

Thiopurine methyltransferase phenotypes and genotypes in Brazilians

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The polymorphism of thiopurine methyltransferase (TPMT) was studied in 306 healthy Brazilians who were classed, on the basis of self-declared colour and ancestry, as Euro-derived ($n = 81$), Afro-derived ($n = 18$) or having interethnic admixture ($n = 204$). TPMT activity (range 0.17–25.93 U) displayed a trimodal distribution of high (> 11.3 U; 9% of individuals), intermediate (5–11.3 U; 9.8%) and low (0.17 U; 0.3%) phenotypes. The occurrence of the TPMT mutations 238G>C, 460G>A and 719A>G was investigated in all individuals with low or intermediate phenotype, and in 43 with high-activity phenotype. None and two mutant alleles were associated with high- or low-activity phenotypes, respectively, whereas one mutant allele was detected in 26 of the 30 intermediate phenotype individuals. The allele frequencies of *TPMT**2, *TPMT**3A and *TPMT**3C did not differ between individuals classed as Euro-derived (0.76%, 2.03% and 2.54%, respectively) or

having interethnic admixture (0.60%, 1.81% and 1.81%, respectively). Furthermore, within each of these groups, the frequencies of *TPMT**3A and *TPMT**3C were not significantly different. *Pharmacogenetics* 13:371–373 © 2003 Lippincott Williams & Wilkins

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Brazil has one of the most heterogeneous populations in the world. Extensive interethnic crosses over the last 500 years, between autochthonous Amerindians, European colonizers and Africans contributed to the gene pool of the present-day approximately 175 million Brazilians. The heterogeneity of the Brazilian population has important implications for pharmacogenetics because extrapolation of data derived from well-defined ethnic groups is clearly not applicable to the majority of Brazilians. Recognition of this fact has stimulated pharmacogenetic studies in the Brazilian population, and we report the first systematic investigation of genetic polymorphism of thiopurine *S*-methyltransferase (TPMT; EC 2.1.1.67) in Brazilians. A seminal study on a Caucasian-American population revealed that the TPMT activity displays a trimodal distribution, with 89% of individuals exhibiting high activity, 11% exhibiting intermediate activity and approximately one in 300 having deficient activity [1]. The genetic basis and the molecular mechanisms underlying TPMT polymorphism have been intensively investigated and, to our knowledge, nine nonfunctional mutant alleles have been described to date [2]. Four of these alleles, namely *TPMT**2 (238G>C), *TPMT**3A (460G>A and 719A>G), *TPMT**3B (460G>A) and *TPMT**3C (719A>G) account for 78–95% of the lower-activity genotypes in different populations [3,4]. In the present study, phenotyping and genotyping procedures were combined to evaluate the polymorphism of TPMT in

306 healthy Brazilian subjects. Preliminary results have been presented previously in abstract form [5].

The study protocol was approved by the Ethics Committee of the Brazilian National Cancer Institute (INCA), and written informed consent was obtained from all volunteers, who were recruited at the INCA blood bank. Previously described polymerase chain reaction-based methods [4] and radiochemical assay [6,7] were employed to determine the TPMT genotype and phenotype, respectively. TPMT activity is reported as units (U), where 1 U corresponds to 1 nmol of 6-methyl-mercaptopurine formed per h/ml red blood cell. The appropriate statistical tests applied to the data sets are indicated in the text. $P < 0.05$ was considered statistically significant.

The demographic characteristics of the study subjects are shown in Table 1. Based on self-declared skin colour and ancestry, 204 (66.7%) subjects were found to have interethnic admixture, the vast majority (197) being mulattoes. Eighteen (5.9%) subjects were classified as Afro-derived, because they referred only black subjects within their ancestries, and 83 (27.1%) were classified as Euro-derived, predominantly of Iberian or Italian ancestries. One subject reported only Arabian ancestry.

