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Genotypic and allelic frequencies analysis of the 19 T>G polymorphism of the UGT1A6 gene in Mexican mestizo population of the state of Puebla

Análisis de las frecuencias genotípicas y alélicas del polimorfismo 19 T>G del gen UGT1A6 en población mestiza mexicana del estado de Puebla

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Abstract

UGT1A6 catalyzes the glucuronidation of several xenobiotics and drugs widely used. Changes in the glucuronidation rate are attributed to inter-personal and inter-ethnic variations, that can impact the expression or enzyme function. Frequencies of genetic polymorphisms of UGT1A6 have been reported in another countries; however, there are no date of frequencies in the Mexican population. The aim of this work was to determine the genotypic and allelic frequencies of UGT1A6 19T>G in a Mexican mestizo population of the state of Puebla and compare them with the frequencies observed in other populations. Peripheral blood DNA was obtained from 60 healthy adults and 19 T>G alleles were identified by the PCR-RFLP technique. Our results were compared with those observed in other ethnic groups, and we observed that our frequencies were lower than those reported in Chinese, Korean, Japanese, Hindu, and Greek populations. These results must be considered to make decisions when choosing the drugs administered to different ethnic groups.

UGT1A6, polymorphisms, Glucuronidation

Resumen

UGT1A6 cataliza la glucuronidación de diversos xenobióticos y fármacos ampliamente utilizados. Hay evidencia de que los cambios en la tasa de glucuronidación se atribuyen a variaciones interpersonales e interétnicas, que pueden impactar la expresión o la funcionalidad de las enzimas. Se han reportado frecuencias de distintos polimorfismos genéticos de UGT1A6; sin embargo, no hay reportes de sus frecuencias en población mexicana. El objetivo de este trabajo fue determinar las frecuencias genotípicas y alélicas de UGT1A6 19T>G en una población mestiza mexicana del estado de Puebla y compararlas con las frecuencias observadas en otras poblaciones. Se obtuvo ADN de sangre periférica de 60 adultos sanos y se identificaron los alelos 19 T>G mediante la técnica de PCR-RFLP. Al comparar los resultados obtenidos con los observados en otros grupos étnicos, se observó que las frecuencias del polimorfismo fueron menores a las reportadas en poblaciones chinas, coreanas, japonesas, hindús, y griegas. Estos resultados deben considerarse al momento de elegir los fármacos y sus dosis y ajustarlos al origen étnico de cada población.

UGT1A6, polimorfismos, Glucuronidación

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Introduction

UDP-glucuronosyl transferases (UGTs) are members of a super family of endoplasmic reticulum enzymes that catalyze the conjugation of glucuronic acid with a nucleophilic substrate. Glucuronic acid conjugates are rapidly removed from hepatocytes to be excreted primarily through bile. Glucuronidation is important for the detoxification and elimination of both endobiotic and xenobiotic compounds [1].

The organisms are constantly exposed to xenobiotic compounds, both natural (secondary metabolites of plants and toxins produced by molds and other organisms), as of anthropogenic origin, drugs, industrial chemicals, pesticides, pyrolysis products of cooked foods, alkaloids, pollutants, etc.

Liposolubility that allows many xenobiotics to be absorbed through the skin, lungs and digestive tract, is an obstacle to their elimination because lipophilic compounds can be easily reabsorbed. Consequently, the removal of lipophilic xenobiotics often depends on their conversion into water-soluble compounds by a process known as biotransformation. Enzyme-catalyzed reactions that produce biotransformation of xenobiotics are divided into two groups.

Phase I reactions comprise hydrolysis, reduction and oxidation. These rections expose or induce a functional group (-OH, NH2, _SH or COOH), and regularly only cause a small increase in hydrophobicity. Phase II biotransformation reactions include glucuronidation, sulfation, acetylation, methylation, glutathione conjugation and conjugation with amino acids (such as glycine, taurine, and glutamic acid).

