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ORIGINAL ARTICLE

Analysis of nucleotide diversity of *NAT2* coding region reveals homogeneity across Native American populations and high intra-population diversity

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N-acetyltransferase 2 (NAT2), an important enzyme in clinical pharmacology, metabolizes antibiotics such as isoniazid and sulfamethoxazole, and catalyzes the transformation of aromatic and heterocyclic amines from the environment and diet into carcinogenic intermediates. Polymorphisms in *NAT2* account for variability in the acetylator phenotype and the pharmacokinetics of metabolized drugs. Native Americans, settled in rural areas and large cities of Latin America, are under-represented in pharmacogenetics studies; therefore, we sequenced the coding region of *NAT2* in 456 chromosomes from 13 populations from the Americas, and two from Siberia, detecting nine substitutions and 11 haplotypes. Variants **4* (37%), **5B* (23%) and **7B* (24%) showed high frequencies. Average frequencies of *fast*, *intermediate* and *slow* acetylators across Native Americans were 18, 56 and 25%, respectively. *NAT2* intra-population genetic diversity for Native Americans is higher than East Asians and similar to the rest of the world, and *NAT2* variants are homogeneously distributed across native populations of the continent.
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Introduction

The *N*-acetyltransferase 2 (NAT2) is an important enzyme in clinical pharmacology. It metabolizes xenobiotic compounds containing aromatic amines by N- or O-acetylation. NAT2 is the metabolizing enzyme of commonly prescribed antibiotics, such as the antituberculosis drug isoniazid (INH)[1] and sulfamethoxazole, prescribed for secondary infections in AIDS patients.[2] NAT2 also catalyzes the transformation of aromatic and heterocyclic amines present in cigarette smoke and overcooked meat into carcinogenic intermediates.[3]

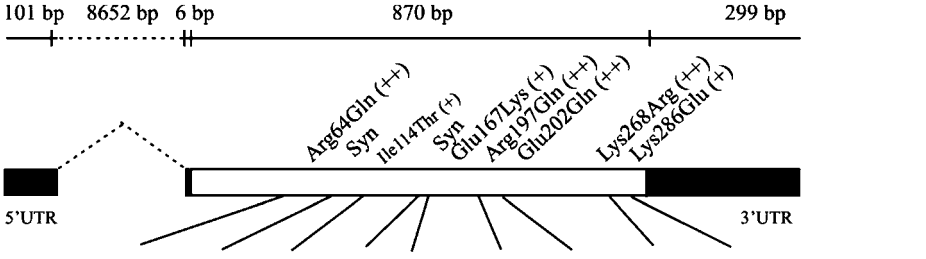
The NAT2 is encoded by *NAT2* (OMIM 243400), a gene located at 8p22. *NAT2* has two exons, the second of which includes a single open-reading frame of 870 bp (Figure 1, Blum *et al*.[5]). Besides the wild-type reference haplotype *NAT2*4* (GenBank NM_000015), 35 variants have been described (http://www.louisville.edu/medschool/pharmacology/NAT2.html), most of which have been associated with impaired metabolic activity.[6–9] The most common substitutions are 191G>A (Arg64Gln), 341T>C (Ile114Thr), 590G>A (Arg197Gln), 803A>G (Lys268Arg), 857G>A (Lys286Glu), 282C>T and 481C>T (Figure 1), which

are unevenly distributed across autochthonous human populations.[10]

NAT2 polymorphism is responsible for variation in the acetylator phenotype. Individuals can be classified as *slow* or *fast* acetylators (or metabolizers) on the basis of their *NAT2* genotype,[11,12] although some authors consider a third *intermediate* category.[13] *Slow*, *intermediate* and *fast* acetylators are defined as carriers of zero, one or two full functioning haplotypes, respectively,[7] although exceptions have been reported, in particular in HIV-infected patients.[14,15]

NAT2 variants are also responsible for variation in pharmacokinetics of INH.[16] Tuberculosis is a major health problem in Latin America and other developing countries, and its therapy, based on INH and other first-line medications, is cheap and effective (http://www.who.int). However, adverse drug reactions such as hepatotoxicity can occur,[17,18] leading to decreased medication adherence and to drug resistance.[19] Whether the incidence of hepatotoxicity differs among *slow* and *fast* acetylators is a controversial topic. Whereas recent studies have suggested that *slow* acetylator status is a risk factor,[20–23] some investigations have shown an opposite trend.[24,25]

