Genetics and Molecular Biology, 34, 1, 11-18 (2011)

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Research Article

Superoxide dismutase, catalase, glutathione peroxidase and gluthatione S-transferases M1 and T1 gene polymorphisms in three Brazilian population groups

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Abstract

Antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX1) reduce the oxidation rates in the organism. Gluthatione S-transferases (GSTs) play a vital role in phase 2 of biotransformation of many substances. Variation in the expression of these enzymes suggests individual differences for the degree of antioxidant protection and geographical differences in the distribution of these variants. We described the distribution frequency of CAT (21A/T), SOD2 (Ala9Val), GPX1 (Pro198Leu), GSTM1 and GSTT1 polymorphisms in three Brazilian population groups: Kayabi Amerindians (n = 60), Kalunga Afro-descendants (n = 72), and an urban mixed population from Federal District (n = 162). Frequencies of the variants observed in Kalunga (18% to 58%) and Federal District (33% to 63%) were similar to those observed in Euro and Afro-descendants, while in Kayabi (3% to 68%), depending on the marker, frequencies were similar to the ones found in different ethnic groups. Except for SOD2 in all population groups studied here, and for GPX1 in Kalunga, the genotypic distributions were in accordance with Hardy-Weinberg Equilibrium. These data can clarify the contribution of different ethnicities in the formation of mixed populations, such as that of Brazil. Moreover, outcomes will be valuable resources for future functional studies and for genetic studies in specific populations. If these studies are designed to comprehensively explore the role of these genetic polymorphisms in the etiology of human diseases they may help to prevent inconsistent genotype-phenotype associations in pharmacogenetic studies.

Key words: antioxidants, PCR-RFLP, gene polymorphisms, Brazilian ethnicities, population genetics, pharmacogenetics.

Received: May 20, 2010; Accepted: August 24, 2010.

Introduction

There has been much interest and research on single nucleotide substitutions (SNPs) in order to understand the maintenance of such polymorphisms in human populations. These data are useful for studying human evolution and the mechanisms that maintain genetic variability in human populations, as well as for identifying genes associated with complex diseases (Nachman and Crowell, 2000). Many potentially significant genetic variants related to oxidative stress have already been identified (Morgenstern, 2004). Several SNPs have been reported to result in changes in the levels or the activities of antioxidant enzymes, which can lead to reduction in protection against oxidative stress (Forsberg *et al.*, 2001). To gain a better understanding of the biological significance of these polymorphisms, studies are required to map their distribution in several ethnicities, since they vary among ethnic groups.

The known superoxide scavenger in mitochondria, manganese superoxide dismutase (MnSOD or SOD2, EC 1.15.1.1), is encoded by a nuclear gene located on chromosome 6q25 (Rosenblum *et al.*, 1996). It is synthesised with a mitochondrial targeting sequence (MTS), which drives its mitochondrial import. In the mitochondrial matrix, the MTS is cleaved, and the mature protein assembles into the active tetramer (Akyol *et al.*, 2005). The cytosine to thymine substitution at nucleotide 47 provokes a valine to alanine (Val9Ala, ref SNP ID: rs179972) substitution in the SOD2 MTS. This, in turn, induces a conformational change which has been reported to change mitochondrial processing efficiency, to affect the transport of SOD2 to the mitochondria, and to decrease SOD2 efficiency against oxida-

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tive stress (Shimoda-Matsubayashi *et al.*, 1996; Akyol *et al.*, 2005). The Ala allele varies among ethnic groups (Zhao *et al.*, 2005) and has been associated with increased risk of different diseases related to oxidative stress and abnormal free radical defence mechanisms (Shimoda-Matsubayashi *et al.*, 1996; Mitrunen *et al.*, 2001; Yen *et al.*, 2003; Olson *et al.*, 2004; Akyol *et al.*, 2005; Choi *et al.*, 2008).

