

# Polymorphisms of the *GSTT1* and *GSTM1* genes in polycystic ovary syndrome

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## SUMMARY

**BACKGROUND:** This study aimed to investigate the deletion polymorphisms of the genes of the glutathione S-transferase family *GSTT1* and *GSTM1* in patients with Polycystic Ovarian Syndrome (PCOS), comparing them with a control population.

**METHODS:** Blood was collected from 219 women (110 with PCOS and 109 controls) and genomic DNA was extracted. For the analysis of polymorphisms, the technique used was multiplex PCR. In the statistical analysis, the chi-square test and multiple logistic regression were used.

**RESULTS:** There is no association between the *GSTM1* null and *GSTT1* null genotypes with PCOS when analyzed separately ( $P = 0.616$  and  $P = 0.188$ ). The analysis of the combined genotypes showed differences between the groups ( $P < 0.05$ ), evidencing that the genotypic combination *GSTT1* positive and *GSTM1* negative is more frequent among patients. In the multivariate analysis, smoking was more frequent in the control group (OR = 0.22; 95% CI - 0.87-0.57;  $P = 0.002$ ) while the presence of a family history of PCOS (OR = 2, 96; 95% CI - 1.54-5.68;  $P = 0.001$ ) was more frequent in women with PCOS.

**CONCLUSIONS:** In the studied sample, the deletion polymorphisms of the *GSTT1* and *GSTM1* genes isolated are not associated with PCOS, but in combination, they may be implicated in the etiology of the condition.

**KEYWORDS:** Multiplex Polymerase Chain Reaction. Polycystic Ovary Syndrome. Polymorphism Genetic.

## INTRODUCTION

Polycystic ovary syndrome (PCOS) is a very common endocrine-metabolic disorder that affects women of reproductive age (18 to 44 years) and one of the main causes of female infertility. Its diagnosis is based

on the Rotterdam criteria, that is characterized by the presence of at least two of the following criteria: chronic anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries,

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characterized by the ultrasonographic examination. High levels of androgens cause hirsutism and acne. There is some insulin resistance that leads to obesity and diabetes type 2<sup>1</sup>.

The syndrome has a multifactorial character; therefore, genetic factors play an important role in its etiology, with emphasis on single-nucleotide polymorphisms (SNPs)<sup>1</sup>. In addition to genetic predisposition, oxidative stress can contribute to the pathogenesis of PCOS, and oxidative stress parameters can be used as biomarkers for the early diagnosis and screening of high-risk groups<sup>2,3</sup>.

Figure 1 illustrates how oxidative stress plays a key role in the physiopathology of PCOS<sup>3</sup>. The family of Glutathione S-transferase enzymes is involved in the detoxification of reactive oxygen species, which are generated in large quantities during oxidative stress. In this sense, polymorphisms in genes of this family can alter the functions of enzymes that act on the metabolism of these molecules and, consequently, be associated with the clinical conditions of PCOS. Such polymorphisms reduce or eliminate the detoxification activities of these enzymes and could increase people's susceptibility to diseases, including PCOS<sup>4</sup>.

Genes *GSTM1* and *GSTT1*, located at 1q13.3<sup>5</sup> and 22q11.2<sup>6</sup>, respectively, belong to the family of GST. Polymorphisms of complete deletion of these genes have already been extensively investigated in type 2 diabetes mellitus (DMT2), a clinical consequence of PCOS<sup>7-13</sup>. However, only two studies have evaluated the polymorphisms of genes *GSTT1* and *GSTM1* in PCOS<sup>14,15</sup>.

