP1*B allele, the effect was the same (Table 2). The percentage of the individuals having the GSTP1 A genotype (AA, AB, or AC) was higher in the cases than in the controls (95.4% vs. 87.1%; OR = 3.1; 95% CI, 1.31–7.35; P = 0.007). At the same time the number of persons being either hetero- or homozygotes for the GSTP1*B allele was decreased among patients with cortical cataract (30.5% vs. 42.1%; OR = 0.6; 95% CI, 0.39–0.94; P = 0.026). Of the 155 patients with cortical cataract, 0.6% were identified as carriers of the GSTP1 BB genotype (Table 1), a portion of which was significantly lower than the 6.4% for the controls, with an OR of 0.09 (95% CI, 0.01–0.73; P = 0.006).

Considering the possible additive effect of different GST loci, the frequencies of combined pheno- and genotypes were also observed. The risk of cortical opacities associated with the GSTM1-positive phenotype increased in carriers of the combined GSTM1- and GSTT1-positive phenotypes (OR = 1.99; 95% CI, 1.30–3.11; P = 0.002) (Table 2). The risk was also higher in carriers of the combined GSTM1-positive and GSTM3 AA genotype (OR = 2.38; 95% CI, 1.51–3.73; P < 0.001). The highest risk of cortical cataract (OR = 2.56) was observed in patients having all three susceptible genotypes (GSTP1-positive/GSTT1-positive/GSTM3 AA) (Table 2). We also found a statistically significant interaction of the GSTP1 A genotype (AA, AB, or AC) with the GSTM1-positive phenotype and the GSTM3 A genotype (AA or AB) in the cortical cataract cases

### Table 1. GST Pheno- and Genotype Frequencies in Senile Cataract Patients and Control Individuals

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Cases (n = 155)</th>
<th>Control (n = 202)</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1 pos</td>
<td>94 (60.6)</td>
<td>91 (45.5)</td>
<td>1.88</td>
<td>1.23–2.94</td>
<td>0.004</td>
</tr>
<tr>
<td>GSTM1 pos/GSTT1 pos</td>
<td>82 (52.9)</td>
<td>73 (36.1)</td>
<td>1.99</td>
<td>1.30–5.11</td>
<td>0.002</td>
</tr>
<tr>
<td>GSTM1 pos/GSTM3 AA</td>
<td>68 (43.9)</td>
<td>50 (24.8)</td>
<td>2.38</td>
<td>1.51–5.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GSTM1 pos/GSTM3 AA (AA or AB)</td>
<td>60 (38.7)</td>
<td>40 (19.8)</td>
<td>2.56</td>
<td>1.59–4.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GSTP1*A</td>
<td>73 (47.4)</td>
<td>64 (31.7)</td>
<td>1.48</td>
<td>1.07–2.05</td>
<td>0.017</td>
</tr>
<tr>
<td>GSTP1 (AA, AB, or AC)</td>
<td>147 (95.4)</td>
<td>176 (87.1)</td>
<td>3.1</td>
<td>1.31–7.35</td>
<td>0.007</td>
</tr>
<tr>
<td>GSTP1 (AA, AB, or AC)/GSTM3 (AA or AB)</td>
<td>146 (94.2)</td>
<td>174 (86.1)</td>
<td>2.61</td>
<td>1.19–5.71</td>
<td>0.014</td>
</tr>
<tr>
<td>GSTP1 AA/GSTM1 pos</td>
<td>50 (32.3)</td>
<td>43 (21.3)</td>
<td>1.76</td>
<td>1.10–2.84</td>
<td>0.021</td>
</tr>
<tr>
<td>GSTP1 AA/GSTM3 AA</td>
<td>62 (40.0)</td>
<td>60 (29.7)</td>
<td>1.58</td>
<td>1.02–2.45</td>
<td>0.042</td>
</tr>
<tr>
<td>GSTP1*B</td>
<td>15.8</td>
<td>24.3</td>
<td>0.59</td>
<td>0.4–0.86</td>
<td>0.006</td>
</tr>
<tr>
<td>GSTP1 (AB, BB, or BC)</td>
<td>47 (30.5)</td>
<td>85 (42.1)</td>
<td>0.6</td>
<td>0.39–0.94</td>
<td>0.026</td>
</tr>
<tr>
<td>GSTP1 BB</td>
<td>1 (0.6)</td>
<td>13 (6.4)</td>
<td>0.09</td>
<td>0.01–0.73</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages. Significant differences from the control group: * OR = 1.88; 95% CI = 1.23–2.88; P = 0.004; † OR = 0.09; 95% CI = 0.01–0.73; P = 0.006; ‡ OR = 0.45; 95% CI = 0.21–0.96; P = 0.034.
Glutathione S-Transferases and Cataract

The results of the present study indicate that polymorphic GSTs may play an important role in cataractogenesis and are possibly associated with a certain type of cataract. GSTM1, GSTM3, GSTT1, and GSTP1 genes do not have a major effect on the occurrence of nuclear, capsular, or mixed type of cataract, but our data suggest that particular allelic variants of polymorphic GSTs are involved in modifying genetic susceptibility to cortical cataract. We found a twofold risk of cortical cataract associated with the GSTM1-positive phenotype. Another locus modifying genetic susceptibility to cortical cataract was GSTP1, where the carriers of the GSTP1*A allele conducted to a threefold higher risk of developing cortical cataract and GSTP1*B allele had an opposite effect: protective against the disease. Although there was only a weak and a statistically nonsignificant overrepresentation of the GSTT1-positive phenotype and the GSTM3 AA genotype in the cortical cataract group, the presence of these two GST variants in GSTM1-positive individuals increased additively the risk of cortical opacity development. Therefore, we can suppose that the presence of the GST T1-1 enzyme and GSTM3 AA genotype also makes individuals more susceptible to cortical cataract. The disease risk was highest in persons having all three predisposing genotypes: GSTM1-positive, GSTT1-positive, and GSTM3 AA.