Glutathione peroxidase 1 (GPX1, EC 1.11.1.9), expressed mainly in erythrocytes (Brigelius-Flohé, 1999), detoxifies hydrogen peroxide and organic hydroperoxides using glutathione in its reduced form (GSH) as co-substrate (Zhao *et al.*, 2005; Ravn-Haren *et al.*, 2006). The GPX1 gene (locus 3p21.3) contains the Pro198Leu SNP (ref SNP ID: rs1050450) whose Leu allele has been implicated in GPX1 activity, which becomes less responsive to stimulation (Zhao *et al.*, 2005). Studies have also associated this variant with increased risk of some kinds of cancer (Ratnasinghe *et al.*, 2000; Hu and Diamond, 2003; Zhao *et al.*, 2005). However, such associations were not consistently observed in all populations studied, since Leu allele frequency varies according to ethnic group (Zhao *et al.*, 2005).

Catalase (CAT, EC 1.11.1.6) is an enzyme whose major role involves controlling H_2O_2 concentrations in human cells, converting H_2O_2 into H_2O and O_2 (Ahn *et al.*, 2006). The CAT gene (locus 11p13) presents an apparently neutral polymorphism, CAT -21A/T (ref SNP ID: rs7943316), located within the promoter region, close to the translational initiation site (Ukkola *et al.*, 2001; Góth and Vitai, 1997; Góth *et al.*, 2004). For this polymorphism, no effects have been reported on catalase expression, catalase activity, or association with disease/pathological changes (Góth *et al.*, 2004). Considering that T allele frequency varies among ethnic groups (Ukkola *et al.*, 2001; Young *et al.*, 2006), studies mapping the distribution of this allele's frequency in several ethnicities can be important to gain a better understanding of its biological significance.

The glutathione S-transferases M1 (GSTM1) and T1 (GSTT1) genes code for the cytosolic enzymes GST-μ (mu) and GST-θ (theta), respectively. These enzymes catalyze reactions involving the conjugation between reduced glutathione (GSH) and a variety of eletrophilic compounds (Cotton *et al.*, 2000; Cho *et al.*, 2005), most of these being xenobiotics or products of oxidative stress (Cotton *et al.*, 2000). The glutathione S-transferase M1 (GSTM1, locus 1p13.3) and T1 (GSTT1, locus 22q11.2) genes may be deleted (null alleles/null genotypes) and these polymorphisms lead to altered GST activity, contributing to inter-individual differences (Hayes and Strange, 2000). Individuals with homozygous deletions do not have detectable GSTT1 or GSTM1 enzyme activity (Landi, 2000), and associations between GSTM1 and/or GSTT1 null genotypes with cardiovascular diseases (Kim *et al.*, 2007) and cancer (Garte *et al.*, 2001; Cha *et al.*, 2007; Hatagima *et al.*, 2008) have been reported. Inter-ethnic differences in the allele frequencies of GST null genotypes have been documented worldwide and some gradients and intra-ethnic differences have already been reported (Cotton *et al.*, 2000; Landi, 2000; Cho *et al.*, 2005).

In Brazil there are few studies that describe these antioxidant polymorphisms. The Brazilian population as a whole is very mixed and heterogeneous, primarily as a result of five centuries of inter-ethnic crosses among Europeans, Africans and Amerindians (Alves-Silva *et al.*, 2000). Samples from four major regions of Brazil (North, Northeast, Southeast and South), verified by genomic comparison in a panel of population-specific alleles, showed that the African contribution ranged from 4 to 34%, and the Amerindian from 0 to 27% (Parra *et al.*, 2002). It is believed that this miscegenation can influence the distribution of certain polymorphisms.

To perform case-control studies analysing the association of certain polymorphisms with the risk of developing certain diseases, it is important to know the frequency distribution of these genes in human populations. Thus, this work aims to describe the distribution of frequencies of CAT (21A/T), SOD2 (Ala9Val), GPX1 (Pro198Leu), GSTM1 and GSTT1 gene polymorphisms in three Brazilian population groups.

