GSTO1 uncommon genetic variants are associated with recurrent miscarriage risk

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Objective: To explore the role of the GSTO1 gene in the pathogenesis of recurrent miscarriage (RM).

Design: Genetic association study.

Setting: Rome, Italy.

Patient(s): 123 women with RM and 130 women without pregnancy complications.

Intervention(s): None.

Main Outcome Measure(s): Genotyping of two single nucleotide polymorphisms (A140D and E208K) and a 3-bp deletion (E155del) of the GSTO1 gene.

Result(s): We found a statistically significant association between GSTO1*E208K variants and RM risk. Specifically, we identified this uncommon genetic variant only in women with RM. None of the women with physiological pregnancies were carriers of K208 allele.

Conclusion(s): GSTO1 has a role in detoxification metabolism, and we hypothesize that a functional variation of GSTO1 is a RM risk factor that interacts with environmental conditions.


Key Words: Arsenic, ascorbate, genetic predisposition, GSTO1, pregnancy loss, phase II enzymes

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Recurrent miscarriage (RM) is a pathologic condition that occurs in 3% to 5% of couples trying to conceive [1]. Different abnormal states cause RM (e.g., chromosomal abnormalities, thrombophilia, metabolic disorders, anatomic anomaly, and immune factors) [2, 3], but half of RM cases cannot currently be explained clinically [4]. Various studies have indicated that genetic polymorphism may be a susceptibility factor for RM with unknown etiology [5–7]. Most of the genetic studies about RM have been based on a gene-candidate approach, through which researchers have analyzed selected variants of genes potentially involved in pregnancy [4].

Cellular detoxification is a relevant aspect of pregnancy, as pregnant women are exposed to a wide variety of chemical compounds as result of maternal medications, lifestyle habits, and occupational or environmental sources [8]. In particular, the placenta plays a key role in the regulation of maternal-to-fetal exchanges of endogenous and xenobiotic molecules [9]. Various studies have highlighted that a complex architecture is present in the placenta’s detoxification systems, where several pregnancy-induced modifications are present in the expression and activity of detoxification enzymes [10, 11]. Genetic polymorphism contributes to the inter-individual variability of detoxification systems: variants in genes encoding for metabolizing enzymes are associated with functional differences in protein expression and activity [12–14]. Moreover, studies have indicated that the genetic polymorphisms of detoxification enzymes are susceptible to multifactorial diseases [15–17].

Regarding RM, several genetic association studies have hypothesized that an important relationship exists

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between disease risk and detoxification genes (18–24). Most of these studies have focused on genes encoding the glutathione S-transferase (GST) enzymes because of their role in cellular detoxification. The GTS enzymes are the main phase II superfamily, and their relevant action is making toxic compounds less dangerous and enabling their excretion (25). Although previous studies have investigated different GST genes, to the best of our knowledge, none have analyzed the role of GSTO1 in RM risk. The GSTO1-1 enzyme presents a different protein structure than the other GST enzymes and catalyzes thiol transferase and reduction reactions not catalyzed by other GSTs (26). Previous genetic association studies revealed that the GSTO1 genetic polymorphism may be associated with disease risk and with an increased susceptibility to toxic compounds such as arsenic (27, 28). Furthermore, a previous immunohistochemistry study found GSTO1-1 in Hofbauer cells of the placenta (29).

Considering the role GSTs play in RM pathogenesis and the potential connection between GSTO1 and disease risk, we analyzed the GSTO1 gene. Specifically, we analyzed three coding variants in a case-control population, considering two nonsynonymous substitutions (A140D, rs4925; E208K, rs11509438) and a 3-base pair (bp) deletion (E155del, rs11509437).

MATERIALS AND METHODS

Study Population

We recruited 123 patients with a history of RM and 130 control women at “San Giovanni Calibita” Fatebenefratelli Hospital. All participants are residents in the Rome area (Latinum in central Italy) and have Italian ancestry. We defined RM as having a history of two or more spontaneous consecutive miscarriages, according to the criteria of the American College of Obstetricians and Gynecologists (30). In accord with this definition, the prevalence of RM is about 5% (1). All our RM patients can be classified as “primary aborters,” and they underwent an extensive clinical investigation to exclude the known RM etiologic factors. The control group consisted of 130 volunteers who had had at least two previous pregnancies and no miscarriage or other pregnancy complications. Further details about inclusion and exclusion criteria were reported in our previous study (23). The study design was approved by the appropriate ethics committee, and all participants gave written, informed consent.

