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# Development and application in Mexican of a method for the identification of polymorphisms of GSTP1

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## Abstract

Glutathione S-transferase (GST) family is formed by several genes coding for a family of enzymes involved in phase II detoxification from xenobiotics through glutathione conjugation, generating water soluble compounds. GSTs have a pharmacological and toxicological interest due, among other facts, to their increased expression in mammalian tumor cells. GSTP1 is polymorphic, having three allelic variants: GSTP1a wild allele, codon 104 to GTC (Val) ATC (Ile) GSTP1b, and codon 113 GSTP1c GCG (Ala) to GTG (Val). A procedure was developed for the identification of polymorphisms by bioinformatic analysis in order to select the primers and enzymes necessary for performing PCR-RFLP, which were BsmI and AclI. The procedure thus developed was applied to 60 Mexican Mestizo healthy individuals, 30 men and 30 women. The resulting frequencies for P1b were P1a/P1a 31.66%; P1a/P1b 59.99% and P1b/P1b 8.33%; while for P1c were P1a/P1a 100%; P1a/P1c and P1c/P1c 0%. These frequencies are obtained in Mexico for the first time.

**Keywords:** GSTP1, Gene polymorphism, PCR-RFLP, Mexican, glutathione S-transferase.

## INTRODUCTION

About 99.9% of the DNA sequence of every individual is shared with each other, it is in the remaining 0.1% where genetic variations are found. This type of variations is known as genetic polymorphisms and they represent differing DNA sequences. The study of polymorphisms has several applications in the medical field as well as in biological research and development (Checa, 2007).

The glutathione S-transferase (GST) family consists of several genes coding for a group of enzymes involved in Phase II detoxification of xenobiotics. It has been shown that this family plays an important role. Due to the wide range of substrates of GSTs, the cell can be protected from oxidative damage through the conjugation of glutathione (GSH) with electrophilic substrates, generating less reactive, more soluble compounds that are easily removed from the cell through specific membrane transporters (Pavanello et al. 2000; Castillo et al. 2007; Guo et al., 2010; Kiran B. et al. 2010; Oliveira et al., 2010; Sailaja et al., 2010). In mammals, these

transporters are classified into six subclasses, Alpha, Mu, Pi, Theta, Omega and Zeta (Hayes et al., 2005; Sailaja et al., 2010).

Gene GSTP1 (n), located on chromosome 11q13, is composed of nine exons, has a length of approximately 3 kb. Being polymorphic, it has three allelic variants: GSTP1a, wild allele; GSTP1b, this polymorphism is located in exon 5, and is the result of a mutation of ATC (Ile) to GTC (Val) at codon 104; GSTP1c, has the same mutation at codon 104 GSTP1b, but has a second mutation located in exon 6, at codon 113 of GCG (Ala) to GTG (Val) (Ali-Osman et al., 1997; Eaton et al. 1999; Young et al., 2001; Castillo et al., 2007; Custodio et al., 2010; Oliveira et al., 2010).

In general, GSTs are of pharmacological and toxicological interest because their expression increases significantly in mammalian tumor cells, so they have been implicated in the resistance of patients to the treatment of different types of cancer. They are also associated with heart disease and neuro-degenerative diseases such as Parkinson's and Alzheimer (Eaton et al., 1999; Hayes et al., 2005; Bolt et al., 2006). Furthermore it has been shown that the presence of polymorphisms in the

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sequences of these genes can alter the activity of these enzymes; this may partly explain the differences in individual susceptibility to develop toxicity or other pathologies as cancer, as a result of exposure to xenobiotics (Eaton et al., 1999, Garcia et al., 2007).

The study of polymorphisms in this family of enzymes can help to understand of the risk of exposure to different xenobiotics in the population, sub-population and individuals. Based on this, the objective of this study was to develop an identification method for gene GSTP1 polymorphisms by PCR-RFLP, and applying it to Mexican Mestizo individuals.

## MATERIALS AND METHODS

### *Development of the procedure*

A review of literature was performed in order to find out the conditions for identifying the P1b polymorphism, i.e. the proper primers and restriction enzyme. In the case of P1c polymorphism, there are no references in the literature on the procedure for the identification of this polymorphism, so we relied on bioinformatic analysis, from the GSTP1 gene sequence obtained from the NCBIdbNucleotide (accession: NG\_012075) and using NCBI/Primer-BLAST.

The primers selected for amplification of P1b (exon 5) were: Forward 5'-ACCCCAGGGCTCTATGGGAA-3', and Reverse 5'-TGAGGGCACAAGAAGCCCT-3', Tm: 62.5, yielding a 176 bp product. For the amplification of P1c (exon 6) the selected primers were: Forward 5'-TGGCAGCTGAAGTGGACAGGATT-3', and Reverse 5'-ATGGCTCACACCTGTGTCCATCT-3 Tm: 64.6, yielding a 332 bp product. To select the enzymes necessary for the identification of polymorphisms, restriction maps were made of the GSTP1 regions that were amplified by PCR, using the Serial Cloner program, version 1.3, resulting in BsmAI for P1b and AcilforP1c. After enzymatic digestion, identification of the polymorphisms is based on the presence of DNA fragments of different sizes, so for exon 5, the 176 bp, 91 bp and 85 bp fragments correspond to heterozygous P1b; the 91 bp and 85 bp fragments correspond to homozygous P1b and a 176 bp fragment corresponds to wild P1a (Wilson et al., 2000). As for polymorphisms in exon 6, the 332 bp fragment corresponds to homozygous P1c; three fragments, of 332 bp, 174 bp and 158 bp correspond to heterozygous P1c; and two fragments, 158 bp and 174 bp, indicate wild P1a.