Material and Methods

Samples

The Brazilian samples analysed in this study were taken from 304 individuals from three different ethnicities: Kayabi (n = 60), Kalunga (n = 72) and Federal District (n = 172). The Kayabi are a Tupi-Guarani Amerindian tribe (Rodrigues, 1994) with a population of about 1,000 found mainly in the Xingu Indigenous National Park (Mato Grosso State). The Kayabi village sampled consisted of 110 individuals living on the banks of the Teles Pires River (11°37'0" S and 55°40'60" W) (Klautau-Guimarães *et al.*, 2005a, b). More details about this tribe can be found in Rodrigues *et al.* (2002). The sample (n = 60) used here was collected in 2000 and consisted of 31 males and 29 females, with a median age of 24.5 years and no first-degree (parent-offspring) relationship. The Kalunga are an Afro-derived Brazilian group with an estimated population of 5,300. This group lives in midwestern Brazil, in a rural area of northeastern Goiás State (15°30' S to 16°03' S ; 47°25' W to 48°12' W) (Oliveira *et al.*, 2002). Historically and numerically, the Kalunga are one of the most important Brazilian Afro-derived populations, known as *quilombos*. The Kalunga live in several subregions with different degrees of isolation. The sample (n = 72) used here was collected in 2001 and 2002 and consisted of 30 males and 42 females from the Vão das Almas and Vão de Muleque subregions, with a median age of 43.3 years and a relationship coefficient of up to 1/16. The Federal District (15°30' S to 16°03' S and 47°25' W to 48°12' W) was founded in 1960, and in 2008 it had an urban population of 2,606,885 (2009

IBGE census). Most of the Federal District population initially consisted of migrants from other regions of Brazil (Queiroz, 2006), and currently almost half of the District's inhabitants are migrants. The sample used here (n = 172) was collected in 2002 and consisted of 71 males and 101 females with a median age of 21.1 years. Based on the subjects' self-declared skin colour, 68.5% were ethnically mixed, 24.7% were white, 1.7% were black, and 5.1% did not declare their colour.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Committee for Health Sciences Faculty Research of the University of Brasília and by the National Commission for Ethics in Research (CONEP). Written informed consent was obtained from all subjects and oral informed consent was obtained in Kayabi village.

Laboratory and statistical procedures

Genomic DNA was isolated from peripheral blood samples collected in Vacutainer tubes containing EDTA using the purification kit GFX (GE Healthcare, Buckinghamshire, England). The samples were stored below -20 °C until analysis. The presence or absence of GSTM1 and GSTT1 genes was detected using PCR amplification according to the methods of Fryer *et al.* (1993) and Kempkes *et al.* (1996). Genotyping of the polymorphisms CAT 21 A/T, SOD2 Val-9Ala (T/C) and GPX1 Pro198Leu (C/T) was done according to Ukkola *et al.* (2001), Mitrunem *et al.* (2001) and Zhao *et al.* (2005), respectively. PCR and restriction endonuclease products were separated by electrophoresis in 10% (GPX1) and 6% (Cat and SOD2) nondenaturing polyacrylamide gels and visualised by staining with silver nitrate.

Allele and genotype frequencies were estimated by gene counting. The goodness of fit of the genotype distribution to the Hardy-Weinberg equilibrium was assessed by exact tests using Genepop 3.4 (Raymond and Rousset, 1995). Values of $p > 0.05$ indicated Hardy-Weinberg equilibrium. Chi-square tests were used to compare the frequencies of the mutant alleles and genotypes with published data. Data for genetic diversity was assessed by comparing the observed and expected heterozygosities, and F_{IS} and F_{ST} (F-statistics) were calculated by Arlequin 3.1.1 (Excoffier and Schneider, 2005).

