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Importance of the identification of the CYP3A4*2 polymorphism for the prescription of pharmacological treatment.

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Abstract

Variability in response to drugs is a problem in clinical practice. The rate of patients who respond adequately to pharmacological therapy is generally around 60. The CYP450 multienzyme complex is a microsomal system located in the endoplasmic reticulum. The enzymes participate in phase I metabolism of xenobiotics. CYP3A4 is the isoenzyme mostly expressed in liver. Its gene is polymorphic, of which CYP3A4*1A is the wild allele and CYP3A4*2 is a polymorphism which consists of the S222P substitution in the amino acid sequence, affecting the activity of the enzyme.
Methods: In this study, we determine the frequency of CYP3A4*2 polymorphism in Mexican individuals. 62 apparently healthy individuals, from Toluca de Lerdo, Mexico. The sample was 3 mL of peripheral blood. The DNA was extracted and PCR-RFLPs were performed.
Results: 58 individuals possess the wild allele in homozygosity CYP3A4*1A/CYP3A4*1A (94%) and 4 individuals were heterozygous CYP3A4*1A/CYP3A4*2 (6%). The homozygous polymorphic CYP3A4*2/CYP3A4*2 was not found in any individual.
Conclusion: The CYP3A4*2 polymorphism is rare in the population studied. Given the low frequency of CYP3A4*2 polymorphisms found in homozygous or heterozygous condition, it is advisable to consider the genotype of the patient before prescribing drugs metabolized by this gene.

Keywords: CYP3A4*2, Polymorphism, metabolism, PCR-RFLPs, Pharmacogenomics.

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Introduction

It is estimated that 99.9% of the DNA sequence of humans is identical and the remaining 0.1% are genetic variations; these are referred to as genetic polymorphisms. The study of these polymorphisms has applications in the field of medicine, since it is known that these variations in the DNA sequence that code for enzymes, can alter their function [1,2].

Pharmacogenomics focuses on the identification of gene variants that encode proteins that participate in the absorption, distribution, metabolism and excretion of drugs. The response to treatment depends not only on the age, gender and physiological state of an individual, but also on their gene load. This is relevant since it is estimated that most of the drugs carry out their optimal therapeutic activity in 50% of the patients who receive standard doses; the rest are under dosed or overdosed and this results either in a lack of response to treatment or in toxicity [3-5].

The cytochrome CYP450 multienzyme complex is a microsomal system that is located in the endoplasmic reticulum of the cell. Enzymes of this family are thiolase-type monooxygenases and consist of hemoproteins in which iron and NADH carry out oxidation, reduction, hydrolysis and hydration reactions in phase I of xenobiotic metabolism to produce polar compounds [6,7]. According to the similarity of the peptides, the cytochrome P450 system is classified into 18 families and 44 subfamilies [8]. The most abundant families in the liver are CYP3A, CYP1A2, CYP3A4, CYP2C, CYP2E1 and CYP2D6 [9].

CYP3A4 is the isoenzyme mostly expressed in the kidney, intestine and liver. It reaches up to 50% of the total CYP3A enzymes in the liver [8-10]. This gene is constitutive and is located in chromosome 7 region 21.1. It has a length of 27 Kb. It is composed of 12 introns and 13 exons, which encode the enzyme classified as polypeptide 4 of subfamily IIIA [11,12]. It is a polymorphic gene with 78 described variants of which CYP3A4*1A is the wild type allele and only the variants

CYP3A4*1B, CYP3A4*2, CYP3A4*4, CYP3A4*5, CYP3A4*18 and recently CYP3A4*22 are relevant, since these affect the activity of the enzyme [8,11,13,14]. The importance of the study of the CYP3A4 family lies in the fact that it is the enzyme responsible for the metabolism of approximately 50% of drugs, having as relevant substrates cortisol, imatinib, testosterone, progesterone, benzo-a-pyrenes, sterigmatocystin and aflatoxins, among others [10,11,15-17].