Genotyping Procedures

For each participant, a sample of buccal cells was collected with an oral swab. We obtained DNA from buccal cells using the phenol/chloroform/isoamyl alcohol method. The GSTO1*A140D (rs4925), GSTO1*E155del (rs11509437), and GSTO1*E208K (rs11509438) genotypes were detected as described elsewhere (31). To ensure the reliability of the results, approximately 15% of the samples were randomly selected and analyzed independently: in all cases, the outcome was concordant. Moreover, the laboratory was not informed of the patients’ diagnoses until the final analysis: patients and controls were identified by progressive numbers.

Among the study population, 245 (97%) of 253 of the samples were analyzed for GSTO1*A140D, 249 (98%) of 253 for GSTO1*E155del, and 243 (96%) of 253 for GSTO1*E208K. It was not possible to assess the genotype of some individuals during the analyses (insufficient DNA/blood sample, genetic analysis failure).

Statistical Analysis

To compare the demographic and clinical characteristics of the patient and control samples, we used Student’s t-test and the chi-square test. The estimation of power to detect an association between GSTO1 variants and RM was evaluated using the Power of Genetic Analysis package (32). The minimum detectable effect with odds ratio (OR) was calculated in a general model, based on an alpha of 0.05 and an RM prevalence of 5% in the general population. With this sample size, we had an 80% power to detect an OR of 1.65 for a 30% risk allele frequency and 2.47 for a 5% risk allele frequency. Allele, genotype, and haplotype frequencies and the Hardy-Weinberg equilibrium were evaluated using SNPsStats (33).

Coefficients of pairwise linkage disequilibrium (LD) among GSTO1 polymorphisms were calculated and graphically displayed using Haploview version 4.2 (34). To account for multiple testing, we used the Single Nucleotide Polymorphism Spectral Decomposition (SNPSpD) program to correct the significance threshold, taking into account the LD between single-nucleotide polymorphisms (SNPs) (35). The experimentwise significance thresholds required to keep the type I error rate at 5% was 0.018. The OR and 95% confidence interval (CI) were calculated to evaluate the association between GSTO1 variants and RM, considering a dominant (one copy of the allele is sufficient to increase the disease risk) and a recessive (two copies of the allele are necessary to increase the disease risk) genetic model. To calculate the P values of genetic associations, we used a logistic regression. The SNP-based and haplotype-based genetic association analyses were performed in SNPSstats. Details about the statistical procedures are available in the article by Sore et al. (33).

RESULTS

Table 1 reported the clinical and demographic characteristics of the study population. No differences were observed for age and smoking habits (P > .05). Table 2 showed the genotype frequencies of GSTO1 gene variants. GSTO1 polymorphisms were in the Hardy-Weinberg equilibrium both in case and control groups (P > .05). Moreover, the GSTO1 frequency distributions were within the range observed in the Italian and European populations. Specifically, considering the data about Europeans in the 1000 Genomes project, the genetic variability of our controls was within that reported for Europeans populations. The previously described LD between GSTO1*E155del and GSTO1*E208K was also confirmed (Fig. 1) (31, 36). Regarding the genetic association analysis, the common polymorphism GSTO1*A140D did not show a statistically significant difference in the case-control population when considering the dominant and the recessive genetic model.
(P > .05). Conversely, the uncommon variants GSTO1*E155del and GSTO1*E208K were detected only in women with RM. Specifically, three RM patients with the GSTO1*del155 allele and four RM patients with the GSTO1*K208 allele were identified (P = .034 and P = .008, respectively). Considering the correction for multiple testing, the outcome of GSTO1*E208K was confirmed as being statistically significant (P < .018).

Regarding the LD between these GSTO1 uncommon variants, we also performed a haplotype-based association analysis (Table 3). The global haplotype association analysis confirmed the association between GSTO1 and RM risk (P = .025).

### DISCUSSION

Because interesting literature has focused on the role of GST gene polymorphisms in RM pathogenesis, we explored the potential association between the GSTO1 gene and disease risk. GSTO1-1 has particular structural and functional characteristics and is involved in more diverse biologic pathways than the other GST enzymes (26). Specifically, the GSTO1 enzyme catalyzes thioltransferase, ascorbate, and S-phenacyl glutathione reductase reactions (37). It is also involved in the reduction steps of arsenic biotransformation (38), and it is implicated in interleukin-1β (IL-1β) activation and the biotransformation of α-haloketones (39, 40).

Comparing the GSTO1 genotype frequencies of our study population with European reference populations, we found no statistically significant differences. However, although the difference was not statistically significant, our study population showed very low E208K frequencies. This may be due to the presence of only women in our samples and to the observed significant association between K208 allele and RM risk.