In this study, we analyzed *NAT2* genetic variation in 12 native and one admixed populations from the Americas and two populations from Siberia, the region that hosted the Pleistocene ancestors of current Native Americans.[26,27] Prior sequencing data for *NAT2* are not available for these ethnic groups. Previous studies genotyped common single-nucleotide polymorphisms (SNPs) identified in individuals from other continents,[28,29] which could lead to ascertainment bias. Therefore, mutations important to define region-specific variation could pass undetected. Because our aim was to identify genetic variants and haplotypes characteristic of the Americas, we sequenced the coding region in each sampled individual. We described the pattern of genetic variation at this locus and tested if haplotype diversity in the American continent fits the population genetics model of isolation-by-distance,[30] which explains the geographical pattern of diversity of neutral loci in part of the continent.[31] American and Siberian *NAT2* haplotype diversities were compared with those of other regions of the world. Finally, we inferred the frequencies of acetylator phenotypes and discussed its pharmacogenetic implications in populations with different demographic history and geographic origin.



Haplotype	**191G>A**	282C>T	**341T>C**	481C>T	499G>A	**590G>A**	607G>C	803A>G	**857G>A**	phenotype
*4	.	.	.	.	.	.	.	.	.	rapid
*5A	.	.	**C**	T	.	.	.	.	.	slow
*5B	.	.	**C**	T	.	.	.	G	.	slow
*6A	.	T	.	.	.	**A**	.	.	.	slow
*7B	.	T	.	.	.	.	.	.	**A**	slow
*7Bnew	.	T	.	.	.	.	C	.	**A**	unknown
*10	.	.	.	.	A	.	.	.	.	unknown
*11	.	.	.	T	.	.	.	.	.	unknown
*12A	.	.	.	.	.	.	.	G	.	rapid
*13	.	T	.	.	.	.	.	.	.	rapid
*14B	**A**	T	.	.	.	.	.	.	.	slow

dbSNP rs	rs1801279	rs1041983	rs1801280	rs1799929	-	rs1799930	-	rs1208	rs1799931
SNP500 [a]	NAT2-04	NAT2-05	NAT2-06	NAT2-07	-	NAT2-08	-	NAT2-01	NAT2-02
	Ex2+197	Ex2+288	Ex2+347	Ex2+487	-	Ex2-580	-	Ex2-367	Ex2-313
EGP[b]	10261	10352	10411	10551	-	10660	-	10873	10927

Figure 1 Representation of human *NAT2* locus and definition of haplotypes. The white box represents the coding sequence. Nucleotide changes identified in this study are indicated along with the haplotypes that carry them as in the official nomenclature (www.louisville.edu/medschool/pharmacology/NAT2.html). Mutations that cause a change in the protein activity are in bold. Amino-acid changes are also shown (++ = conservative, + = moderately conservative; Grantham[4]). [a]*SNP500Cancer* Database (http://snp500cancer.nci.nih.gov/home.cfm) and [b]NIEHS Environmental Genome Project (http://egp.gs.washington.edu).

Results

Molecular variation, haplotypes and distribution of NAT2 variants
In a sequence analysis of 900 bp that includes the coding region as well as part of the 3′ UTR (Untranslated Region) of *NAT2* on 456 chromosomes, nine substitutions were observed (Figure 1), eight of which were reported previously (http://www.louisville.edu/medschool/pharmacology/NAT2.html): the non-synonymous substitutions 191G>A, 341T>C, 499G>A, 590G>A, 803A>G and 857G>A, and the silent substitutions 282C>T and 481C>T. Functional studies have determined that mutations 341T>C and 590G>A reduce the expression or activity of *NAT2*, and mutations 191G>A, and 857G>A, and in minor part 590G>A, reduce the protein stability.[32,9] Despite their phenotypic effect, 341T>C, 590G>A and A857G>A are common substitutions.

We inferred 11 different haplotypes (Figure 1). The non-synonymous 607G>C substitution, detected for the first time in a Coyaima individual, was observed on the background of the slow-function haplotype **7B*, and thus the haplotype was called **7Bnew*. Haplotype frequencies determined in the studied populations are in Table I of Supplementary Information.