The CYP3A4*2 polymorphism consists of a substitution of a thymine by a cytosine at position 673 (T673C) of exon 7 of the nucleotide sequence of the gene, resulting in the change of a serine (S) by a proline (P) at position 222 (S222P) in the amino acid sequence. This change induces the formation of a turn in the helix of the protein, resulting in quantitative differences in the activity of the enzyme.

It has been reported that CYP3A4*2 participates in the metabolism of Imatinib, a drug frequently used in the treatment of patients with chronic myeloid leukemia. It can interfere with the effectiveness of treatment [11-18].

At present, the growing knowledge of the molecular mechanisms of health-disease processes is giving rise to the clinical practice of a new personalized medication for each individual, based on their gene load [19]. The presence or absence of polymorphisms is very relevant to response to treatment. Consequently, the objective of this study was to know the frequency of the CYP3A4*2 polymorphism in inhabitants of Toluca de Lerdo, Mexico, with the purpose of being considered for a personalized prescription of treatment.

Materials and Methods

Study group

The investigation was of a transversal nature. It was carried out in a group of apparently healthy students of the Autonomous University of the State of Mexico. Participation was by invitation and voluntary, those who accepted signed an informed consent letter. A 3 mL sample of peripheral blood in a Vacutainer tube with heparin was drawn from each participant.

Ethical considerations

The authors agree that all the research was carried out in compliance with government policies and the Declaration of Helsinki (1964). This study was approved by the Research Ethics Committee of the Medical Sciences Research Center of the Universidad Autónoma del Estado de México.

DNA extraction

DNA extraction was performed from whole blood with the Quick-gDNA Mini Prep Kit (Zymo Research). The DNA was quantified using a NanoPhotometer (Implen). Additionally, DNA quality and integrity were verified by horizontal electrophoresis on 1% agarose.

Genotyping

The identification of the CYP3A4*2 polymorphism was performed by PCR-RFLPs, according to Reyes et al., (2004), which consists of the amplification of the region of interest by endpoint PCR. The mixture had a final volume of 25 µL, containing 5 µL 5x PCR Buffer (Promega), 4 µL of 25 mM MgCl2 (Promega), 1 µL dNTPs Mix 10 mM (Fermentas), 10.7 µL of molecular biology grade H2O, 0.3 µL 5U/µL Taq Polymerase (Promega), 2 µL of the DNA template and 1 µL of the CYP3A4*2 primer. Forward: 5'-CCT GTT GCA TGC ATA GAC-3 'and 1 µL of the CYP3A4*2 primer. Reverse: 5'-GAT GAT GGT CAC ACA TAT C-3 ', both with a concentration of 30 pm/µL. The conditions used in this amplification were: 5 minutes at 95°C, denaturation 45 seconds at 95°C, alignment 45 seconds at 58°C, extension 45 seconds at 72°C and a final elongation of 5 minutes at 72°C, for 35 cycles [18].

The PCR products were digested with restriction enzyme MnII. The reaction mixture had a final volume of 20 µL, containing 7 µL molecular biology H2O, 2 µL 10X Buffer G (Thermo Scientific), 1 µL of the enzyme MnII 5U/µL (Thermo Scientific) and 10 µL DNA product of PCR. The reaction was incubated at 37°C for 12 hours and inactivated at 65°C for 15 minutes. Digestion products were verified by horizontal electrophoresis, in 1.5% agarose.

The identification of the polymorphism was based on the presence of DNA fragments of different sizes. A 357bp fragment corresponds to the wild-type allele CYP3A4*1A/CYP3A4*1A (T/T). A 213 bp fragment and a 144 bp fragment correspond to a heterozygous allele CYP3A4*1A/CYP3A4*2 (T/C). While the presence of a single 213 bp fragment corresponds to the polymorphism in homozygous condition CYP3A4*2/CYP3A4*2 (C/C). Additionally, a 9 bp product difficult to identify due to its size is generated in the wild and polymorphic allele [18].

