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Short Communication

Genetic variability of glutathione S-transferase enzymes in human populations: Functional inter-ethnic differences in detoxification systems

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ABSTRACT

Glutathione S-Transferase enzymes (GSTs) constitute the principal Phase II superfamily which plays a key role in cellular detoxification and in other biological processes. Studies of GSTs have revealed that genetic polymorphisms are present in these enzymes and that some of these are Loss-of-Function (LoF) variants, which affect enzymatic functions and are related to different aspects of human health.

The aim of this study was to analyze functional genetic differences in GST enzymes among human populations. Attention was focused on LoF polymorphisms of GSTA1, GSTM1, GSTO1, GSTO2, GSTP1 and GSTT1 genes. These LoF variants were analyzed in 668 individuals belonging to six human groups with different ethnic backgrounds: Amhara and Oromo from Ethiopia; Colorado and Cayapa Amerindians and African Ecuadorians from Ecuador; and one sample from central Italy. The HapMap database was used to compare our data with reference populations and to analyze the haplotype and Linkage Disequilibrium diversity in different ethnic groups.

Our results highlighted that ethnicity strongly affects the genetic variability of GST enzymes. In particular, GST haplotypes/variants with functional impact showed significant differences in human populations, according to their ethnic background. These data underline that human populations have different structures in detoxification genes, suggesting that these ethnic differences influence disease risk or response to drugs and therefore have implications for genetic association studies involving GST enzymes.

In conclusion, our investigation provides data about the distribution of important LoF variants in GST genes in human populations. This information may be useful for designing and interpreting genetic association studies.

1. Introduction

Genes and environment are included in an "action-reaction" mechanism, which determines in the expression of the human phenotypes (Li et al., 2012). Genetic predisposition may strongly affect not only a person's susceptibility to toxic compounds but also their response to drug response (Mroziewicz and Tyndale, 2010). Therefore, the study of human genetics helps to elucidate many aspects of human health. For example, the study of genes involved in cellular detoxification may be useful for analyzing the interaction between genetics and the environment (Piacentini et al., 2010). Indeed, detoxification enzymes are directly involved in interactions between living organisms and their environments (diet, climate, and lifestyle) (Lampe, 2007). The cellular detoxification mechanism is broken down into three phases: Phase I (oxidation, reduction and hydrolysis), Phase II (conjugation), and Phase III (excretion) (Omiecinski et al., 2011). The enzymes involved in the detoxification processes showed significant inter-ethnic and inter-individual differences in their efficiency (Polimanti et al., 2011a). These differences in the enzymatic systems are due to genetic, and environmental factors and may explain the ethnic diversity observed in the susceptibility to exposure to some xenobiotic compounds (Thier et al., 2003). In particular, the investigation of ethnic differences in variants associated with significant alterations in the coding-proteins, called Loss-of-Function (LoF), may contribute to our understanding at the population level the genetic predisposition to the disease or to drug response.

Among detoxification enzymes, glutathione S-transferases (GSTs) are multi-functional proteins that constitute the principal superfamily

Abbreviations: GSTs, Glutathione S-Transferases; LoF, Loss-of-Function; MAPEG, Membrane-Associated Proteins involved in Eicosanoid and Glutathione metabolism; GSTA, GST alpha class; GSTM, GST mu class; GSTP, GST pi class; GSTT, GST theta class; GSTK, GST kappa class; GSTZ, GST zeta class; GSTO, GST omega class; CNVs, copy number variants; X^2, chi-square test; LD, Linkage Disequilibrium; ASW, African ancestry in Southwest USA; CEU, Utah residents with Northern and Western European ancestry from the CEPH collection; CHB, Han Chinese in Beijing, China; CHD, Chinese in Metropolitan Denver, Colorado; GIH, Gujarati Indians in Houston, Texas; JPT, Japanese in Tokyo, Japan; LWK, Luhya in Webuye, Kenya; MEX, Mexican ancestry in Los Angeles, California; MKK, Maasai in Kinyawa, Kenya; TSI, Tuscan in Italy; YRI, Yoruban in Ibadan, Nigeria; IL-1β, interleukin-1β.