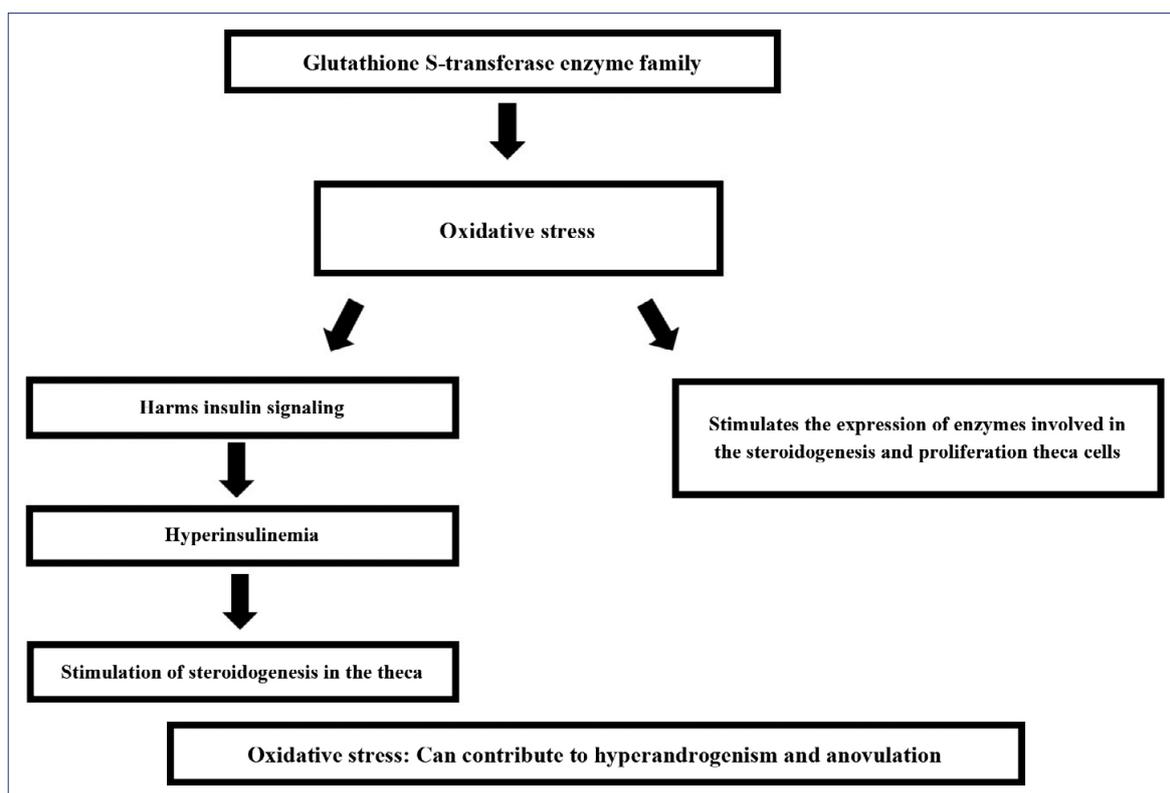
Thus, based on the evidence presented, the objective of this study was to investigate the deletion polymorphisms of the genes *GSTT1* and *GSTM1* in patients with a PCOS and in a control group.

## METHODS

The project was approved by the Research Ethics Committee of the Federal University of Triângulo Mineiro (UFTM), under protocol number 1,796, and all participants signed the Informed Consent Form.

The study had the participation of 219 women (110 patients with PCOS and 109 controls), who were being followed-up in the outpatient clinic of General Gynecology, Endocrine Gynecology, and Endocrinology of the Maria da Glória Outpatient Clinic, of

**FIGURE 1.** FLOWCHART ILLUSTRATING THE ROLE OF THE GLUTATHIONE S-TRANSFERASE ENZYME FAMILY IN OXIDATIVE STRESS, A PROCESS THAT CAN CONTRIBUTE TO HYPERANDROGENISM AND ANOVULATION, ALL CLINICAL CHARACTERISTICS OF PCOS.



UFTM, Uberaba, MG, Brasil, at the moment of the study, constituting a sample by convenience. The patients with PCOS were diagnosed with the condition based on the Rotterdam criteria, and the control group was comprised of women of reproductive age, without a history or sign of PCOS. As the exclusion criteria, we did not evaluate patients with Cushing's syndrome, 21-hydroxylase deficiency, thyroid dysfunction, hyperprolactinemia, diabetes, androgen-secreting tumors, antiandrogens, statins, glucocorticoids, or infertility medications.

We collected 8 mL of peripheral blood, in vacuum tubes with EDTA; the DNA was extracted using the salting-out technique<sup>16</sup>.