The TPMT activity ranged from 0.17–25.9 U (median,

Table 1 Demographic characteristics and thiopurine methyltransferase activity of the Brazilian sample population

Group	n (%)	Age (years)		Activity (U)	
		Median	Range	Median	Range
Study population	306 (100)	32	18–58	17.1	0.17–25.93
Male	188 (61.4)	32	18–57	17.0	0.17–25.93
Female	118 (38.6)	32	19–58	17.2	6.70–24.12
Euro-derived	83 (27.1)	36	20–58	17.3	7.56–23.59
Afro-derived	18 (5.9)	31	20–42	15.6	10.89–20.51
Interethnic admixture	204 (66.7)	32	18–57	17.1	0.17–25.93
Arabian-derived	1 (0.3)	44 ^a	NA	18.9 ^a	NA

^aSingle value. NA, not applicable.

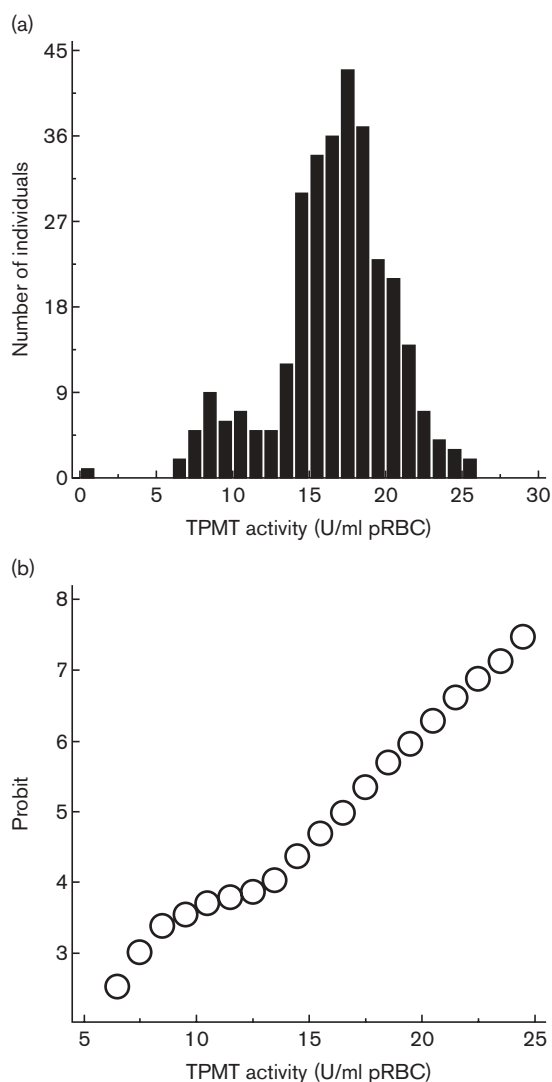
17.1, 95% confidence interval 9.3–24.1) (Table 1). No significant differences in the mean values were observed when comparing gender (Mann–Whitney test) or ethnicity (ANOVA). The frequency distribution of the TPMT activity is best fit by assuming the existence of two normally distributed populations (Fig. 1a). Because one subject was found to be TPMT deficient, a trimodal distribution can be defined (Fig. 1a). Probit plot indicates a cut-off value of approximately 12 U separating high- and intermediate-activity phenotypes (Fig. 1b). However, because no subjects with activity higher than 11.3 were found carrying mutant alleles (data not shown), we adopted 11.3 U as the cut-off value. Accordingly, 275 individuals (89.9%) were classified as high-activity phenotype, 30 (9.8%) as intermediate-activity and one individual (0.3%) as low-activity. A trimodal distribution of TPMT activity, with a cut-off point between high- and intermediate-activity estimated to correspond to 9.5 U, was previously reported for a sample of healthy, uremic and organ transplanted Brazilians [8]. In the latter study, TPMT genotyping was not performed.