The cofactors for these reactions act on functional groups that are in the xenobiotic or are induced or exposed during phase I of the biotransformation. Almost all phase II biotransformation reactions result in a large increase in the hydrophobicity of the xenobiotic, thus favoring its excretion. Phase II of biotransformation of xenobiotics may or may not be preceded by phase I [2].

UGTs are expressed in liver, lung, bile ducts, stomach, colon, kidney and brain [3, 4]. It has been observed that multiple compounds induce the activity of UGTs in humans. UGT 1A6 is the only isoform of UGT1A that is expressed in lung, is responsible for the detoxification of carcinogens such as benzo [a] pyrene (BaP) of tobacco smoke and 4 (Methylnitrosamino) -1- (3-pyridyl) -1-butanone (NNK, figure 1) [5-8].

Xenobiotic metabolism enzymes are highly polymorphic [9], single-nucleotide variations (SNPs) can induce changes in the phenotype, if they are located in non-coding regions they can alter genetic expression, while being in coding regions can alter the function of proteins by an amino acid change.

Genetic polymorphisms in UGTs have been associated with risk of suffering from different types of cancer, it has also been observed that some polymorphic variants are associated with reduction in plasma drug concentrations, which could affect the pharmacokinetics of medications. The allelic variant UGT1A6 19 T> G has been associated with an increased risk of lung cancer in the Chinese population, so it has been proposed as a marker of genetic susceptibility [10].

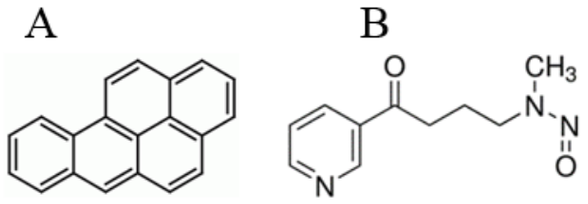

Figure 1 Chemical structure of: A) benzo [a] pyrene and B) 4 (Methylnitrosamino) -1- (3-pyridyl) -1-butanone, both compounds are metabolized for excretion by the enzyme UGT1A6

UGT1A6 also participates in the metabolism of valproic acid (VPA, figure 2), an antiepileptic drug consumed particularly in less developed countries. The antiepileptic effects of VPA require stable plasma concentrations, which are associated with genetic polymorphisms that affect the pharmacokinetics and pharmacodynamics of VPA.

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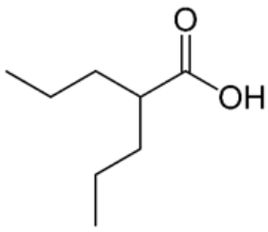

Figure 2 Chemical structure of valproic acid (di-n-propylacetic acid)

Polymorphisms 19T> G / 541A> G / 552A> C in UGT1A6, have been associated with an increase in enzymatic activity. In patients with single nucleotide polymorphisms (SNPs), an increase in glucuronidation of VPA is observed. Patients with double heterozygosity at nucleotide positions 19T> G / 541A> G / 552A> C, have significantly lower plasma VPA concentrations than those with wild genotype or single heterozygosity. Consequently, these patients required higher daily doses of VPA in the following treatment to increase plasma VPA concentrations and prevent seizures [11].

Given the clinical importance of the 19T> G polymorphism in the UGT1A6 gene (figure 3), the objective of this work is to investigate its genotypic and allelic frequencies in the Mexican mestizo population of the state of Puebla and compare them with the frequencies observed in other populations. This information will help to understand the genetic susceptibility to diseases due to exposure to xenobiotics, as well as the therapeutic efficacy of drugs metabolized by UGT1A6, in this ethnic group.