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of Phase II enzymes (Frova, 2006). These enzymes are involved in a number of catalytic processes, such as reactive electrophiles' detoxification, in biosynthesis of leukotrienes, prostaglandins, testosterone and progesterone, and tyrosine degradation. These proteins also play a role as non-enzymatic modulatory elements (Hayes et al., 2005). This enzymatic superfamily is composed of three different families: mitochondrial, microsomal (or MAPEG, Membrane-Associated Proteins involved in Eicosanoid and Glutathione metabolism), and cytosolic. The cytosolic family is the most abundant and the cytosolic GSTs are classified in seven classes based on chromosomal location and on sequence similarity: alpha (GSTA), mu (GSTM), pi (GSTP), theta (GSTT), kappa (GSTK), zeta (GSTZ) and omega (GSTO). Each cytosolic class is usually constituted by multiple members and is located in a specific chromosomal cluster. Numerous variants have been identified in GST genes, and some of these polymorphisms can be classified as LoF because they are associated with significant alterations in the enzymatic functions (Fuciarelli et al., 2009; Josephy, 2010). Several studies have explored whether the LoF variants of GSTs are significantly associated with disease risk, highlighting positive outcomes for different types of cancers (Di Pietro et al., 2010) and for other common disorders, such as neurologic (Piacentini et al., 2012a, 2012b), cardio-vascular (Polimanti et al., 2011b), pregnancy-related (Polimanti et al., 2012), and allergic diseases (Piacentini et al., 2012c). These studies point out that great inter-ethnic diversity is present in the allele frequencies of some LoF variants of GST genes and that it is possible to observe that ethnicity significantly influences these GST-disease associations (Piacentini et al., 2011). To understand the variability of GST genes in worldwide populations, different studies have analyzed LoF variants in different ethnic groups, confirming that human demographic history affects GST gene distribution (Polimanti et al., 2011c). Unfortunately, most of these studies have focused their attention on the copy number variants (CNVs) of GSTM1 and GSTT1 genes, leaving the other LoF GST variants poorly investigated (Gaspar et al., 2002; Polimanti et al., 2011a).

The aim of this study was to analyze functional genetic differences in GST enzymes among human populations. Attention was focused on LoF polymorphisms of GSTA1 (rs3957357), GSTM1 (CNV), GSTO1 (rs4925, rs11509437, rs11509438), GSTO2 (rs156697), GSTP1 (rs1695) and GSTT1 (CNV) genes that were investigated in populations with African, American and European origins. The selection of variants was based on their functional impacts and on their implication in human disease. To provide a comprehensive analysis of human diversity, our data were compared with the genetic information available in the HapMap project (International HapMap 3 Consortium et al., 2010). Moreover, HapMap data were used to analyzed the ethnic differences in the structures of the investigated GST genes.

2. Materials and methods

2.1. Subjects

A total of 668 unrelated adult individuals of both sexes have been typed: Amhara ($n=100$), Oromo ($n=97$), Cayapas ($n=114$), Colorados ($n=78$), African Ecuadorians ($n=159$), and Italians ($n=120$). 5–10 ml of peripheral blood from each subject was collected by venipuncture and stored in heparinized. Each donor was asked to supply name, birthplace, language and ethnicity for three generations, in order to allow us to determine the extent of recent admixture. Further information about these human groups is available in previous studies (De Angelis et al., 2012; De Stefano et al., 2002; Polimanti et al., 2010).

2.2. Genotyping

Genotyping of rs3957357 (GSTA1*-69C/T), rs4925 (GSTO1*A140D), rs156697 (GSTO2*N142D) and rs1695 (GSTP1*I105V) was performed using the PCR-restriction fragment length polymorphism (RFLP) method. rs11509437 (GSTO1*E155del) and rs11509438 (GSTO1*E208K) were typed using the confronting two-pair primer and the allele-specific methods, respectively. Genotyping of GSTM1 and GSTT1 CNVs was carried out by a Multiplex PCR reaction. Methodologies have been described in our previous studies (Piacentini et al., 2010; Polimanti et al., 2010).