The network in Figure 2 represents the mutational relationships between *NAT2* haplotypes and groups of haplotypes (*i.e.* haplogroups) and shows how each haplotype is distributed across groups of populations. For this analysis, we included data from this study and haplotype frequencies obtained from the literature (see the legend of Figure 2 for references). Although the network encompasses a region of only 900 bp, it contains loops, suggesting that recombination or recurrent mutations have influenced the pattern of diversity in the coding region of *NAT2*. One *NAT2* sequence of chimpanzee was used to root the network. Based on our survey and sequencing data from the *SNP500Cancer* database and from Patin *et al.*,[33] we identified 13 substitutions between the chimpanzee and the ancestral human haplotype **4*, 12 of which fixed and one polymorphic in chimpanzee. This haplotype is modal among East Asians and Native Americans. Haplogroup **5* (defined by 341T>C) shows high frequencies, especially in Europe and in the Hispanic sample, and includes the haplotype **5B*, the common and ubiquitous slow-function haplotype with frequency of 23.2% among our sample of the American continent. Haplogroup **7* (defined by 857G>A) is typical of Asian and Native American populations, but is rare in Africa and Europe. Haplogroup **6* (defined by 590G>A) is common in Eurasia and Africa, but rare among Native Americans, and therefore, when observed at moderated frequencies, could be used as indicator of post-Columbian admixture. Altogether, the haplotype distribution shown in Figure 2 is consistent with previous descriptions of allele distribution of *NAT2*.[10,36,37]

The frequencies of different haplotypes in each population of this study are represented in Figure 3. The non-marginal frequencies of haplogroup **6* – an indicator of

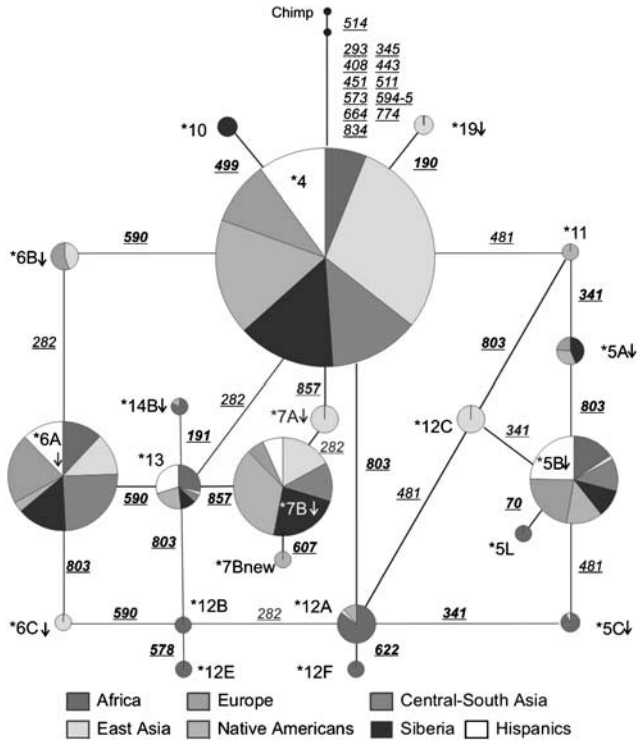


Figure 2 Network of haplotypes. Underlined numbers indicate mutations (non-synonymous in bold) and vertical arrows identify slow function variants. Different colors represent different groups of populations: Africans (African ancestry plus African: $n=24$ from *SNP500Cancer*: http://snp500cancer.nci.nih.gov; Bantu, $n=10$;[33] Bakola, $n=10$[33]); East Asia (Japan, $n=48$;[34] Korea, $n=1000$;[35] Pacific Rim, $n=24$ from *SNP500Cancer*); Europe (United States European ancestry, $n=31$ from *SNP500Cancer*; Ashkenazi, $n=10$;[33] French, $n=10$;[33] Saami, $n=10$;[33] Sardinians, $n=6$[33]); Central-South Asia (Gujarati, $n=10$ and Thai, $n=14$[33]); Native Americans (data from this study); black: Siberians (data from this study) and white: Hispanics (admixed $n=23$ from *SNP500Cancer*).

recent admixture from Europe or Africa, among the Coyaima (5/26 chromosomes, 19.2%), Cree (3/28 chromosomes, 10.7%) and Maya (3/32 chromosomes, 9.4%) – suggest the presence of admixture for these Native American populations. In particular, the Coyaima population displays similar frequencies of *NAT2*4* and *NAT2*6A* to the Hispanics from *SNP500Cancer*, and exhibit the haplotype *NAT2*14*, which is typical from Africa.[38] The observed frequency (9.4%) of the haplogroup **6* in the Maya sample is also consistent with evidence of European admixture in this population, estimated using 377 microsatellites.[39]

Interestingly, the *'admixed'* urban sample of tuberculosis patients from the shantytown of Las Pampas (LIM) shows haplotype frequencies typical for Native South-American populations and, in particular, is very similar to that of the native sample of Tayacaja (TAY, Tarazona-Santos *et al.*[40]), from the rural area of the Peruvian Central Andes (F_{ST}: 0.001; $P>0.05$).