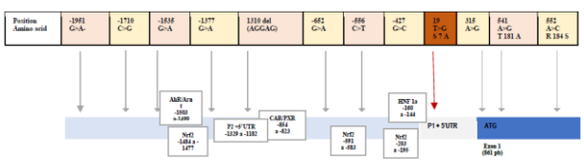

Figure 3 Genetic polymorphisms identified in exon 1 and regulatory region 5 '(-2052pb) of the UGT1A6 gene. The location of the experimentally identified transcription factor binding sites (for the Ah / Aril receptor translocating nuclear receptor and Nrf2 receptor [12-13] or by computer homology analysis (nuclear hepatocyte factor 1α and CAR / PXR) is shown. , MatInspector, [14] The position of the gene is given according to the first nucleotide predicted for the start codon of UGT1A6 (nucleotide 108, 262 of the GenBank sequence with reference NG_002601.1). Modified from Krishnaswamy S. et al. 2005 [15]

Methods

Sampling of individuals

60 samples of 4 mL of peripheral blood were collected from healthy individuals in lilac vacuette tubes with EDTA (Greiner bio-one 454209H) 45 women and 15 unrelated men among them between 16 and 87 years, inhabitants of the state of Puebla with three lines ascending generational born within Mexican territory, who signed an informed consent and completed a questionnaire with their demographic data. The institutional ethics committee reviewed and approved the research protocol.

DNA purification

The DNA was purified with the sucrose and sodium perchlorate method described by Daly et al. 1996 [16]. Cell nuclei were isolated by adding 36 mL of lysis buffer (320 mM sucrose, 5 mM MgCl2, 1% Triton X-100, 10 mM Tris-HCl, pH 7.4) to 4 mL of blood. The mixture was vigorously stirred in a vortex and centrifuged at 2000g for 20 minutes at 4 ° C. The button was resuspended in 2 ml of suspension buffer (150 mM NaCl, 60 mM EDTA, 1% SDS, 400 mM Tris-HCl, pH 7.4) and 0.5 mL of 5M sodium perchlorate (NaClO4). This suspension was mixed by rotation 15 minutes at room temperature and subsequently incubated at 65 ° for 30 minutes. 2 mL of cold chloroform (at -20 ° C) was added and mixed again by rotation at room temperature for 10 minutes followed by centrifugation at 1400g for 10 minutes.

The aqueous phase containing the DNA (the highest phase) was transferred to a 15 mL tube and 2 volumes of cold ethanol (at 4 ° C) were added. The tube was inverted several times to precipitate the DNA and transfer it to a 2 mL Eppendorf tube. The DNA was washed twice with 70% ethanol and allowed to dry at room temperature in a laminar flow hood, once dry, 200 µL of TE buffer (10 mM tris-HCl, 1 mM EDTA pH 7.4) was added and incubated 16 hours at 60 ° C to resuspend it, subsequently its concentration was quantified on a NanoDrop 1000 (Thermo Scientific) spectrophotometer and stored at -20 ° C until used for genotyping of the 19 T>G polymorphism of the UGT1A6 gene.

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Genotyping of UGT1A6 19 T>G by PCR-RFLP

Genotyping was performed using the Restriction Fragment Length Polymorphism (PCR-RFLP) technique. The amplification of the polymorphic segments by Polymerase Chain Reaction (PCR) was carried out in a Bio-Rad T100 thermocycler, placing 100 ng of genomic DNA, 1X Taq buffer, 1 unit of Taq Polymerase (Qiagen Cat No. 201203), 50 µM dNTPs (Qiagen Cat. No. 201901), 500 nM oligonucleotides described by Nagar S. et al 2014 Forward F-51 5 'GAT TTG GAG AGT GAA AAC TCT TT 3' and Reverse R 184 5 ' CAG GCA CCA CCA CTA CAA TCT C 3 '[17].

The conditions used for amplification were the following: a first denaturation step at 94 ° C for three minutes, followed by 40 cycles of denaturation at 94 ° C for 30 seconds, alignment at 58 ° C for 30 seconds, extension at 72 ° C for 30 seconds and one last step of 72 ° C for 3 minutes. An amplicon of 237bp was obtained.

