



Original Article

Molecular Analysis of the Glutathione S-Transferase System in Patients with Depression

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ABSTRACT

Depression is defined as a mood disorder in which changes in temperament occur making the patient sad, lacking energy and, at a severe stage, with suicidal thoughts. Depression is a multifactorial tantrum that may be associated with changes in neurotransmitters, social or genetic factors. It is suggested that oxidative stress may be associated with worsening of depression in patients not treated with antidepressants. In addition, it is known that oxidative stress is favored in situations where the individual presents a compromise in the function of antioxidant enzymes, such as GSTP1. The objective of the present study was to verify if the GSTP1 gene polymorphism confers genetic susceptibility to depression. PCR-RFLP technique was used to analyze polymorphisms. The samples were collected at the Brain Institute Clinic were divided into two groups, case and control, for later study of the heterozygous genotypes (Ile/Val), wild homozygote (Ile/Ile) and mutant homozygote (Val/Val). The results suggest that there was no statistically significant correlation between GSTP1 polymorphism and the risk of developing depression ($p = 0.1835$). In this sense, more studies should be carried out in search of more consistent results, thus allowing more accurate correlation regarding the role of this polymorphism with the susceptibility to depression.

1. Introduction

Depression is defined by the *World Health Organization* - WHO (2012) as a mood disorder in which the individual presents with poor self-esteem, sleep or appetite disorders, loss of interest or pleasure in life, feelings of guilt, lack of concentration, feeling tired and, mainly, sadness. The *Diagnostic and Statistical Manual of Mental Disorders V* classifies this disorder in disorders: non-specific depressive, dysthymia and major depressive disorder, highlights the major depressive disorder, also called depression, as one of the mental illnesses with the greatest degree of disability (WHO, 2012).

Depression is a non-transmissible disease causing functional disability to individual. Thus, the depression has been considered a public health problem as well as an economic one (WHO, 2012). Epidemiological data suggest that depression affects approximately 350 million people worldwide. The prediction is that, until 2030, this disorder becomes a main concern among the fundamental, mainly, in the

Brazil (10.4%) and the USA (8.3%), which present highest prevalence rate for this disease.

Socioeconomic factors, including abuse, education and income, may impact the higher rate of depression among individuals (Máximo, 2010). It is not proven that the rate of depression is higher in countries where women have a lower socioeconomic power than men in countries that have this inequality, but there is an account that, in countries with that name, the prevalence of depression is more common in women than in men (Albert, 2015). Despite this, a depression can also occur in children, adolescents and the elderly, as consequences and sequelae can be severe (Albert, 2015; Cyranowski et al., 2000).

Depression is manifest in mild, moderate and severe episodes and the form of treatment is based on the use of medications and psychotherapeutic follow-up (WHO, 2012). Studies suggest that the development of depression is associated with changes in neurotransmitters (serotonin and norepinephrine), adverse events that occur in a person's life

(stress, loss of a loved one, pregnancy and some trauma), or genetic factors (Kennedy et al., 2016; Martin et al., 2017; Oluboka et al., 2017).

The suggestion of genetic factors is contradicted by the presence of the disease in people with no family history (Kennedy et al., 2016). This duality draws the attention of the scientific world and makes the human genome very attractive for studies. Researches involving genetic polymorphism and its correlation with depression have been the subject of research. Study in the New Zealand population, for example, suggest that deletion of 44 base pairs in the 5HTT promoter region results in a reduction in transcriptional activity that may be associated with an increased risk of developing depression (Capsi et al., 2003). Results of other studies with the polymorphic genes HTR1A and HTR1B indicate that serotonergic system receptors are compromised and this may also be associated with the development of depression (Mekli et al., 2011).