Results

Table 1 shows the distribution of the antioxidant enzymes, GSTM1 and GSTT1 variant allele frequencies in the three Brazilian population groups studied in comparison with frequencies obtained in other populations. The results revealed differences in the three populations, primarily for allele frequency of $GPX1^{*T}$ (Kayabi = 3%, Kalunga = 18% and Federal District = 33%).

Genotypic frequencies and results of Hardy-Weinberg Equilibrium analysis, as well as Heterogeneity tests for SOD2, CAT, GPX1 and GST polymorphisms are summarised in Table 2. For the SOD2 locus, the results indicated a significant deviation from Hardy-Weinberg equilibrium (HWE) in all population groups studied; this was also denoted for the GPX1 locus in Kalunga. For the SOD2 locus, this was due to a heterozygote excess: F_{is} = -0.27 (Kalunga), F_{is} = -0.29 (Kayabi) and F_{is} = -0.67 (Federal District). For GPX1, the results were compatible with a homozygote excess in Kalunga (F_{IS} = 0.26). The other markers analysed, as well as GPX1 in Kayabi and Federal District, were in accordance with HWE. The distributions of CAT, SOD and GPX1 genotypes are significantly different among the ethnic groups studied ($p < 0.0001$). Based on the F-statistics, these Brazilian population groups showed a moderate degree of genetic differentiation ($F_{ST} = 0.10$).

Discussion

Populations of the world vary considerably in their predisposition to diseases and in allele frequencies at pharmacogenetically important loci, probably as a result of genetic drift, and also because of adaptation to local selective factors such as climate and available nutrients (Suarez-Kurtz, 2004). Our study describes the frequency distribution of polymorphisms in three different Brazilian groups for SOD2, CAT, GPX1, GSTM1 and GSTT1, these being loci of pharmacological relevance.

The CAT^{T} allele frequency in Kalunga was homogeneous and similar to that observed in Europeans (Ukkola *et al.*, 2001), whereas $SOD2^{C}$ allele frequency was homogeneous to those observed in U.S. and Canadian populations (Ambrosone *et al.*, 1999; Knight *et al.*, 2004). These values corroborate previous findings suggesting admixture of the Kalunga population with Europeans or Euro-descendants. A strong male contribution from Europeans in forming the Kalunga population was indicated by Y chromosome studies (Ribeiro *et al.*, 2009).

The allele frequency of $GPX1^{T}$ was lower in Kalunga than in the Federal District. In fact, the frequency observed in Kalunga was neither close to nor homogeneous with any frequency of other studied populations. Even though the $GPX1^{T}$ frequency determined in Kalunga was low, it was twice that observed for Asians, an ethnic group that has been reported to have the lowest frequencies for the Pro198Leu GPX1 polymorphism (Bastaki *et al.*, 2006). $GSTM1^{*0}$ frequency in Kalunga was homogenous and in agreement with African-Brazilians from São Paulo (Gattás *et al.*, 2004), Porto Alegre (Kvitko *et al.*, 2006) and Africans (Rebbeck, 1997). Concerning the frequency of $GSTT1^{*0}$, it was homogenous and in agreement with that of African-Brazilians from Porto Alegre (Kvitko *et al.*, 2006) and Afro-descendants (Fujihara *et al.*, 2009). The highest contribution in forming the Kalunga population had al-

Table 1 - Distribution of antioxidant enzymes, GSTM1 and GSTT1 variant allele frequencies in the Kalunga, Kayabi and Federal District populations in comparison with the frequencies obtained in other populations.

