Clinical Study

Association of GST Genes Polymorphisms with Asthma in Tunisian Children

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Background. A positive association between genetic polymorphism and asthma may not be extrapolated from one ethnic group to another based on intra- and interethic allelic and genotype frequencies differences. Objective. We assessed whether polymorphisms of GST genes (GSTM1, GSTTI, and GSTP1) are associated with asthma and atopy among Tunisian children. Methods. 112 unrelated healthy individuals and 105 asthmatic (73 atopic and 32 nonatopic) children were studied. Genotyping the polymorphisms in the GSTTI and GSTM1 genes was performed using the multiplex PCR. The GSTP1 Ile105Val polymorphism was determined using PCR-RFLP. Results. GSTM1 null genotype was significantly associated with the increased risk of asthma (P = .002). Asthmatic children had a higher prevalence of the GSTP1Ile105 allele than the control group (43.8% and 33.5%, respectively; P = .002). Also, the presence of the GSTP1 homozygote Val/Val was less common in subjects with asthma than in control group. We have found that GSTTI null genotype (GSTTI∗0/*0) was significantly associated with atopy (P = .008). Conclusion. Polymorphisms within genes of the GST superfamily were associated with risk of asthma and atopy in Tunisia.

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1. INTRODUCTION

Asthma is a chronic disease characterized by reversible airflow obstruction and airway inflammation that affect many people. There is evidence that a genetic predisposition may also alter the capability of the airway to protect itself against inhaled toxic substances from the environment [1, 2].

Several candidate genes were implicated in the development of atopy and asthma. It has been reported that prevalence of these candidate genes can vary considerably by ethnicity [3, 4]. Furthermore, data from given studies suggest that a positive association between genetic polymorphism and asthma or atopy may not be extrapolated from one ethnic group to another based on intra- and interethic allelic and genotype frequencies difference.

In North Africa, and especially in Tunisia, research data on this subject is absent. That is why we selected among asthmatic candidate genes three genes that have been known to manifest remarkable inter- and intraethnic differences [5]. These genes are GSTTI, GSTM1, and GSTP1, which are the code names for enzymes belonging to the glutathione S-transferase (GST) super family. In humans, GSTs represent a large and diverse super family of enzymes, with at least 13 GST enzymes belonging to five different families: mu, theta, alpha, pi, and gamma. The GSTM1, GSTTI, and GSTP1 belong, respectively, to the GSTMu, GSTtheta, and GSTpi categories of enzymes. GSTs are known to play an important role in the functioning of antioxidant defences through reactive oxygen species (ROS) metabolism, in the repairing of damaged ROS and in the detoxification of several xenobiotics such as carcinogens found in tobacco smoking [6, 7]. The role played by GSTs may be especially important in response to oxidative stress [6, 8]. Common homozygote deletion polymorphisms of the GSTM1 and GSTTI genes, as well as the GSTP1Ile105 polymorphism, have been known to abolish enzymes activity and increase susceptibility to oxidative stress [6, 8]. Studies have so far reported contradictory results regarding any association between GST gene polymorphisms and asthma and/or atopy. In fact, some have reported the presence of an association between GSTP1VAL105Ie polymorphisms and bronchial hyper responsiveness (BHR), asthma and atopy [9–16]. However, other studies have found no such evidence of any association between polymorphism and asthma or atopy [17]. In several studies, null alleles in the GSTTI and GSTM1 genes (GSTTI∗0 and GSTM1∗0) were associated with childhood asthma [10, 11, 18, 19] but this finding was contradicted in other studies [17].
2 Mediators of Inflammation

Although there is a high incidence of asthmatic diseases in Tunisia, no data has been reported in this country. In this study we assess whether polymorphisms of GST genes previously found to be associated with asthma and atopy in Caucasian and Asiatic subjects are also to be associated with asthma and atopy in Tunisian children.

2. SUBJECTS AND METHODS

2.1. Study subjects

A total of 105 asthmatic children ranging from ages of 5 to 16 years (mean 11.5) were enrolled in this study along with 112 control individuals (aged 5 to 16 years, mean 9.5). None had any recent illnesses requiring treatment and no history of chronic diseases. All lived in the countryside near Tunis, in a town called Ariana. This region is generally considered to be representative of the general Tunisian population. Our national ethics committee approved the study.

2.2. Total IgE and Prick test assays

Atopy was defined by the skin sensitivity to specific allergens (skin reaction with a mean weal diameter \( \geq 3 \) mm larger than 3 mm) to be representative of the general Tunisian population. Our national ethics committee approved the study.

