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Relationship between *GSTM1* and *GSTT1* polymorphisms and schizophrenia: A case–control study in a Tunisian population

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ABSTRACT

There is substantial evidence found in the literature that supports the fact that the presence of oxidative stress may play an important role in the pathophysiology of schizophrenia. The glutathione S-transferases (GSTs) forms one of the major detoxifying groups of enzymes responsible for eliminating products of oxidative stress. Interindividual differences observed in the metabolism of xenobiotics have been attributed to the genetic polymorphism of genes coding for enzymes involved in detoxification. Thus, in this study we investigated the association of glutathione S-transferase Mu-1 (*GSTM1*) and glutathione S-transferase theta-1 (*GSTT1*) gene deletion polymorphisms and schizophrenia in a Tunisian population. A case–control study including 138 schizophrenic patients and 123 healthy controls was enrolled. The *GSTM1* and *GSTT1* polymorphisms were analyzed by multiplex polymerase chain reaction (PCR). No association was found between the *GSTM1* genotype and schizophrenia, whereas the prevalence of the *GSTT1* active genotype was significantly higher in the schizophrenic patients (57.2%) than in the controls (45.5%) with (OR = 0.6, IC 0.37–0.99, $p = 0.039$). Thus, we noted a significant association between schizophrenia and *GSTT1* active genotype. Furthermore, the combination of the *GSTM1* and *GSTT1* null genotypes showed a non-significant trend to an increased risk of schizophrenia. The present finding indicated that *GSTT1* seems to be a candidate gene for susceptibility to schizophrenia in at least Tunisian population.

1. Introduction

Schizophrenia is a devastating psychiatric disease with a complex genetic etiology (Sullivan et al., 2003). The high genetic risk for schizophrenia has led to considerable research efforts, including linkage studies, aiming at the identification of susceptibility genes (Mowry et al., 2004). Several lines of evidence suggest that oxidative stress may be involved in this disease (Marchbanks et al., 2003; Prabakaran et al., 2004; Yao et al., 2001). Glutathione S-transferases (GSTs) constitute a superfamily of ubiquitous, multifunctional enzymes which play a key role in phase II cellular detoxification and are generally considered to be antioxidant enzymes (Rushmore and Pickett, 1993). The glutathione S-transferase Mu-1 (*GSTM1*) gene, coding for cytosolic GST class mu enzyme, is located on chromosome 1p13.3 (Pearson et al., 1993). A deletion of *GSTM1* polymorphism in the homozygous state (*GSTM1* null) leads to a total absence of a functional gene product (Seidegard et al., 1988). The glutathione S-transferase theta-1(*GSTT1*) gene is located on chromosome 22q11.2 (Webb et al., 1996). The inactivating homozygous deletion polymorphism in this gene was also described (Pemble et al., 1994). Several studies have reported that the *GSTM1* and *GSTT1* null genotypes, which lead to a lack of functional protein, are correlated with an increase susceptibility to numerous diseases associated with oxidative stress such as cancers (Abbas et al., 2004; Parl, 2005; Saadat and Saadat, 2001), asthma (Saadat et al., 2004a), cataracts (Saadat et al., 2004b) and diabetes (Amer et al., 2011; Gönül et al., 2012). These findings are interesting within the context of the hypothesis that neurodegeneration due to oxidative metabolites in the central nerve system may contribute to the development of schizophrenia (Wood et al., 2009). However, few numbers of studies with conflicting results concerning the association between *GSTM1* and *GSTT1* polymorphisms and schizophrenia were conducted. Indeed, the null genotype of *GSTM1* was reported to be associated with

Abbreviations: CI, confidence interval; dNTP, deoxy-nucleotide-tri phosphate; DSM-IV-TR, diagnostic and statistical manual of mental disorders; EDTA, ethylenediaminetetraacetic acid; *GCLC*, glutamate cysteine ligase catalytic; *GSTM1*, glutathione S-transferase Mu-1; GSTs, glutathione S-transferases; *GSTT1*, glutathione S-transferase theta-1; MgCl2, magnesium chloride; NS, no significant; OR, odds ratio; PCR, polymerase chain reaction; SD, standard deviation.

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Table 1
The socio-demographic characteristics of schizophrenic patients and healthy controls.

Variables	Schizophrenic patients ($n=138$)	Controls ($n=123$)	Statistic tests
Mean age ± SD (years)	32.67 ± 7.44	31.28 ± 5.28	NS
Gender: *n* (%)			
Male	120 (86.9%)	99 (80.5%)	
Female	18 (13.0)	24 (19.5%)	NS
Smoking status: *n* (%)			
Smokers	83 (60.1%)	61 (49.6%)	NS
Non-smokers	55 (39.8%)	62 (50.4%)	
Educational level: *n* (%)			
Primary school	66 (47.8%)	22 (17.9%)	$\chi^2=27.11$ (df = 2)
Secondary school	55 (39.8%)	70 (56.9%)	$p<0.001$*
University	17 (12.3%)	31 (25.2%)	
Marital status: *n* (%)			
Single	88 (63.8%)	66 (53.6%)	$\chi^2=15.31$ (df = 2)
Married	40 (29.0%)	57 (46.3%)	$p<0.001$
Divorced	10 (7.2%)	0 (0%)	

* $p<0.05$, df: degrees of freedom, NS: not significant, SD: standard deviation.

schizophrenia in Japanese and Korean populations (Harada et al., 2001; Pae et al., 2004). The *GSTT1* null genotype was associated with a significantly reduced risk of developing schizophrenia (Saadat et al., 2007). Gravina et al. (2011) showed that the combination of the absence of *GSTM1* gene and the presence of *GSTT1* gene represents a risk factor for schizophrenia. Therefore, the purpose of this study was to investigate the relationship between *GSTM1* and *GSTT1* polymorphisms and schizophrenia in a Tunisian population.