In the present study, the three TPMT mutations (238G>C, 460G>A and 719A>G) most prevalent in other populations were investigated in 74 individuals, comprising the 31 with low- or intermediate-activity phenotype and 43 with high activity. The latter included six individuals with TPMT activity in the range of 11.3–13.0 U, plus 37 randomly selected subjects with activities higher than 13 U. No mutations were detected within the high-activity group, whereas two mutations (460G>A, heterozygously and 719A>G, homozygously) were detected in the individual with low TPMT activity, denoting the genotype *TPMT**3A/**3C*. One mutant allele was carried by 26 of the 30 intermediate phenotype individuals (data not shown). Taken together, these results indicate that alleles *TPMT**2, *TPMT**3A and *TPMT**3C are detected in 87.1% (27/31) of the individuals with low or intermediate TPMT activity. This level of phenotype–genotype concordance is within the range (78–95%) reported for

other populations [3,4]. In our study, the mutations 238G>C, 460G>A and 719A>G were not detected in four individuals with intermediate activity. This apparent discordance between phenotype and genotype might be due to one of the rarer mutations lying on the open reading frame of the TPMT gene, to variable number of tandem repeats on its promoter, previously reported but not examined in this study, or to yet unidentified mutations [9,10]. Moreover, the possibility that intermediate activity can be observed in the absence of genetic TPMT deficiency should not be overlooked.

Table 2 shows the observed frequencies of *TPMT**2, *TPMT**3A and *TPMT**3C in the general study population, in Euro-derived individuals and in those having interethnic admixture. No variant alleles were detected in Afro-derived subjects, probably because of their small representation ($n = 18$) in the population studied. The frequencies of the variant alleles *TPMT**2, *TPMT**3A and *TPMT**3C did not differ significantly among the three groups in Table 2 for the present study ($P = 0.99$, Fisher exact test). It is noteworthy that the frequencies of the *TPMT**2 allele in the study population (0.82%), in Euro-derived (0.60%) and in individuals having interethnic admixture (0.76%) are approximately two- to three-fold higher than those previously reported for Caucasians and African-Americans (Table 2). The data in Table 2 for the present study also shows that, for each population of the three Brazilian groups, the frequencies of *TPMT**3A and *TPMT**3C were not different ($P = 0.88$, Fisher exact test). This contrasts with the wide differences in the frequencies of these alleles in other ethnic groups (Table 2, other studies). Thus, among Caucasians, *TPMT**3A is three- to five-fold more frequent than *TPMT**3C [3,9,11] whereas, in Africans, *TPMT**3A is not detected and *TPMT**3C frequency is 5–7% [12,13]. In African-Americans, these two alleles coexist, but *TPMT**3C is three-fold more frequent than *TPMT**3A [3]. This latter result was ascribed to the estimated 25% penetrance of Caucasian genes within

Fig. 1



Frequency distribution histogram of (a) thiopurine methyltransferase (TPMT) activity and (b) probit plot of 306 healthy, unrelated Brazilian volunteers.

Table 2 Frequency of variant thiopurine methyltransferase (TPMT) alleles in different ethnic populations

Population	Alleles (%)		
	TPMT*2	TPMT*3A	TPMT*3C
Present study			
Study population	0.82	1.63	2.12
Interethnic admixture	0.76	2.03	2.54
Euro-derived	0.60	1.81	1.81
Other studies			
Caucasians ^a	0.2–0.5	3.2–5.7	0.2–1.0
African-Americans ^b	0.4	0.8	2.4
Africans ^c	0	0	7.6–10.9

References: ^aCaucasians [3,9,11], ^bAfrican-Americans [3] and ^cAfricans [11,12].

African-Americans [3,13]. Accordingly, the similar frequencies of *TPMT*3A* and *TPMT*3C* observed in our study can be attributed to the extensive interethnic crossings, with a high level of genetic admixture, that is characteristic of the Brazilian population [14].

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