The analysis of the deletion polymorphisms of genes *GSTM1* and *GSTT1* was carried out using the multiplex PCR technique. The primers for the polymorphism of the gene *GSTM1* were sense: 5' GAA CTC CCT GAA AAG CTA AAG C 3' and antisense: 5'GTT GGG CTC AAA TAT ACG GTG G 3' (219 bp) and of gene *GSTT1* sense: 5' TTC CTT ACT GGT CCT CAC ATC TC 3' and antisense: 5' TCA CCG GAT CAT GGC CAG CA 3' (480 bp). The sequence of primers for gene *CYP1A1* was sense: 5' GAA CTG CCA CTT CAG CTG TCT 3' and antisense: 5' CAG CTG CAT TTG GAA GTG CTC 3' (312 bp), and this gene was used as an internal positive control for the reaction.

The PCR reaction was carried out on a final volume of 30 µl, containing 0.2 mM dNTP, 1x PCR buffer, 3 pmol of each primer, 1 unit of Taq DNA Polymerase, and approximately 100 ng of genomic DNA. The PCR amplification consisted of an initial stage at 94 °C for 5 minutes, followed by 35 cycles at 94 °C for 2 minutes (denaturation), 59°C for 1 minute (annealing), 72 °C for 1 minute (extension), and a final extension at 72 °C for 10 minutes. The PCR products were applied in 1.5% agarose gel, stained using GelRed™, and analyzed under ultraviolet light for genotyping.

We used the chi-square test to compare data from the case and control groups. The multiple logistic regression model was used to determine the effects of risk factors (smoking, family history of PCOS, alcoholism, and presence of polymorphisms) in PCOS. The results of the logistic regression analysis were presented in odds-ratio (OR) and with a confidence interval of 95% (95% CI). For all analyzes, the level of significance was set at 5% ( $p \leq 0.05$ ).

## RESULTS

The mean age of the patients and the control group was 26 years ( $\pm 7.54$ ) and 31 years ( $\pm 9.25$ ), respectively. Regarding the risk factors, the data show that 13.8% of the participants are smokers, something that was more frequent in the control group. With regard to alcoholism, it was observed that 27.8% of the women studied had this habit, but no significant differences were found between the groups. Observing the family history of PCOS, 29.3% of the participants had relatives with PCOS, with a significant difference between the groups, and the patients had a greater number of relatives with PCOS (67.6%) compared to the control group (32.4%).

In Table 1 it is possible to see the frequency of the deletion polymorphisms of genes *GSTT1* and *GSTM1* in women with PCOS and in the control group. No significant differences were observed between the groups when they were analyzed individually.

The analysis of the combined genotypes showed

**TABLE 1.** GENOTYPIC DISTRIBUTION OF POLYMORPHISMS *GSTT1* AND *GSTM1* IN PATIENTS WITH PCOS AND CONTROLS.

Genes	Total	Patients	Controls	P
	n (%)	n (%)	n (%)	
<i>GSTT1</i>	219 (100)	110 (50.2)	109 (49.8)	0.616
[+]	162 (100)	83 (51.2)	79 (48.8)	
[-]	57 (100)	27 (47.4)	30 (52.6)	
<i>GSTM1</i>	219 (100)	110 (50.2)	109 (49.8)	0.188
[+]	129 (100)	60 (46.5)	69 (53.5)	
[-]	90 (100)	50 (55.6)	40 (44.4)	

Note: Values of  $P \leq 0.05$  are significant; [+]: Presence of the gene; [-]: Deletion of the gene.

differences between the group of patients and the controls ( $\chi^2=11.534$ ,  $p < 0.05$ ) (Table 2). This analysis shows that the combination of genotypes *GSTT1 +/GSTM1-* is more frequent in patients.

The multivariate analysis carried out to determine

**TABLE 2.** FREQUENCY OF THE GENOTYPES *GSTT1* AND *GSTM1* COMBINED IN PATIENTS AND CONTROLS.

Genotypes	Patient n (%)	Control n (%)	P
			<0.05
<i>GSTT1 +/GSTM1 +</i>	44 (40)	52 (47.7)	
<i>GSTT1 +/GSTM1 -</i>	38 (34.5)	17 (15.6)	
<i>GSTT1 -/GSTM1 +</i>	16 (14.5)	27 (24.8)	
<i>GSTT1 -/GSTM1 -</i>	12 (10.9)	13 (11.9)	

Note: Values of  $P \leq 0.05$  are significant; [+]: Presence of the gene; [-]: Deletion of the gene.

the effects of risk factors (smoking, family history of PCOS, alcoholism, and presence of polymorphisms) in PCOS showed that smokers were more frequent in the control group (odds ratio=0.22; CI 95% - 0.87-0.57; p=0.002), while a family history of PCOS (OR=2.96; 95% CI: 1,54-5,68; p=0.001) was more frequent among women with the syndrome, with a significant difference between the groups. The variables of alcoholism and deletion polymorphisms of genes *GSTT1* and *GSTM1* presented no statistically significant differences between the groups analyzed.