Results

Socio-demographic characteristics

The study group consisted of 62 apparently healthy individuals, originally from Toluca de Lerdo, Mexico, of which 42 individuals (67.7%) were women and 20 individuals (32.3%) were men. In an age range of 18 to 30 years.

Genotyping

The allelic frequencies of the study group were as follows: 58 individuals possess the wild-type allele CYP3A4*1A/CYP3A4*1A (T/T), which represents 94%. Four individuals possess the heterozygous allele CYP3A4*1A/CYP3A4*2 (T/C), meaning a 6%. While the homozygous polymorphic CYP3A4*2/CYP3A4*2 (C/C) allele was not identified in any individual of the study group. Detail of the data is presented in Table 1.

Discussion

Like other populations in the world, Mexicans constitute a mestizo population, made up of ancestral Amerindian and European origins, and in a smaller proportion of Africans [20]. CYP3A4 is the most important drug metabolizing enzyme in humans, due to its prominent expression in the liver and intestine and its broad substrate specificity, which includes drugs from most therapeutic categories and many endogenous substances [20,21].

Table 1. *Allelic frequencies.*

Allele	n	Frequency (%)
CYP3A4*1A/CYP3A4*1A (T/T)	58	94
CYP3A4*1A/CYP3A4*2 (T/C)	4	6
CYP3A4*2/CYP3A4*2 (C/C)	0	0

Reyes et al. (2004), studied 69 individuals from Mexico. They found only the wild allele (T/T) [10]. While Reyes et al. (2008), reported the frequency of CYP3A4*2 in a population of northern Mexico, where they only identified one individual with the heterozygous allele (T/C) and the rest with the wild version (T/T) [14]. Merging the previous data with the results of this research, the average frequency of this polymorphism is <3%. Regarding the distribution of the CYP3A4*2 polymorphism in other regions of the world, similar results are reported. Sata et al. (2000), determined the frequency of this polymorphism in 162 individuals from three different ethnic groups, namely Caucasian from Finland, African-American and Chinese. They found only 2.7% of the heterozygous allele in the first group and 0% in the two remaining groups [18]. While Garsa et al. (2005), when studying two ethnic groups, the first conformed by 95 European individuals and the second one by 95 Africans found in both groups a frequency of 0% for the CYP3A4*2 polymorphism [22]. In this same line, Cavaco et al. (2013), conducted the study of 100 individuals from the south of Portugal and reported a frequency of 4.5% for the heterozygous allele (T/C) [23]. The results of the CYP3A4*2 polymorphism frequency reported here are similar to those reported in other populations. They are relevant in terms of their application to a pharmacogenomic approach.

The study of polymorphisms of CYP450 genes is relevant in the area of health. Particularly the CYP3A4 gene is important for high participation in the metabolism of antineoplastic drugs (vinblastine, imatinib, paclitaxel, sterigmatocystin, cyclophosphamide, doxorubicin, etoposide), immunosuppressants (ciclosporin), hormones (cortisol, testosterone, progesterone) and other xenobiotics (benzo-a-pyrene and aflatoxins) [10,11,15,17,24-26]. In addition to this, it has been determined that the CYP3A4*2 polymorphism affects the activity of the enzyme [18], used nifedipine as a substrate. They analyzed the enzymatic activity of the protein encoded by CYP3A4*2, their results showed a 7.5-fold decrease in the metabolism activity with this substrate. This decrease in enzymatic activity is important, since it can influence the metabolism of a large number of xenobiotics and drugs, resulting in an increase in the concentration of these molecules in blood, causing toxicity for the patient, especially when antineoplastic drugs are used [24-26].

Conclusion

It is important to note that the metabolism of a xenobiotic is carried out in most cases by a set of enzymes of Phase I and II of the metabolism and not individually, so it is necessary to study the combination of different allelic versions. The information generated from the study of these polymorphisms is relevant since it allows us to know the frequencies of these variations in a population, clarify their influence on health and monitor environmental risk or drugs to which an individual or a population may be exposed. This allows the promotion of pharmacogenetic studies that impact personalized treatment.

Conflict of Interest

The authors declare that they have no conflict of interest.

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