2.3. DNA isolation

The genomic DNA for genotyping was isolated from 10 ml peripheral blood lymphocytes which were collected, using a salting-out DNA extraction procedure [19], in an EDTA-containing vacutainer.

2.4. GSTM1 and GSTT1 genotypes

The GSTM1 and GSTT1 null genotypes were detected using a multiplex PCR method [20]. Briefly, 100 ng of DNA were amplified in a 50 ll multiplex reaction mixture containing 0.90 pmol of each of the following GSTM1 primers (GSTM1-F: TCACGGGATCATGGCCAGCA and GSTM1-R: TCACCGGATCATGGCCAGCA) and GSTT1 primers (GSTT1-F: GAACCTCCTGAAAAAGCTAAAGC and GSTT1-R: GTTGGGCTAAATATACGGTG). As an internal control, the ALBUMIN gene was also amplified with 0.2 pmol of each primer (AlbF: GCCCTCTGCTAACAAGTCTTAC and AlbR: GCCCTAAAAAGAAA-

Table 1: The clinical characteristics.

<table>
<thead>
<tr>
<th>Phenotypes associated</th>
<th>Cases n (%)</th>
<th>Controls n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhinitis</td>
<td>15 (14.28)</td>
<td>0</td>
</tr>
<tr>
<td>Sinusitis</td>
<td>7 (6.66)</td>
<td>0</td>
</tr>
<tr>
<td>Dermatitis</td>
<td>5 (4.76)</td>
<td>0</td>
</tr>
<tr>
<td>RGO*</td>
<td>8 (7.61)</td>
<td>0</td>
</tr>
<tr>
<td>Asthma, dermatitis, and rhinitis</td>
<td>7 (6.66)</td>
<td>0</td>
</tr>
<tr>
<td>Asthma, sinusitis, and rhinitis</td>
<td>5 (4.76)</td>
<td>0</td>
</tr>
</tbody>
</table>

* RGO: gastro-esophageal reflux.
** FEVI: forced expiratory volume in 1 second.
*** FVC: forced vital capacity; ND, not determined.
Table 2: Association of genotype profile between asthmatics and controls. NS: $P$ value > .05.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosomal location</th>
<th>Polymorphisms</th>
<th>Genotypes</th>
<th>(n = 105) (%)</th>
<th>(n = 112) (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1</td>
<td>1p13</td>
<td>Null allele</td>
<td>Null</td>
<td>79</td>
<td>70.7</td>
<td>53</td>
<td>50.2</td>
<td>2.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>WT</td>
<td>33</td>
<td>29.3</td>
<td>52</td>
<td>49.8</td>
<td></td>
</tr>
<tr>
<td>GSTT1</td>
<td>22q11</td>
<td>Null allele</td>
<td>Null</td>
<td>42</td>
<td>37.5</td>
<td>31</td>
<td>29.5</td>
<td>1.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>WT</td>
<td>70</td>
<td>62.5</td>
<td>74</td>
<td>70.5</td>
<td></td>
</tr>
<tr>
<td>GSTP1</td>
<td>11q13</td>
<td>Ile105Val</td>
<td>Ile/Ile</td>
<td>49</td>
<td>43.8</td>
<td>35</td>
<td>34.4</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ile/Val</td>
<td>51</td>
<td>45.5</td>
<td>48</td>
<td>47.1</td>
<td>1.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Val/Val</td>
<td>12</td>
<td>10.7</td>
<td>20</td>
<td>18.6</td>
<td>2.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A(Ile)</td>
<td>149</td>
<td>66.5</td>
<td>118</td>
<td>56.2</td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G(Val)</td>
<td>75</td>
<td>33.5</td>
<td>92</td>
<td>43.8</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Association between genotype profile and atopic asthma. NS: $P$ value > .05.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosomal location</th>
<th>Polymorphisms</th>
<th>Genotypes</th>
<th>(n = 73) %</th>
<th>(n = 32) %</th>
<th>OR</th>
<th>95% CI</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1</td>
<td>1p13</td>
<td>Null allele</td>
<td>Null</td>
<td>36</td>
<td>49.3</td>
<td>19</td>
<td>59.4</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>WT</td>
<td>37</td>
<td>50.7</td>
<td>25</td>
<td>78.1</td>
<td>3.74</td>
</tr>
<tr>
<td>GSTT1</td>
<td>22q11</td>
<td>Null allele</td>
<td>Null</td>
<td>36</td>
<td>49.31</td>
<td>7</td>
<td>21.8</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>WT</td>
<td>37</td>
<td>50.69</td>
<td>25</td>
<td>78.12</td>
<td>3.74</td>
</tr>
<tr>
<td>GSTP1</td>
<td>11q13</td>
<td>Ile105Val</td>
<td>Ile/Ile</td>
<td>31</td>
<td>42.5</td>
<td>13</td>
<td>43.0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ile/Val</td>
<td>34</td>
<td>46.50</td>
<td>12</td>
<td>40.0</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Val/Val</td>
<td>08</td>
<td>11.00</td>
<td>07</td>
<td>23.3</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A(Ile)</td>
<td>95</td>
<td>65</td>
<td>38</td>
<td>59.4</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G(Val)</td>
<td>51</td>
<td>35</td>
<td>26</td>
<td>40.6</td>
<td>1.27</td>
</tr>
</tbody>
</table>