## DISCUSSION

Glutathione S-transferases (GSTs) are a superfamily of detoxifying enzymes involved in maintaining cellular integrity, oxidative stress, and protection against DNA damage. Many polymorphisms have been reported to occur in GST coding genes, which can reduce their efficiency and increase the risk of certain human diseases, such as cancer<sup>4</sup>.

A study explored the association between PCOS and oxidative stress and examined the relationship between biomarkers of oxidative stress and insulin parameters, concluding that oxidative stress may be a contributing factor for future risk of cardiovascular disease in these women, in addition to known characteristics such as dyslipidemia and obesity<sup>17</sup>. Besides obesity, there is evidence that sex hormones, particularly estradiol, in PCOS can contribute to increased oxidative stress<sup>2</sup>.

In the present study, no association was found between the deletion of genes *GSTT1* and *GSTM1* and PCOS in isolation, only in combination. A previous study suggests that the combined effect of *CYP1A1* and the mutant genotypes *GSTM1* and *GSTT1* confers an increased risk of the syndrome in Indian women<sup>14</sup>. Another study analyzed parameters of oxidative stress and GST polymorphisms in non-obese adolescents, with normal levels of insulin, and recently diagnosed with PCOS. Regarding the molecular data, girls with PCOS, carriers of the null *GSTM1* genotype showed significantly lower testosterone in comparison with those carriers of the active *GSTM1* genotype<sup>15</sup>.

Another study investigated the four most common polymorphisms of GST (*GSTM1*, T1, A1, and P1) in epithelial ovarian cancer and found that these can affect both the susceptibility to and the progression of this type of cancer<sup>18</sup>.

Polymorphisms in genes *GSTT1* and *GSTM1* were

analyzed in gynecological conditions such as endometriosis<sup>19-22</sup> and pre-eclampsia<sup>23,24</sup>, with divergent results (Table 3), even in populations of the same country of origin. The discrepancy between the results may be due to the particularities of the samples investigated in regarding their ethnicity and genetic background. It is also important to highlight that, in addition to PCOS, endometriosis is one of the main causes of female infertility.

DMT2 is highly prevalent in patients with PCOS<sup>25</sup>.

**TABLE 3.** STUDIES THAT EVALUATED POLYMORPHISMS IN THE GENES *GSTT1* AND *GSTM1* IN GYNECOLOGICAL CONDITIONS AND THE PRESENCE (YES) OR NO PRESENCE OF ASSOCIATION, ACCORDING TO THE CONDITION INVESTIGATED.

Study/Country of origin	Sample	Association
Henidi <i>et al.</i> <sup>19</sup> / Tunisia	105 women with endometriosis 150 controls	Yes
Kubiszkeski <i>et al.</i> <sup>20</sup> / Brasil	121 women with endometriosis 97 Controls	No - <i>GSTM1</i> Yes - <i>GSTT1</i>
Tuo <i>et al.</i> <sup>21</sup> /China	262 women with endometriosis 275 controls	No
Batista <i>et al.</i> <sup>22</sup> / Brasil	49 women with endometriosis 83 controls	No
Guan <i>et al.</i> <sup>23</sup> /China	232 women with pre-eclampsia 293 controls	Yes
Slijivancanin Jakovljevic <i>et al.</i> <sup>24</sup> /Serbia	50 women with pre-eclampsia 50 controls	Yes

Seven studies investigated the polymorphisms in the genes *GSTT1* and *GSTM1* in patients with DMT2 and positively associated them with the condition, either individually or in combination<sup>7-13</sup>. One of these studies was carried out with 120 Brazilian patients with DMT2 and it suggests that the null *GSTM1* and *GSTT1* genotypes may contribute to the clinical course of the disease<sup>10</sup>. A meta-analysis of 25 studies concluded that the null *GSTM1* and *GSTT1* genotypes increase the risk of DMT2 alone, in combination, or with respect to ethnicity<sup>26</sup>.