Although all individuals in mixed cataract group had cortical opacities, no association of this subgroup with the GST variants was found. It can be explained in two ways: first, the processes leading to formation of cortical and mixed opacities are different and second, cortical opacities in mixed cataract group may be of "secondary" origin. Our results suggest that the GSTM1, GSTP1, and probably also the GSTT1 and GSTM3 loci are involved in modifying cataract risk. The hypothesis is supported by the distribution of GSTs in the lens. A study by Huang et al. has shown that class Mu and Pi isoenzymes are abundantly expressed in the human lens, with the Pi isoenzyme predominating. The presence of GST T1-1 and GST M3-3 enzymes in the human lens has not been studied, but class Theta enzymes are supposed to be present in the dog lens. The highest GST activity in the human lens occurs in the peripheral and equatorial cortex, whereas very low activity is present in the posterior cortex, and no measurable activity can be detected in the nucleus and lens epithelium. The described activity distribution in the human lens is in a good accordance with our results where association between GSTs and development of lens opacities was found only in cortical cataract cases. These results support the hypothesis that different mechanisms may be implicated during development of different types of cataract.

The exact molecular mechanisms, by which the expressing genotypes of GSTM1 and GSTT1 loci conduct to increased cortical cataract risk, remain to be elucidated in subsequent studies. Although GSTs are generally recognized as detoxifying enzymes, they may also be involved in generation and activation of toxic compounds. Possibly, during cataractogenesis the GST M1-1 and GST T1-1 enzymes take part in activation and formation of some toxic metabolites derived from nutrition, drug metabolism, or environmental pollution. The toxic metabolites formed can induce changes in the protein struc-
ture, thus favoring aggregation of lens proteins and promoting the development of cataract.\textsuperscript{1,3} The mechanism by which the GSTM3 AA genotype in GSTM1-positive individuals brings about increased cataract risk is supposedly connected with either linkage disequilibrium between the GSTM1 and GSTM3 loci\textsuperscript{10} or different biological effects of two GSTM3 alleles. Although polymorphism occurs in the sixth intron of the GSTM3 gene, a 3-bp deletion in the GSTM3*B allele creates a recognition motif for the YY1 transcription factor,\textsuperscript{52} and possibly the expression of the GSTM3*A and GSTM3*B alleles or even the GSTM1 gene is regulated differently.

The GST P1-1 isoenzyme is prevalent among all the GSTs expressed in the human lens.\textsuperscript{35} We found a significant over-representation of the GSTP1*A allele, as well as individuals having the GSTP1 A genotype (AA, AB, or AC) among cortical cataract patients compared with the controls. The frequency of GSTP1*A homozygotes was also higher in patients with cortical opacities (51\% vs. 42.6\%), but the difference did not reach the level of significance. In contrast to GSTP1*A allele, a considerable protective role of GSTP*B allele against cortical cataract was observed, having the highest effect in individuals being homozygous for the allele. A different effect of the GSTP1*A and GSTP1*B alleles on disease susceptibility may be caused by different catalytic properties of the corresponding isoenzymes. The previous studies have shown that the two different GSTP1 isoenzymes with isoleucine or valine at position 105 (corresponding to the GSTP1*A and GSTP1*B allele products, respectively), differ significantly from each other in respect of catalytic properties. The mechanism by which the GSTP1*A allele, on the one hand, and the GSTM1-positive, GSTT1-positive, and GSTM3 AA genotypes, on the other, conduct to increased risk of cortical cataract are probably different, because we have not found any additive effect of the GSTM1, GSTT1, and GSTM3 loci on genetic predisposition caused by GSTP1 A genotype.

The different influence of particular allelic forms of polymorphic GSTs may be caused by their different ability to resist the oxidative damage and function in the conditions of oxidative stress. As a matter of fact, the GST activity is significantly decreased in cataractous lenses compared with normal clear lenses,\textsuperscript{34} and a large interindividual variation in oxidative stress. As a matter of fact, the GST activity is significantly decreased in cataractous lenses compared with normal clear lenses,\textsuperscript{34} and a large interindividual variation in oxidative stress.

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4. Huang QL, Lou MF, Straatsma BR, et al. Distribution and activity of glutathione S-transferase in normal human lenses and in catarac-

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versus ~50\% in whites.\textsuperscript{38,39} At the same time, the risk of developing cortical cataract is also higher in black populations than in Caucasians.\textsuperscript{40} To establish the role of GSTM1 and possibly other GSTs in the distribution of cataract in different populations further case-control studies are required.

Recently, an association between homozygous deletion of GSTM1 and cataracts was found in a Japanese population, but no association was found in an Italian population.\textsuperscript{18,19} It is not clear why our results differ from these two studies. The apparent discrepancy could be explained by the possibility that the contrasting results reflect differences in genetic (including GST polymorphism), nutritional, and environmental backgrounds of the three populations studied.

The relationship between polymorphic GSTs with other genetic and cataractogenic environmental factors is highly complicated. A number of studies, including the present one, suggest that evaluating the role of a particular GST gene in any disease susceptibility, the whole pattern of different biotransformation enzymes should be taken into account as much as possible, because multiple detoxification enzymes may be involved in the metabolism of a given compound,\textsuperscript{22} and forming metabolites may be affected differently. Extensive research is required to ascertain how exactly the GST genotype affects the individual susceptibility to cataract and which detoxifying en-
zymes and environmental factors are responsible for cataractogenesis.

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