There are other factors related to the development of depression, for example genetic polymorphisms (Kiyohara; Yoshimasu, 2009; Opmeer et al., 2013). According to Bieleński et al. (2017), the Val158Met polymorphism compromises the activities of the Catechol-O-methyl Transferase (COMT) enzyme and thus increases the chance of developing depression, but later studies contradict these results (Massat et al., 2005). There are also study of polymorphisms for genes encoding norepinephrine-NET Transporters and Spermine/Spermidine N1-Acetyl Transferase (SSTA) enzyme showing relationship of these modifications to predisposition to depression (Kiyohara; Yoshimasu, 2009; Sequeira et al., 2006).

Other study of association of polymorphic genes with the pathophysiology of depression are being performed. It is known that depression is related to immuno-inflammatory processes (Glucocorticoids and cytokines, for example) and to oxidative stress (Kopschina Feltes et al., 2017; Maes et al., 2011; Zunszain et al., 2012) and, in this sense, a polymorphism of interest for these studies involves S-transferases Glutathione (GST). Considering the genetic alterations, it is known that there are polymorphic genes that compromise the action of enzymes linked to the body's defense system against oxidative stress (Zhang; Yao, 2013). In depression, this oxidative stress is involved in the worsening of the disease and its development (Chunga et al., 2014).

This family of phase II metabolic enzymes mainly acts in the detoxification of xenobiotics, such as carcinogenic substances and toxic to the body, avoiding oxidative stress (Huber; Almeida; De Fátima, 2008; Leme et al., 2010). The GSTs are divided into nine classes: *alpha*, *mu*, *pi*, *omega*, *theta*, *sigma*, *zeta*, *delta* (cytosolic GSTs) and *kappa* (mitochondrial GST) (Pavarino et al., 2013). Among these, the GSTP1 enzyme, of the class *pi*, that is codified from the gene *GSTP1* located in the chromosome 11q13 stands out (Dragovic et al., 2014).

The enzyme GSTP1 is found abundantly in the liver, brain, lung, muscles, red blood cells, placenta and human plasma and has a high efficacy in the fight against many carcinogens and the metabolism of antitumor (Dragovic et al., 2014). Therefore, the polymorphic *GSTP1* gene has been of great interest to researchers in the field.

One of the functional polymorphisms that can occur in the *GSTP1* gene is the exchange of adenine nucleotide by guanine at codon 105. This change results in the exchange of the amino acid Isoleucine (Ile) by Valine (Val) and, thus, the gene *GSTP1* is characterized by the wild genotypes (Ile/Ile), heterozygous (Ile/Val) or mutant homozygote (Val/Val) (Harries et al., 1997). These SNPs (*Single Nucleotide Polymorphism*) cause a decrease in the enzymatic activity and, consequently, decreases the glutathione conjugation, increases the level of oxidative stress and causes DNA damage (KARLSON et al., 2007). Studies suggest that this increase in oxidative stress may be related to the development of psychiatric disorders; however, these studies are still scarce. Therefore, the

objective of this study is to evaluate the association of *GSTP1* gene polymorphism with susceptibility to depression in a population of Central Brazil.

2. Material and methods

2.1. Obtaining samples

The present study, performed in 2015, was composed of 24 samples (convenience) from individuals with depression, medicated with Escitalopram, from patients attending and diagnosed in *Brain Institute Clinic, Brazil*, second The Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 2013). A total of 60 healthy subjects were selected from the general population with no history of drug abuse and psychiatric or psychotic disorders. Participants were between 18 and 65 years of age and patients with depression were being treated with escitalopram for at least one year. Samples of 10 ml of peripheral blood from all 84 patients (case-control) were collected in a vacuum tube and heparinized. The samples were centrifuged and the leukocyte ring was stored in cryotubes at a temperature of -20°C for further DNA extraction. All participants in the experiment signed the informed consent form. This study was approved by the Ethics in Research Committee at the Federal University of Goiás, Brazil (No. 039/13 dated February 25, 2013) was obtained.