Our genetic analysis indicated a potential association between two uncommon variants of the GSTO1 gene, GSTO1*E155del and GSTO1*E208K. Previous studies have highlighted that these genetic variants have a high impact on protein function and that they can be considered markers for GSTO1-related diseases (25, 27, 41). In our study population, we identified the mutant allele only in RM patients; this may underline a high association between GSTO1 deficiency and the RM development.

### TABLE 1

Demographic, clinical, and genetic characteristics of the study population.

<table>
<thead>
<tr>
<th></th>
<th>Women with RM (n = 123)</th>
<th>Control women (n = 130)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (min–max)</td>
<td>36 (24–44)</td>
<td>37 (22–45)</td>
<td>.180</td>
</tr>
<tr>
<td>Previous miscarriage</td>
<td>2 (2–10)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>median (min–max)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of live births, median (min–max)</td>
<td>0</td>
<td>2 (2–4)</td>
<td></td>
</tr>
<tr>
<td>Smokers, %</td>
<td>16%</td>
<td>20%</td>
<td>.441</td>
</tr>
</tbody>
</table>

* Student’s t-test and chi-square test as appropriate.


### TABLE 2

Genotype frequencies of GSTO1 gene polymorphism in women with recurrent miscarriage and in healthy women.

<table>
<thead>
<tr>
<th></th>
<th>Women with RM</th>
<th>Control women</th>
<th>P value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTO1*A140D, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A140/A140</td>
<td>58 (48)</td>
<td>59 (48)</td>
<td>.492</td>
<td>D: 0.99 (0.60–1.63)</td>
</tr>
<tr>
<td>A140/D140</td>
<td>52 (43)</td>
<td>48 (41)</td>
<td>R: 0.63 (0.28–1.41)</td>
<td></td>
</tr>
<tr>
<td>D140/D140</td>
<td>11 (9)</td>
<td>17 (11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTO1*E155del, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E155/E155</td>
<td>116 (98)</td>
<td>130 (100)</td>
<td>.034</td>
<td>NA</td>
</tr>
<tr>
<td>E155/del155</td>
<td>3 (2)</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTO1*E208K, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E208/E208</td>
<td>116 (96)</td>
<td>122 (100)</td>
<td>.008</td>
<td>NA</td>
</tr>
<tr>
<td>E208/K208</td>
<td>5 (4)</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: D = dominant genetic model; NA = not estimable; R = recessive genetic model; RM = recurrent miscarriage.


### TABLE 3

Haplotype frequency of GSTO1 gene in the study population.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Women with RM</th>
<th>Control women</th>
</tr>
</thead>
<tbody>
<tr>
<td>A140D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E155del</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E208K</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Global haplotype association P-value :.025. RM = recurrent miscarriage.

Considering the biologic function of the GSTO1 enzyme, two scenarios can be assumed. The first is linked to the ascorbate reductase activity of GSTO1–1. Because the role of oxidative stress as a risk factor for RM is well known, other investigators have hypothesized that an intake of antioxidant vitamins such as ascorbate may reduce the risk of miscarriage (42). Despite the little information available about the impact of vitamins on RM risk, some studies have indicated that levels of ascorbate are significantly associated with birth outcomes (43–45). Therefore, genetic defects in genes involved in ascorbate recycling may be associated with an increased susceptibility to oxidative stress.

The second scenario is based on the involvement of GSTO1–1 in the arsenic biotransformation process. GSTO1*E155del and GSTO1*E208K can be associated with an increased susceptibility to arsenic-related adverse events (28). Some evidence has widely demonstrated the strong relationship between arsenic exposure and pregnancy loss. In vivo experiments have indicated that arsenic exposure is associated with embryo-fetal development abnormalities, aberrant placental vasculogenesis, and placental insufficiency (46, 47). Moreover, molecular investigation has indicated that arsenic exposure in pregnancy enhances placental inflammatory responses, reduces placental T cells, and alters cord blood cytokines (48). A number of epidemiologic reports have indicated that areas with high arsenic concentrations in groundwater have significantly higher rates of fetal loss and infant deaths than unexposed areas (49–54); however, arsenic is an ubiquitous metalloid, and humans are widely exposed via the ingestion of drinking water contaminated with naturally occurring arsenic (55). In individuals exposed to low arsenic concentrations, the presence of functional genetic variants in arsenic-metabolism enzymes can increase individual susceptibility to arsenic-related adverse effects. Regarding our study population, previous reports have found arsenic contamination in the Latium, Italy, drinking water from extensive volcanic activity (56). Consequently, our results suggest that the functional variation of GSTO1 is a genetic RM risk factor that interacts with the environmental arsenic concentration.

In conclusion, we provide new insight into the pathogenesis of RM, proposing a scenario based on gene–environment interaction. Further studies on arsenic-metabolism genes, arsenic levels, and RM can confirm our hypothesis.

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