Genetics markers	Kalunga			Kayabi			Federal District		
	Frequencies	n	References	Frequencies	n	References	Frequencies	n	References
CAT^T	0.52	72	*	0.26	60	*	0.38	172	*
	0.58	244	1 (χ^2_2 = 2.28; p = 0.3198)	0.31	100	9 (χ^2_2 = 2.67; p = 0.2631)	0.58	245	1 (χ^2_2 = 1.02; p = 0.6004)
	0.51	72	*	0.68	60	*	0.40	172	*
	0.50	110	2 (χ^2_2 = 1.09; p = 0.5798)	0.49	372	3 (χ^2_2 = 7.03; p = 0.0293)	0.41	135	13 (χ^2_2 = 9.12; p = 0.0104)
$SOD2^C$	0.49	372	3 (χ^2_2 = 3.36; p = 0.1863)	0.56	196	10 (χ^2_2 = 12.5; p = 0.0019)	0.47	135	14 (χ^2_2 = 21.53; p = 0.0000)
				0.41	370	11 (χ^2_2 = 8.09; p = 0.0175)			
$GPX1^T$	0.18	72	*	0.03	60		0.33	172	*
	0.08	122	4 (χ^2_2 = 109.12; p = 0.0000)	0.08	122	4 (χ^2_2 = 148.7; p = 0.0000)	0.33	517	15 (χ^2_2 = 0.12; p = 0.9436)
	0.53	72	*	0.55	60	*	0.63	172	*
	0.57	137	5 (χ^2_1 = 0.57; p = 0.4502)	0.52	26	12 (χ^2_1 = 0.08; p = 0.7732)	0.60	521	16 (χ^2_1 = 0.81; p = 0.3681)
$GSTM1^{*0}$	0.58	100	6 (χ^2_1 = 0.75; p = 0.3864)				0.61	190	17 (χ^2_1 = 0.26; p = 0.6101)
	0.47	69	7 (χ^2_1 = 0.69; p = 0.4061)						
	0.58	72	6 (χ^2_1 = 0.56; p = 0.4542)	0.45	60	*	0.49	172	*
	0.60	134	8 (χ^2_1 = 0.11; p = 0.7401)	0.42	67	12 (χ^2_1 = 0.09; p = 1.000)	0.49	190	17 (χ^2_1 = 0.002; p = 0.9643)
$GSTT1^{*0}$							0.49	135	14 (χ^2_1 = 0; p = 1.000)
							0.47	233	5 (χ^2_1 = 0.24; p = 0.6242)
							0.37	135	13 (χ^2_1 = 5.4; p = 0.0201)

*Present study; 1- Ukkola *et al.*, 2001; 2- Ambrosone *et al.*,1999; 3- Knight *et al.*, 2004; 4- Bastaki *et al.*, 2006; 5- Gattás *et al.*, 2004; 6- Kvitko *et al.*, 2006; 7- Rebbeck, 1997; 8- Fujihara *et al.*, 2009; 9- Young *et al.*, 2006; 10- Akyol *et al.*, 2005; 11- Bica *et al.*, 2007; 12- Gaspar *et al.*, 2002; 13- Akimoto *et al.*, 2010; 14- Miranda-Vilela *et al.*, 2010; 15- Hu and Diamond, 2003 16 - Santovito *et al.*, 2008; 17- Maciel *et al.*, 2009.

ready been observed to be of African origin, as seen in a study of classical markers and polymorphisms of Alu insertion (Pedrosa and Oliveira, unpublished data).

In the Kayabi group, the frequencies of $GSTM1^{*0}$ (55%) and $GSTT1^{*0}$ (45%) were homogeneous and similar to Wai Wai Amerindians (52%) and Aché Amerindians (42%), respectively (Gaspar *et al.*, 2002). The $SOD2^C$ frequency was higher and non-homogeneous compared to that observed in Euro-descendants (41% to 56%) (Knight *et al.*, 2004; Akyol *et al.*, 2005; Bica *et al.*, 2007). For the other polymorphisms (GPX1 and CAT), the values were similar to those described for Asians, being homogeneous for CAT^T allele frequency (Young *et al.*, 2006) and non-homogeneous for allele frequency of $GPX1^T$ (Bastaki *et al.*, 2006). It has been reported that the Kayabi live in an area which has received intense migration due to gold prospecting, and they have consequently become somewhat mixed (Klautau-Guimarães *et al.*, 2005a). Because this is the first description of SOD2 Val9Ala, GPX1 Pro198Leu and CAT 21A/T polymorphisms in an Amerindian Brazilian group, this recent miscegenation should be taken into account, given that it can influence the distribution of certain polymorphisms, contributing to deviations from Hardy-Weinberg Equilibrium.