2. Methods

2.1. Subjects

One hundred thirty-eight (120 males and 18 females) patients with schizophrenia and 123 (99 males and 24 females) healthy controls were involved in this case–control study. The socio-demographic characteristics of the study subjects are summarized in Table 1. Patients were recruited from consecutive admissions at the psychiatric department of the Teaching Hospital of Monastir (Tunisia). They had DSM-IV-TR criteria for schizophrenia and based on the structured Clinical interview for DSM-IV-TR (American Psychiatric Association, 2000). Inclusion criteria of patients were being aged between 18 and 60 years, and having chronic schizophrenia. These patients had no other psychiatric disorders; including major depression, schizoaffective disorder, substance abuse (except tobacco consumption), or mental retardation. The healthy controls were recruited from the blood center of the Teaching Hospital of Monastir; they had no personal or family history of major psychiatric disorders including schizophrenia, bipolar disorder or major depression. Their current mental status and personal or family history of mental disorder were assessed by unstructured interviews. This study was approved by the local ethical committee. All subjects were signed informed consent after a full explanation of the study.

2.2. DNA preparation

For genotyping, 4 ml blood were drawn into an EDTA tube and stored at −20 °C until DNA extraction was carried out. Genomic DNA was isolated from whole peripheral blood using the standard salt precipitation method (Miller et al., 1988).

2.3. Multiplex PCR

To examine the polymorphisms of *GSTM1* and *GSTT1*, a simultaneous amplification of genes of interest in the same reaction was performed using a multiplex polymerase chain reaction (PCR) as described in the literature (Arand et al., 1996).

PCR for albumin was also performed as an internal control. The primer pairs for each gene were 5′-GAACTCCCTGAAAAGCTAAAGC-3′ and 5′-GTTGGGCTCAAATATACGGTGG-3′ for *GSTM1*, 5′-TTCCTTACTG GTCCTCACATCTC-3′ and 5'-TCACCGGATC ATGGCCAGCA-3' for *GSTT1* and 5′-GCCCTCTGCTAACAAGTCCTAC-3′ and 5′-GCCCTAAAAAGAAAA TCGCCAATC-3′ for the albumin gene.

The PCR buffer contained 100 ng DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, primers of *GSTM1* at 3 μg/ml each, primers of *GSTT1* at 1 μg/ml each, and albumin primers at 1 μg/ml each, 4 μl 5× colorless GoTaq Flexi Buffer, and 1.5 U GoTaq Flexi DNA polymerase (Promega, USA) in a final volume of 20 μl. PCRs were carried out with a thermal cycler Mastercycler Personal (Eppendorf, Germany). The PCR program consisted of 30 cycles of 94 °C for 30 s, 64 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 5 min. The initial pretreatment was carried out at 95 °C for 5 min. The multiplex PCR products containing fragments of 215 bp (indicating the presence of GSTM1), 480 bp (*GSTT1*), and 350 bp (albumin) were separated by electrophoresis on 2% agarose gel stained with ethidium bromide and visualized by ultraviolet detection (Fig. 1).

Subjects were categorized as having either an active [heterozygous deletion with GSTM1 (*GSTM1*+/− and *GSTM1*+/+) or *GSTT1* (*GSTT1*+/− and *GSTT1*+/+)] or null (homozygous deletion with *GSTM1*−/− and *GSTT1*−/−) genotypes. For both *GSTM1* and *GSTT1*, heterozygous and active homozygous genotypes were pooled for statistical analysis because previous studies reported that they present similar enzyme activities (Tkacova et al., 2004) and expression levels (Bell et al., 1993).

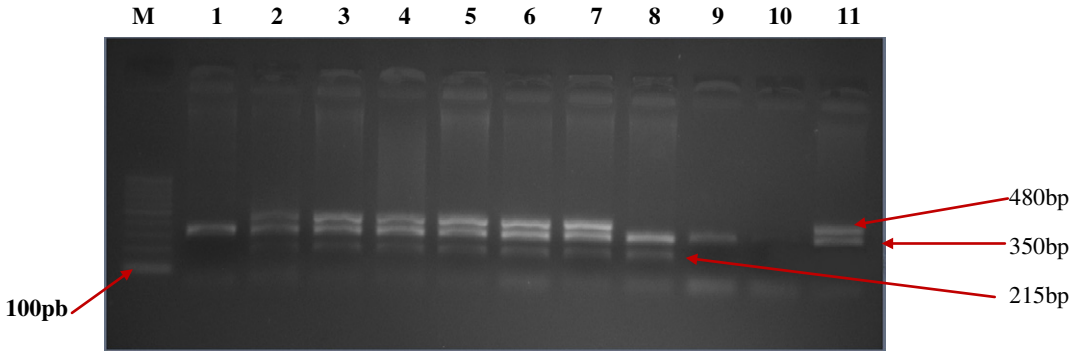

Fig. 1. Genotyping of GSTM1 and GSTT1 by multiplex PCR. M: 100 bp DNA marker. Lanes 1, 9 and 10: GSTM1 and GSTT1 null genotypes, albumin as an internal control (350 bp). Lanes 2–7: GSTM1 active (215 bp)/GSTT1 active (480 bp) genotypes. Lane 8: GSTM1 active/GSTT1 null genotypes. Lane 11: GSTM1 null/GSTT1 active genotypes. Null: (−/−), active (−/+) or (+/+).