2.2. DNA extraction and quantification

For extraction and purification of the DNA, the commercial kit *Illustra Blood Genomic Prep Mini Spin Kit* (GE Healthcare®, USA) was used, following the protocol suggested by the manufacturer. After extraction the samples were labeled and quantified using the *Epoch spectrophotometer* (Biotek®). Subsequently, these were stored at -20°C for further genotyping. For the PCR analysis, we used 50ng/uL DNA.

2.3. Genotyping of samples using the PCR-RFLP technique

DNA amplification was done by the Polymerase Chain Reaction - Restriction-Fragment-Length-Polymorphism (PCR-RFLP). The thermocycling conditions and the primers was based on the protocol proposed by Harries et al. (1997). PCR conditions: initial denaturation at 94°C for 5 minutes, followed by 35 cycles containing the following steps: denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, elongation at 72°C for 30 seconds, finalized by a final elongation step at 72°C for 5 minutes, with final maintenance at 4°C.

Analysis of the presence of the 176bp DNA fragment in the samples was done using the agarose gel (3.0%) stained with ethidium bromide. The PCR product was subjected to restriction with the use of the enzyme Alw26I according to the manufacturer's suggested protocol (Thermo Scientific®) for further identification of the genotypes. The enzymatic restriction was 16h at 37°C followed by 20 minutes at 65°C.

The SNP studied in the present work presents an A-to-G exchange in codon 105 that results in the exchange of the encoded amino acid, passing from isoleucine to valine (Figure 1).

The RFLP visualization was performed in polyacrylamide gel at (12%) and, for identification of the genotype, stained in silver solution. For the differentiation of the genotypes, the information described in (Table 1) was considered.

2.4. Statistical analysis

The evaluation of the balance between the genotypic proportions and the genetic frequencies was made from the *Hardy-Weinberg Equilibrium Test* performed through the *Arlequin software* (version 3.5.1.2). Statistical analyzes were performed using the *BioEstat® software* (version 5.3) to compare the genotype frequencies between the case and control groups. The chi-square test (χ^2) was used. Finally, to

assess the risk of a particular genotype and the susceptibility to the development of schizophrenia in relation to the control group, the *Odds Ratio (OR)* was calculated, with a 95% confidence interval. The value of $p < 0.05$ was considered statistically significant.

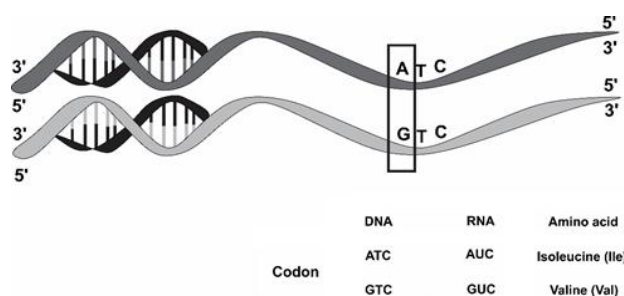


Figure 1 - Exchange of bases in the genetic sequence of the *GSTP1* gene resulting in the amino acid exchange in the final protein.

Table 1 - *GSTP1* gene genotypes

Genotypes	Phenotypes	DNA bases
Wild Type	Ile/Ile	176 bp
Heterozygous	Ile/Val	176 bp e 91 bp, 85 bp
Mutant	Val/Val	91 bp, 85 bp

Ile: Isoleucine Amino acid; Val: Valine Amino acid; bp: base pairs.

3. Results

3.1. PCR-RFLP reading on polyacrylamide gel

The set of primers used in the conventional PCR technique are annealed to the genetic sequence in order to allow the amplification of a 176 bp fragment observed in all samples in the PCR reading. The band combinations observed after RFLP show bands of 176 bp, 91 bp and 85 bp (Figure 2).