The restriction with the FastDigest HhaI enzyme (Thermo Scientific FD1854) was incubated for 12 h at 37 ° C. Digestion products were separated by horizontal electrophoresis on 3% agarose gels containing a fluorescent nucleic acid dye with UV light (SmartGlow Pre-Stain E4500-PS Accuris).

The wild allele (19 T) does not have a cut-off site for the HhaI enzyme so that in a homozygous condition a single band of 237 bp is observed, the mutant allele (19 G) does have the cut-off site, so in condition homozygous, two bands are observed, one of 165 bp and another of 72 bp, in the heterozygous individuals three bands are observed 237 bp, 165 bp and 72 bp (figure 4).

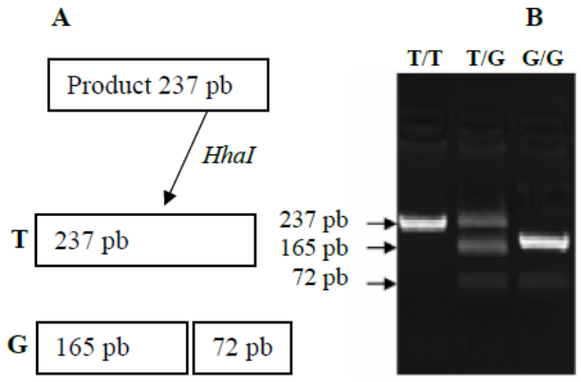

Figure 4 Determination of the UGT1A6 19T>G polymorphism by the PCR-RFLP assay. A) the 237 bp PCR amplification product (amplicon) digested with the HhaI endonuclease for the identification of the 19 T> G alleles by restriction patterns. B) The wild allele (19 T) does not have a cut-off site for the HhaI enzyme, so in a homozygous condition a single band of 237 bp is observed, the mutant allele (19 G) does show the cut-off site, so in Homozygous condition two bands are observed, one of 165 bp and another of 72 bp, in heterozygous condition T / G three bands are observed, modified from Nagar S. et al 2004 [17]

Results

This study included 60 healthy individuals, 75% women and 25% men between 16 and 87 years, inhabitants of the state of Puebla. The DNA amplification segment that was obtained by PCR had a size of 237 bp, in Figure 5, its sequence is shown in the mRNA transcript [18] delimited by the hybridization position of the oligonucleotide primers we used (PCR in silico), the position of the SNP is also observed.

The allelic and genotypic frequencies of UGT1A6 19 T>G identified by PCR-RFLP in the mestizo population of the state of Puebla are reported in Table 1. The most common genotypic frequency was the wild homozygous (T / T) 0.683 (41 individuals) , followed by heterozygous (T / G) with a genotypic frequency of 0.30 (18 individuals), only one homozygous mutant individual (G / G), genotypic frequency 0.016 was observed. The frequency of the mutant allele (G) in our population was 0.166 and is below that reported for other populations: In the Chinese Dong ethnicity a frequency of 0.17 [19] was reported, in the Chinese ethnicity She 0.189 [19], in the Korean population 0.2 [20], in the Japanese population 0.226 [21], in the Chinese ethnic group Han 0.38 [19], in the Hindu population 0.356 [22] and the population that reported the highest frequency of the mutant allele was the Greek with 0.37 [2. 3].

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gtttagggaaaatacctaggagccctgtgatttggagagtgaaaactctttattaccgttgtt
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agtgctgggcaagtttacttttttttctgatgtttcctacaactaaaaataaattaataaatttata
taaattctatttaaaaaaaaaaaaa

Figure 5 Human UGT1A6 UDP glucuronosyl transferase messenger RNA transcript obtained from GenBank, NCBI sequence reference: NM_001072.3 2495 bp [18], the yellow amplified segment, delimited by the turquoise priming oligonucleotides and the polymorphism of a single nucleotide 19 T>G in red color