The Federal District urban population group presented CAT^T and $GPX1^T$ allele frequencies that were similar to those described for Europeans (Ukkola *et al.*, 2001; Hu and Diamond, 2003). For the SOD2 polymorphism, our values were close to other District Federal samples, but it was non-homogeneous with them (Akimoto *et al.*, 2010;

Table 2 - Hardy-Weinberg Equilibrium and Heterogeneity tests for Catalase, SOD2, GPX1, GSTM1 and GSTT1 polymorphism in Brazilian groups.

Genetic markers	Kalunga (n = 72)	Kayabi (n = 60)	Federal District (n = 172)	Heterogeneity test
Catalase				
AA	15 (0.21)	32 (0.53)	25 (0.15)	
AT	39 (0.54)	25 (0.42)	81 (0.47)	$\chi^2_{(g.l=4)} = 47.44$ p < 0.0001
TT	18 (0.25)	3 (0.05)	66 (0.38)	
Hardy-Weinberg test	p = 0.6369	p = 0.7387	p = 1.0000	
SOD2				
TT	12 (0.17)	2 (0.03)	34 (0.20)	
CT	46 (0.64)	34 (0.57)	138 (0.80)	$\chi^2_{(g.l=4)} = 72.48$ p < 0.0001
CC	14 (0.19)	24 (0.40)	0 (0.0)	
Hardy-Weinberg test	p* = 0.0333	p* = 0.0340	p* = 0.0000	
GPX1				
CC	51 (0.71)	57 (0.95)	80 (0.47)	
CT	16 (0.22)	2 (0.03)	69 (0.40)	$\chi^2_{(g.l=4)} = 45.58$ p < 0.0001
TT	5 (0.07)	1 (0.02)	23 (0.13)	
Hardy-Weinberg test	p* = 0.0426	p = 0.0507	p = 0.2302	
GSTM1				
GSTM1 (+)	52 (0.72)	42 (0.70)	68 (0.395)	$\chi^2_{(g.l=2)} = 3.87$ p = 0.1444
GSTM1 null	20 (0.28)	18 (0.30)	104 (0.605)	
GSTT1				
GSTT1 (+)	48 (0.67)	48 (0.80)	130 (0.76)	$\chi^2_{(g.l=2)} = 3.37$ p = 0.1854
GSTT1 null	24 (0.33)	12 (0.20)	42 (0.24)	

*p < 0.05.

Miranda-Vilela *et al.*, 2010). Regarding the frequency of the null allele of GSTM1 (63%), this was homogeneous and similar to that observed in Euro-descendants (Santovito *et al.*, 2008) and African-Brazilians from Curitiba (Maciel *et al.*, 2009).

The observed *GSTT1*^{*0} frequency in the Federal District group was similar and homogeneous to that for African Brazilians from Curitiba (Maciel *et al.*, 2009), the other sample from the Federal District (Miranda-Vilela *et al.*, 2010) and European-Brazilians from São Paulo (Gattás *et al.*, 2004). These results denote the participation of African descendants in the formation of the Federal District population and corroborate the estimate based on autosomal STR analyses indicating a genetic contribution of more than 39% by sub-Saharan Africans (Godinho *et al.*, 2008). Moreover, the distribution of these GST polymorphisms in this sample reflects the history of the creation of the new Capital in the 1960s. It was formed by people from all regions of Brazil (Queiroz, 2006) and this very diverse origin suggests that it may be the most representative sample-group of the Brazilian population as a whole.