3. STATISTICAL ANALYSIS

Association analysis in our case-control study was performed using standard Chi-squared test (Epistat statistical package, Epi Info Version 6) to detect differences in genotypes and alleles distribution among our groups.

Correction for multiple comparisons was performed, and only the value of corrected $P < .05$ was considered to be significant.

4. RESULTS

4.1. Case-control analysis

The association between GST genotype and susceptibility was studied in 105 unrelated asthmatic children residing in the northern part of Tunisia, using a control group of 112 healthy children.

Table 1 summarizes the clinical characteristics of subjects conducted in this study. In total, 35% of asthmatic children and 22% of nonasthmatic children were passive smokers. With an average age of 11.5 (ranging between 5 and 16 years), 70.32% of the asthmatic children were diagnosed as atopic.

Table 2 summarizes the data found regarding the genotype frequencies for the RFLP in the GSTP1 gene, as well as the homozygous deletions of the GSTM1 and GSTT1 genes. Genotype frequencies (GSTP1, GSTM1, and GSTT1) were within the Hardy-Weinberg equilibrium for control population.

We found that GSTM1 null genotype was significantly associated with increased risk of asthma ($P = .002$). Indeed, the GSTM1 null genotype was present among 70.7% of the asthmatic children and among 50.2% of the control group.

As for the GSTP1, the homozygote GSTP1 Val/Val genotype was less common among the asthmatic patients than in the control group (10.7% versus 18.6%, $P = .04$). Subjects with the GSTP1Val/GSTP1Val genotype registered a 2.33 fold lower risk of asthma than those with the GSTP1Ile/Ile genotype (OR = 2.33, 95% CL 0.94–5.87). Between both study samples, there was a significant difference in the frequency of the GSTP1 alleles ($P = .02$): asthmatic children had a higher prevalence of the GSTP1Ile allele than those in the control group (43.8% and 33.5%, resp.).

The presence of the GSTT1 null polymorphism was compared in both sample groups. The difference showed to be nonsignificant ($P > .05$) between controls and asthmatics, 29.5% and 37.5%, respectively, see (Table 2).

4.2. GST genes and atopy

Table 3 summarizes the association between GST genes and atopy.
The GSTTI null genotype (GSTTI*0/*0) was significantly higher in atopic asthmatic cases than in nonatopic asthmatic subjects \((P = .008)\). As for the GSTMI, there was a 1.5-fold increased risk of atopic asthma in individuals with the GSTMI null genotype \((OR = 1.5; 95\% CI, 0.6–3.83)\), but this increase was not significant. No significant associations have been found between atopy and GSTPI polymorphism in present study.

4.3. Polymorphisms of glutathione S-transferase M1, T1, and P1 in a Tunisian control population

Human cytosolic GSTs have been well documented; they are polymorphic and have ethnic-dependent polymorphism frequencies. Compared with research carried out in other countries, the distribution of the GSTMI null genotype and the GSTTI null among our group of control was found to be, respectively, 50.2% and 29.5% (Table 4). Their GSTPI polymorphism frequencies for the Ile/Ile genotype registered at 34.4%, at 47.1% for the Ile/Val genotype, and at 18.6% for the Val/Val genotype (Table 2). The Ile allele frequency for this particular group was set at 0.562.

5. DISCUSSION

The glutathione S-transferase (GST) super family of enzymes has a vital role in phase II of biotransformation of xenobiotics and in protection of cells from reactive oxygen species (ROS) by its ability to utilize substrates of a wide range of products of oxidative stress [6]. Oxidative stress was reported to be the key component of inflammation. Inflammation was considered a characteristic of asthma disease when it attacked airways. So defect in detoxifying ROS may influence the development and severity of asthma.