### *DNA extraction and PCR-RFLP*

DNA extraction was performed using the Quick-g DNA MiniPrep Kit (Zymo Research). PCR was carried out with a final volume of 25  $\mu$ L of a mixture containing 2  $\mu$ L template DNA, 5  $\mu$ L of 5x PCR buffer (Promega), 1.5 $\mu$ L 2

mM MgCl<sub>2</sub> (Promega), 0.1  $\mu$ L of 5U/ $\mu$ L Taq Polymerase (Promega), 0.5 $\mu$ L of 50 mM dNTPs (Fermentas) and 13.9  $\mu$ L of molecular biology grade H<sub>2</sub>O. The conditions for PCR were: 5 min at 94°C, 1 min denaturation at 94°C, alignment 1 min at 59°C, extension 1 min at 72°C and a final elongation of 5 min at 72°C, for 35 cycles. The digestions were performed with enzymes BsmAI for P1b and Acil for P1c (both New England Biolabs). The digestion mixture consisted of: 5  $\mu$ L molecular biology grade H<sub>2</sub>O, 1.5 $\mu$ L 1X NE Buffer, 1 $\mu$ L of 10 U/ $\mu$ L enzyme and 7.5 $\mu$ L DNA PCR product; total volume of 15 $\mu$ L. BsmAI was incubated at 55°C for 12 hours and Acil at 37°C for 12 hours. Both PCR products and digestions were checked by horizontal electrophoresis using a 2% agarose gel.

Once the procedure was developed and standardized, it was applied to a group of 60 Mexican Mestizo individuals, apparently healthy students of the School of Chemistry (UAEMEX, Toluca, Mexico), of which 30 were men and 30 women, with an age range of 18-25 years. All volunteered to participate and signed an informed consent letter; a 3 mL sample of peripheral blood was drawn from each individual in a tube with heparin (Vacutainer) and kept refrigerated until processing.

## RESULTS

The frequencies obtained in the group studied were as follows. For P1b genotypes (exon 5): 31.66% P1a/P1a (wild), 59.99% P1a/P1b (heterozygous) and 8.33% P1b/P1b (homozygous). For P1c genotypes (exon 6): 100% P1a/P1a (wild), 0% P1a/P1c (heterozygous) and 0% P1c/P1c (homozygous). Classifying by sex in our group, we found that men and women most frequent genotype P1a/P1b with 63.33% and 56.66% respectively, followed by P1a/P1a wild genotype with a frequency of 30% and 33% respectively; and finally P1b/P1b genotype with 10% in women and 6.66% in men. While P1c, genotype was present in wild P1a/P1a 100% of individuals. The detailed results are shown in Table 1.

## DISCUSSION

The frequencies of polymorphisms of exon 5 of the GSTP1 gene have different values in the examined group, P1a/P1b heterozygous genotype being the most frequent, followed by the wild P1a/P1a genotype and finally homozygous P1b/P1b. This type of distribution in which the heterozygous genotype is higher or has a larger distribution than homozygous wild genotypes, occurs when natural selection acts against both homozygotes, increasing the fitness of heterozygotes, as many diseases are naturally recessive (sickle cell anemia, Wilson's disease, among others), so it is unlikely that this genotype is susceptible to them and

**Table 1.** Genotype frequencies of GSTP1 polymorphisms

| Exon | Polymorphism (Genotype) | Men<br>n=30<br>(%) | Women<br>n=30<br>(%) | Total<br>n=60<br>(%) |
|------|-------------------------|--------------------|----------------------|----------------------|
| 5    | P1a/P1a                 | 30                 | 33.33                | 31.66                |
|      | P1a/P1b                 | 63.33              | 56.66                | 59.99                |
|      | P1b/P1b                 | 6.66               | 10                   | 8.33                 |
| 6    | P1a/P1a                 | 100                | 100                  | 100                  |
|      | P1a/P1c                 | 0                  | 0                    | 0                    |
|      | P1c/P1c                 | 0                  | 0                    | 0                    |

consequently the population will be polymorphic, with each heterozygote generation providing 50% of their gametes with each allele. This may explain the wide distribution of heterozygous polymorphism in our study group, because although P1a/P1b heterozygous polymorphism provide a decrease in enzyme activity and can make it more likely to develop toxicity or other conditions such as cancer, it may work in conjunction with other enzymes such as GSTT1, GSTM1, CYP1A1, CYPB1 in the biotransformation of xenobiotics (Leite et al., 2007, Oliveira et al. 2010).

In regard to GSTP1c polymorphisms (exon 6), the only genotype identified was wild P1a/P1a. In order to be considered a polymorphism, GSTP1c must have a population distribution greater than 2%. This not being the case, our findings may be related to the size of sample, which could account for the absence of P1c polymorphisms, both heterozygous and homozygous, P1a/P1c and P1c/P1c. We believe that in order to identify P1c it would be necessary to increase the number of individuals in the sample.

The development and application of molecular procedures such as the one here reported (PCR-RFLP) for the identification of polymorphisms in GSTP1, is a way to determine and quantify the geographic distribution and genetic variability of the population, allowing direct study of the genome of individuals in a relatively quick and easy way, offering the possibility to be incorporated into the polymorphic gene battery and to be considered, on the one hand, to identify populations or groups at risk of developing toxicity or other conditions such as cancer, and secondly, to select personalized treatments, as these polymorphisms influence the response to treatment.

## CONCLUSIONS

A reliable procedure was developed for the identification of polymorphisms in GSTP1. The application of this procedure to a group of Mexican Mestizo individuals, allowed us to determine that the polymorphism P1a/P1b

is the most common, whereas wild P1c allele was the only one present. These results show the frequencies of these polymorphisms in a group of Mexican Mestizo individuals, something that had not been previously done.

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