The considerable range of variation in human populations may reflect, in part, distinct processes of natural selection and adaptation to variable environmental conditions (Barreiro *et al.*, 2008). Deviations from Hardy Weinberg Equilibrium may be explained by natural selection or recent ethnic admixture. Population growth and positive selection increase the proportion of rare alleles (*i.e.*, alleles with low frequency), whereas balancing selection and population substructure increases the proportion of intermediate frequency alleles (Serre and Hudson, 2006). Natural selection can act at the level of genes, if particular genotypes allow for increased fitness in specific environments (Barreiro *et al.*, 2008).

Although the cytosine to thymine substitution in the SOD2 gene has been reported to decrease SOD2 efficiency against oxidative stress (Shimoda-Matsubayashi *et al.*, 1996; Akyol *et al.*, 2005), a study conducted with Federal District athletes showed that SOD2 heterozygotes presented less tissue and DNA damages, as well as lower lipid peroxidation indices (Miranda-Vilela *et al.*, 2009), indicating that SOD2 heterozygosis can favor defense against oxidative stress. Furthermore, our results are in accordance with other studies obtained by our research group with other population groups from the Federal District (Akimoto *et al.*, 2010; Miranda-Vilela *et al.*, 2009, 2010).

Genes under positive selection are thought to have an important role in human survival and to affect complex phenotypes of medical relevance. Indeed, as reported for negative selection, nonsynonymous SNPs showing signs of

positive selection are observed more frequently than expected in genes involved in disease (Barreiro *et al.*, 2008). Many indigenous people in Latin America still live in isolated environments where conditions are harsh. Contact with workers in mining and exploration projects affects indigenous people's health (Montenegro and Stephens, 2006). Tuberculosis constitutes a major health problem among the indigenous people of the upper Rio Negro in Brazil (Buchillet and Gazin, 1998) and a pattern of moderate endemism with a prevalence of previous HBV (Hepatitis B virus) infection of 55.7% and 49.5% was observed for two indigenous groups of Pará, Brazil (Nunes *et al.*, 2007).

Similarly, the Kalunga population lives in very poor conditions in remote settlements in the mountains on both sides of the Paraná River. The majority of the individuals live at low socioeconomic and education levels, with poor hygiene and crowded conditions. Also, the majority of them live basically on subsistence agriculture or cattle-raising, and their houses have no sewage system or tap water service. Rates of 80%, 30% and 0.5% were found for HAV (Hepatitis A virus), HBV (Hepatitis B virus) and HCV (Hepatitis C virus) infections, respectively (Matos *et al.*, 2009). In the presented contexts, it is likely that in the Kayabi and Kalunga population groups, heterozygotes have a selective advantage in the global aspect of diseases, thus increasing their frequency in these populations. Neverthelesss, selection in another area of the SOD2 gene or in another unknown gene located in the close vicinity of the SOD2 gene should also be taken into account.

Deviation from Hardy-Weinberg Equilibrium was also detected for the GPX1 polymorphism in Kalunga, which showed excess of CC homozygotes. This was also observed in a study on Asians/Pacific Islanders (Bastaki *et al.*, 2006). As the Leu allele ($GPX1^T$) has been implicated in effects on GPX1 activity, which becomes less responsive to stimulation (Zhao *et al.*, 2005), these results are expected, mainly because this population lives in precarious conditions.

To conclude, we think that the SNPs described in this report will be valuable resources for future functional studies and for specific population genetic studies designed to comprehensively explore the role of these genetic polymorphisms in the etiology of human diseases. It is necessary to characterize genetic variation among different population groups when assessing disease risk. The differences in allelic frequencies observed among samples emphasize the importance of being careful in planning epidemiological studies.

Acknowledgments

This work was supported by the Universidade de Brasília, the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and by the Fundação de Empreendimentos Científicos e Tecnológicos (FINATEC).

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Associate Editor: Francisco Mauro Salzano