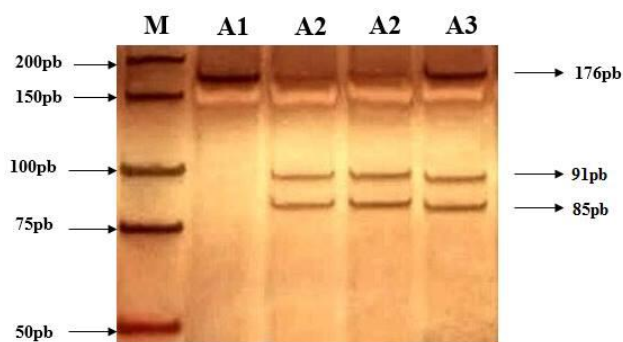


Figure 2 - Genotyping on polyacrylamide gel. M: molecular marker (25bp); bp: base pairs; A1: Wild (176bp); A2: Mutant (91, 85bp); A3: Heterozygous (176, 91, 85bp).

3.2. Genotype analyzes

A total of 84 individuals (24 cases and 60 controls) were genotyped for the SNP polymorphism of the *GSTP1* gene. In the patients in the case group the percentage of heterozygous, wild and mutant individuals was 45.8%, 45.8% and 8.3% respectively. For the control group, the percentage of heterozygous, wild and mutant individuals was 65%, 28.3% and 6.6%, respectively (Table 2). According to the χ^2 test there is no statistically significant difference between the genotypic proportions observed in the case and control groups, since, the value of $p = 0.2593$.

3.3. Analysis of the Hardy-Weinberg

The values obtained by the *Hardy-Weinberg Equilibrium Test* suggest that the genotypic distribution of *GSTP1* polymorphism in the control group is not in equilibrium (Table 3).

Table 2 - Genotypic frequencies of case and control samples

Genotypes	Case (%)	Control (%)	Genotype frequency (%)	χ^2	GL	P
Heterozygous	11 (45.8%)	39 (65%)	59.52%	2.7	2	0.25
Wild Type	11 (45.8%)	17 (28.3%)	33.33%			
Mutant	2 (8.4%)	4 (6.7%)	7.15%			
Total	24	60	100%			

χ^2 : chi-square; GL: degree of freedom; p: probability

Table 3 - *Hardy-Weinberg Equilibrium Test* for the case group and control

Genotype (<i>GSTP1</i>)	Obs.	Exp.	χ^2 (1 D.F.)	p-value
CASE				
ILE/ILE (Wild Type)	11	11.34	0.11 (1)	0.74
ILE/VAL (Heterozygous)	11	10.32		
VAL/VAL (Mutant)	2	2.34		
Total	24	24		
Alleles	Frequency			
ILE (Wild Type)	0.69			
VAL (Mutant)	0.31			
CONTROL				
ILE/ILE (Wild Type)	17	22.20	7.95 (1)	0.005*
ILE/VAL (Heterozygous)	39	28.59		
VAL/VAL (Mutant)	4	9.21		
Total	60			
Alleles	Frequency			
ILE (Wild Type)	0.61			
VAL (Mutant)	0.39			

Observed, Exp. - Expected; DF - Degree of Freedom.

3.4. Analysis of Odds Ratio (OR)

The *Odds Ratio (OR)* was performed to assess whether there is an association between the polymorphism found in the *GSTP1* gene and the risk of developing Depression. The OR value obtained was 0.4672, the value of $p = 0.20$, with 95% CI. From these results it was concluded that there is no association between the polymorphism and the development of the disease.

Table 4 - *Odds Ratio (95% CI)* for association of polymorphism with the development of depression.

Genotype	Case		Control		OR	IC 95%	p
	GR	GNR	GR	GNR			
Ile/Val	11	11	39	17	0.4359	0.1585 - 1.19	0.1722
Val/Val	2	11	4	17	0.7727	0.1204 - 4.95	0.8488
Ile/Val + Val/Val	13	11	43	17	0.4672	0.1754 - 1.24	0.2

Ile: Amino acid Isoleucine; Val: Amino Acid Valine; GR: risk group; GNR: Group does not risk; OR: Odds ratio; CI: confidence interval; p-value < 0.05*