The results of our works suggest the presence of associations of GSTMI, T1, and P1 with childhood asthma and atopy. In comparing asthmatic children to healthy controls we have demonstrated a significant association between subjects lacking GSTMI activity and asthma \((P = .002)\). Numerous studies have demonstrated a significant association between subjects lacking GSTMI activity and the risk of developing a form of lung disease [30–32]. For asthmatics, the association with GSTMI null genotype has been reported in Caucasian population [11, 18, 33, 34] but not in Asiatic groups [35].

As for the GSTPI, we have also found significant differences between our two study samples regarding the genotype frequencies of the GSTPIIle105Val polymorphisms. Indeed, asthmatic children have low frequency of GSTPIVal allele compared with healthy children \((P = .002)\). The defensive role of the GSTPI in cases of asthma was reported in several studies [9, 12–15]. It was reported that the presence of GSTPI Val/Val genotype conferred a sixfold lower risk of asthma than did GSTPI Ile/Ile and that the frequency of GSTPI Val/Val genotype correlated negatively with severity of airway dysfunction [9]. Aynacioglu et al. [12] have also reported that the frequency of GSTPI Val homozygote was significantly lower in the group of patients with asthma than in the control individuals \((3.8\% ~versus ~12.1\%, ~P = .01)\). On the other hand, a recent study [11] has found that the GSTPI Val/Val was more prevalent among asthmatic subjects than the control group \((22.8\% ~and ~7.8\%, ~respectively)\) and that subjects with the GSTPI homozygous Val/Val genotype had a 3.55-fold increased risk of having atopic asthma compared to nonatopic asthma \((OR = 3.55; 95\% CI, 1.10–12.56)\), see [11]. Although, the GSTPI-derived enzyme contributes more than 90% of GST activity [36], it has been found by many studies [9, 12–15] and confirmed by our finding that it protects children against developing asthma disease. Nevertheless, it has been reported that the Val105 variant has higher catalytic efficiency for polycyclic aromatic hydrocarbon diol epoxides but its efficiency for 1-chloro-2,4-dinitrobenzene is lower compared to the Ile105 variant [37]. Therefore, it seems to be possible that GSTPI plays a role in asthma disease by modulation of ROS production.

In this study, for GSTTI null genotype, significant association was found between atopic asthmatic children and nonatopic asthmatic children \((P = .008)\), see (Table 2). While our findings are substantiated by several studies on Caucasians populations [11, 33], other studies were unable to establish this association [17, 18, 34, 38]. Several studies have suggested that individuals with the GSTTI*0/*0 (GSTTI null) genotype may be more susceptible to genotoxic damage and lung diseases than individuals with the GSTTI gene [30, 39].

Contradictory results were found in regards to GSTTI, GSTPI, and GSTMI [11, 17, 34, 35, 39]; ethnicity is the most important reason for these differences. That is why we have taken into consideration inter- and intrerethenic characteristics in analyzing our findings.

In control populations with intra- and interethnic differences, frequent genetic deletion polymorphisms of GSTMI \((GSTMI*0/*0)\) and GSTTI \((GSTTI*0/*0)\) have been reported [5]. The distribution of the GSTMI null genotype and GSTTI null of our healthy control sample was found to be 50.2% and 29.5%, respectively, see (Table 2).
The frequency of \textit{GSTM1} null genotype in our healthy controls (50.2%) seems to be within the frequency range reported for the Caucasian populations (Table 4). In fact, the \textit{GSTM1} deletion frequencies range, respectively, from 50.4% to 58.00%, 49% to 63%, and 20% to 33% in Caucasian, Asiatic, and African control groups [5].

Regarding \textit{GSTT1} null type, the frequency of deletion genotype in our Tunisian control group is set at 29.5%. Like the Egyptian population, Tunisia has a slightly higher frequency than that registered by Caucasian Europeans (between 13% and 22.3%) and Africans (between 19% and 26%). The frequency of the deletion genotype in the Tunisian population is closer to that of the Caucasian Americans (10%–36%) and is considerably lower than that reported for the Asiatic populations (45% to 53%).

In conclusion, we have demonstrated that polymorphisms of \textit{GST} genes previously found to be associated with asthma and atopy in Caucasian and Asiatic subjects are also associated with asthma and atopy in Tunisian children. Therefore, \textit{GST} genotypes may be useful in order to optimize future treatment of asthma in the cases of patients with a risk profile.

\section*{ABBREVIATIONS}

\begin{itemize}
  \item \textbf{GST:} Glutathione-S-transferase
  \item \textbf{OR:} Odds ratio
  \item \textbf{ROS:} Reactive oxygen species
  \item \textbf{PCR-RFLP:} Restriction length polymorphism
  \item \textbf{PCR:} Polymerase chain reaction
\end{itemize}

\section*{ACKNOWLEDGMENTS}

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\section*{REFERENCES}


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