In this study, the multiple logistic regression model showed an increased frequency of smokers in the control group, which may be a peculiar characteristic of the sample. The increased frequency of PCOS in the study group is associated with the multifactorial nature of the condition, which demonstrates the contribution of genetic factors in its etiology.

Recently, a multicenter study was published in Brasil that could guide public strategies specific for primary and secondary prevention of metabolic and reproductive comorbidities in the population with

PCOS in the country. This information is extremely relevant in view of the extension of our country associated with a population of mixed ethnic origin and marked variation of diet and cultural characteristics. These characteristics provide a unique opportunity to investigate the association of factors related to lifestyle, environment, genetics, epigenetics, and the phenotypic expression of PCOS<sup>27</sup>.

One limitation of our study was the sample analyzed, considered small for research with genetic polymorphisms. However, the scarcity of studies published on polymorphisms in the genes *GSTM1* and *GSTT1* in PCOS compromised the comparison with the results found herein but emphasizes the need for additional studies regarding the contribution of these polymorphisms to the development of this endocrinopathy.

It is also important to highlight that the lack of association in our study may be due to the ethnicity and genetic background of the population investigated. Another interesting approach, in subsequent studies, would be to examine whether there is an association between comorbidities such as DM2, central obesity, and cardiovascular disease and the polymorphisms analyzed. However, this would require dividing the patients into subgroups, which might not have statistical significance.

It is also worth mentioning that there are environmental, behavioral, and psychological factors that can interfere with the phenotype of PCOS<sup>28</sup>, and that the sample does not reflect the community, since they were

patients who sought care. Therefore, further studies are needed in order to minimize this sample bias. Thus, the lack of association of the gene polymorphisms studied and the syndrome should also be explored.

## CONCLUSIONS

The deletion polymorphisms of genes *GSTT1* and *GSTM1* in isolation are not associated with PCOS; however, in combination, they may be involved in the etiology of the condition in the sample investigated.

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### Author's Contribution

Mariana Mendes Porto Azevedo, Alessandra Bernadete Trovó de Marqui: concept, data collection and analysis, writing; Bruna Tavares Bacalá, Marly Aparecida Spadotto Balarin, Marco Fábio Prata Lima, Mariana Kefalas Oliveira Gomes, Elisabete Aparecida Mantovani Rodrigues de Resende: data collection and analysis. Mariangela Torreglosa Ruiz Cintra: project design, data collection and analysis, article writing.

All authors participated in the final analysis of the article.

## RESUMO

**OBJETIVO:** Este estudo teve como objetivo investigar os polimorfismos de deleção dos genes da família glutationa S-transferase *GSTT1* e *GSTM1* em pacientes com síndrome dos ovários policísticos (SOP), comparando-as com uma população controle.

**MÉTODOS:** Foi colhido sangue de 219 mulheres (110 com SOP e 109 controles) e extraído o DNA genômico. Para análise dos polimorfismos, a técnica empregada foi PCR multiplex. Na análise estatística foi utilizado o teste do qui-quadrado e regressão logística múltipla.

**RESULTADOS:** Não há associação dos genótipos *GSTM1* nulo e *GSTT1* nulo com SOP quando analisados isoladamente ( $p=0,616$  e  $p=0,188$ ). A análise dos genótipos combinados mostrou diferenças entre os grupos ( $p<0,05$ ), evidenciando que a combinação genotípica *GSTT1* positivo e *GSTM1* negativo é mais frequente entre as pacientes. Na análise multivariada, o hábito tabagista foi mais frequente no grupo controle (OR=0,22; IC 95% - 0,87-0,57;  $p=0,002$ ), enquanto que a presença do histórico de SOP familiar (OR=2,96; IC 95% - 1,54-5,68;  $p=0,001$ ) foi mais frequente nas mulheres com SOP.

**CONCLUSÕES:** Na casuística estudada, os polimorfismos de deleção dos genes *GSTT1* e *GSTM1* isolados não estão associados a SOP, mas em combinação podem estar implicados na etiologia da condição.

**PALAVRAS-CHAVE:** Reação em cadeia da polimerase multiplex. Síndrome do ovário policístico. Polimorfismo genético.

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