4. Discussion

GSTs are important enzymes involved in this process of defense against oxidizing agents and protect the body against the attacks of free radicals and xenobiotic metabolites through their detoxification. The brain is one of the parts of the body with less protection against oxidative stress in counterpart is the most produces free radicals due to its high metabolic activity (Maes et al., 2011). According to a study conducted in postmortem tissues, among the GST classes found *GSTP1* was found in higher concentrations in the brain and heart (Rowe; Nieves; Listowsky, 1997). The *GSTP1* enzyme belongs to the GST family and has SNPs polymorphisms in different regions of

the gene. The focus of this study is the SNP of codon 105, amino acid 313, where the nucleotide modification of Adenine for Guanine occurs. As previously mentioned, this polymorphism entails the exchange of an amino acid in the final protein, resulting in conformational change of the catalytic site and in the loss of enzymatic affinity with its substrates leading to a failure in defense against oxidative stress. Therefore, studies in this sense are necessary (Johansson et al., 1998).

Our results show that the proportion of individuals with wild genotype, heterozygous and mutant, in the case group, are 45.8%, 45.8% and 8.4%, respectively. While in the control group these proportions are 28.3% (wild), 65% (heterozygous) and 6.6% (mutant). Considering *p-value* ($p > 0.05$), it is implicitly evident that there is no statistically different difference between case and control groups. Despite this it is worth noting that the proportion (wild type) of heterozygous and wild individuals in the case group was the same, which differs from the values found in other studies where the frequency of wild individuals is usually higher than the frequency of heterozygotes and mutants (Rossini et al., 2002; Sharma et al., 2014). This contradiction between results can be justified due to the difference of ethnicities, in cases of studies carried out in other countries, or even the multiethnicity present within the Brazilian population, or even by the reduced number of samples in our study (Rossini et al., 2002). In addition, it is noted that genotypic distribution in the control group is not in the *Hardy-Weinberg Equilibrium*, which may indicate consanguinity, population stratification or may be, again, due to the small number of samples.

In the *OR* analysis were considered as risk genotype heterozygous and mutant individuals, due to the presence of at least one mutated allele. This is because individuals who have at least one of the mutated alleles present a decrease in enzyme activity (Beeghly et al., 2006). The *OR* was calculated by comparing the wild genotype, not considered risk. *OR* values were found to be less than 1, indicating that there is no increased risk of developing depression.

The polymorphism of *GSTP1* has already been studied in relation to the development of other diseases. The presence of the polymorphism increases the susceptibility to the development of cancer, as in other articles on cancer there is no correlation with the polymorphism (Ge et al., 2013; Leichsenring et al., 2006). The mutant genotype of the *GSTP1* gene may be considered good for antitumor treatment, since the low enzymatic activity promotes the accumulation of the drug compounds that act on the tumor. Study performed with Caucasian patients found a significant correlation between *GSTP1* polymorphism and late-onset Alzheimer's disease (Bernardini et al., 2005).

The literature found associating the *GSTP1* polymorphism and depression is scarce, as well as, studies associating this polymorphism with other mental disorders such as schizophrenia and bipolar disorder. In the studies performed with Central Italian patient, the *GSTP1* polymorphism was related to the development of Schizophrenia, but in none of these the result obtained was significant (Gravina et al., 2011).

It should be considered that the results obtained in this work need to be confirmed with more experiments and studies, since we had a limiting factor that was the size of the sample group. The aspects to be analyzed should be increased, age, weight, sex and addictions should be taken into account for the study of association of polymorphism. More work on the association of *GSTP1* polymorphism with the development of depression is necessary, mainly due to the shortage of articles in this area, which may be the first to perform this association.

5. Conclusion

In the present study it is suggested that there is no association between the *GSTP1* gene polymorphism and the

development of depression, that is, the mutant genotype has no association with the development of depression. However, more genetic studies are needed to analyze the association between *GSTP1* polymorphism and the development of depression. In addition, it is important to note that there are few studies on the population of Goiás in both genetics and mental disorders.

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