2.3. Statistical analysis

Allele frequencies were computed by the genotype-counting method. Hardy–Weinberg equilibrium was evaluated using the chi-square (χ^2) test. Population comparisons and AMOVA were performed by Arlequin 3.5.1.2 (Excoffier and Lischer, 2010). Pairwise F_{ST} differences and F_{ST} P values were calculated to analyze the inter-population differences. One hundred ten permutations of individuals between populations were computed to test the significance of distances and 0.05 was the minimum P value of a test to be considered as significant. To compare the GST allelic frequencies in worldwide populations, correspondence analysis was utilized (Greenacre, 1992). Linkage disequilibrium (LD) analysis was performed by Haploview version 3.2 (Barrett et al., 2005) and graphically displayed using Haploview linkage disequilibrium plots. To identify the functional impact of the analyzed SNPs, FASTSNP (Function Analysis and Selection Tool for SNPs) was used (Yuan et al., 2006).

3. Results

Table 1 shows the genotype frequencies of GST LoF variants observed in the six human groups considered. Genotype distributions were in

Table 1
Genotype frequencies of GST LoF variants in worldwide populations.

	African Ecuadorians $n=159$	Amhara $n=100$	Cayapas $n=114$	Colorados $n=78$	Italians $n=120$	Oromo $n=97$
GSTA1*-69C/T						
C/C (%)	84 (53)	55 (55)	46 (41)	43 (55)	54 (45)	48 (50)
C/T (%)	65 (41)	35 (35)	62 (54)	34 (44)	47 (39)	43 (44)
T/T (%)	10 (6)	10 (10)	6 (5)	1 (1)	19 (16)	6 (6)
GSTM1						
Positive (%)	87 (55)	50 (50)	92 (81)	37 (47)	61 (51)	60 (62)
Null (%)	72 (45)	50 (50)	22 (19)	41 (53)	59 (49)	37 (38)
GSTO1*A140D						
A/A (%)	123 (77)	62 (62)	108 (95)	64 (82)	54 (45)	54 (56)
A/D (%)	31 (20)	35 (35)	6 (5)	14 (18)	62 (52)	35 (36)
D/D (%)	5 (3)	3 (3)	0 (0)	0 (0)	4 (3)	8 (8)
GSTO1*E155del						
E/E (%)	148 (93)	95 (95)	98 (86)	69 (88)	109 (91)	94 (97)
E/del (%)	11 (7)	5 (5)	16 (14)	9 (12)	11 (9)	3 (3)
GSTO1*E208K						
E/E (%)	145 (91)	95 (95)	95 (83)	70 (90)	106 (88)	93 (96)
E/K (%)	14 (9)	5 (5)	19 (17)	8 (10)	14 (12)	4 (4)
GSTO2*N142D						
N/N (%)	27 (15)	15 (15)	98 (86)	64 (82)	51 (43)	17 (18)
N/D (%)	89 (60)	56 (56)	15 (13)	13 (17)	58 (48)	45 (46)
D/D (%)	43 (25)	29 (29)	1 (1)	1 (1)	11 (9)	35 (36)
GSTP1*I105V						
I/I (%)	55 (35)	64 (64)	45 (40)	32 (41)	63 (53)	60 (62)
I/V (%)	66 (41)	29 (29)	41 (36)	27 (35)	47 (39)	31 (32)
V/V (%)	38 (24)	7 (7)	28 (24)	19 (24)	10 (8)	6 (6)
GSTT1						
Positive (%)	143 (90)	61 (61)	111 (97)	68 (87)	86 (72)	63 (65)
Null (%)	16 (10)	39 (39)	3 (3)	10 (13)	34 (28)	34 (35)