Country	N	Frequencies Genotypic			Allelic Frequencies		Appointment
		T/T	T/G	G/G	T	G	
Puebla Mexico	**60**	**0.683 (41)**	**0.30 (18)**	**0.016 (1)**	**0.834**	**0.166**	**This studio**
China	531 (Han)	0.586 (311)	0.352 (187)	0.062 (33)	0.762	0.238	[19]
	268 (Dong)	0.687 (184)	0.287 (77)	0.026 (7)	0.83	0.17	[19]
	259 (She)	0.654 (167)	0.332 (86)	0.023 (6)	0.811	0.189	[19]
Korea	50	0.64 (32)	0.32 (16)	0.04 (2)	0.8	0.2	[20]
Japan	195	0.6 (117)	0.348 (68)	0.051 (10)	0.774	0.226	[21]
India	80	0.45 (36)	0.388 (31)	0.162 (13)	0.644	0.356	[22]
Greece	134	0.381 (51)	0.492 (66)	0.127 (17)	0.63	0.37	[23]

Table 1 Genotype and allelic frequencies of AUG1A6 19 T>G observed in this population and reported in previous work in different populations

Population genetic balance analysis using an X^2 test

All frequencies of the analyzed locus are in Hardy Weinberg equilibrium by presenting values of X2 lower than the critical value (the critical value of X2 for the analysis with two degrees of freedom and an $\alpha = 0.05$ is 3.84), which indicates that the genetic composition of our population remains in equilibrium, that is, natural selection is not acting on it and therefore an evolutionary change is not being generated.

Discussion

UGT1A6 is an enzyme that catalyzes the glucuronidation of various substrates including environmental pollutants such as BaP and drugs such as VPA, acetaminophen and other non-steroidal anti-inflammatory drugs. There is evidence to support the idea that changes in the glucuronidation rate are attributed to interpersonal and inter-ethnic variations, which may impact the expression or functionality of enzymes [24].

About a dozen polymorphisms have been identified in exon 1 of the UGT1A6 gene and its adjacent 5 'regulatory region [14], in this study the frequency of 19 T 19G polymorphism was analyzed in 60 mestizo individuals from the state of Puebla and It was observed that the incidence of the mutant allele is lower than that reported for other populations. Although the 19 T>G polymorphism is not located in the coding region but in the 5'UTR region, it has been observed that when combined with the 541 A>G and 552 A>G polymorphisms, it increases the risk of lung cancer due to its Participation in the metabolism of carcinogenic compounds such as BaP and NNK [10]. On the other hand, it has been reported that it increases the enzymatic activity, modifying the pharmacokinetics of some medications, reducing the plasma time of compounds metabolized by this enzyme [11].

Unlike other reported populations, in our population we observed few individuals carrying the G allele in a homozygous condition, indicating low risk of loss of medication functionality due to an increase in glucuronidation rate.

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It is important to mention that in the metabolism of drugs and other xenobiotics, in addition to UGT1A6, other highly polymorphic enzymes such as cytochrome P450 and Sulfotransferases participate, so it is necessary to evaluate the additive effect conferred by allelic variations in metabolic enzymes. Determining the allelic frequency of these variants in our population will serve to explain their role in the disposition and toxicity of medications and other compounds, generating useful pharmacogenetic information to predict therapeutic results.

Conclusion

Our work is the first to report genotypic and allelic frequencies of the 19 T>G polymorphism of the UGT1A6 enzyme in the Mexican population. The frequency we found for the mutant allele (0.166) is lower than that reported in other populations (Chinese, Korean, Japanese, Hindu and Greek). Due to the size of the population sample (n = 60) and the low frequency of this allele, we only find it in a homozygous condition in an individual. Studies are needed to estimate genetic frequencies with a greater number of individuals, as well as patients suffering from diseases that have been associated with this polymorphism in other populations.

Studying how genetic variations between individuals affect the metabolism of drugs producing a variable response with respect to their efficacy or adverse effects profile will allow us to make decisions when choosing the administered drugs and adjust them to the ethnic origin of a given population.

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