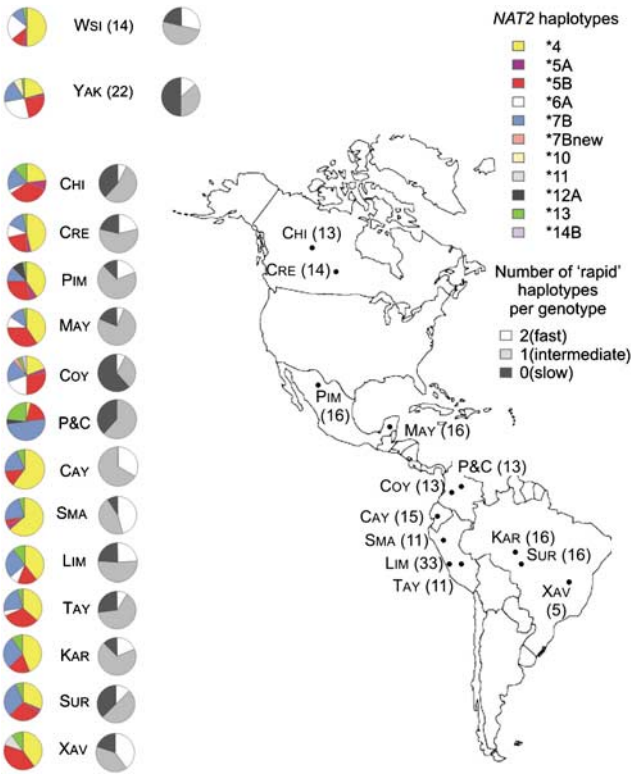


Figure 3 Geographic distribution of samples, haplotype frequencies and number of full function copies of *NAT2* haplotypes in Siberians and populations from the American continent. Population codes: CHI: Chypewian; CRE: Cree; PI: Pima; MAY: Maya; COY: Coyaima; P&C: Piapoco and Curripaco; CAY: Cayapa; SMA: San Martín; TAY: Tayacaja; LIM: Las Pampas, Lima; KAR: Karitiana; SUR: Surui and XAV: Xavante. In parentheses number of individuals genotyped per population.

Population structure

Table 1 shows estimators of intra-population genetic diversity. We also calculated genetic diversity indexes for 13 populations from the literature for which *NAT2* coding region was sequenced (Table 1). Although sequencing allows to detect new polymorphisms, most of these screenings only identified the known common mutations 282C>T, 341T>C, 481C>T, 590G>A, 803A>G and 857G>A. The exception were the rare 190C>T in seven individuals from a large Korean sample ($n=2000$), and two Bantu (70T>A and 578C>T) and one Bakola (622T>C) from Africa (Figure 2). The comparison across major geographic regions of the world shows that Native Americans have higher *NAT2* intra-population genetic diversity than East Asians, and similar values to populations from other continents (Table 1).

The F_{ST} (*i.e.* the among-population component of genetic variance) calculated from worldwide populations included in Table 1 is 0.15 ($P<0.01$), and is similar to the average value estimated across the genome.[44,45] For the American continent, the differentiation among populations for *NAT2* haplotype frequencies is low ($F_{ST}=0.03$, $P<0.01$).

Geographic distribution of NAT2 *genetic diversity*

To describe the geographic pattern of genetic diversity at *NAT2* in the American continent, we used the spatial autocorrelation analysis on the populations of this study. The spatial autocorrelation coefficients test whether the similarity among haplotypes or samples of haplotypes collected from different locations depends upon geographic distances. The scatterplot of the autocorrelation indexes against classes of geographic distance among populations (*i.e.* correlogram) can be informative with respect to the evolutionary processes generating it.[46] Three analyses were performed: (1) among all observed haplotypes, considering their frequencies and differences in the number of substitutions between them (Figure 4a), using the autocorrelation index for DNA analysis (AIDA) algorithm;[47] (2) considering separately the frequencies of each of the common haplotypes **4, *5, *6* and **7* (Figures 4b and c); and (3) considering separately frequencies of inferred phenotypic classes (*fast*, *intermediate* and *slow* acetylators; Figure 4d). Analyses of haplotype and phenotype frequencies (2 and 3) were performed by the classical spatial autocorrelation analysis.[48] The observed correlograms were not compatible with a simple isolation-by-distance model,[46] whereby an asymptotically decreasing shape is expected. An association between geographic and genetic distances appeared significant only for few classes of distances. In particular, the high frequencies of slow function haplotypes in Colombian populations (COY and P&C) with respect to their low frequencies in CAY and SMA (Figure 3) account for the negative autocorrelation observed on the second distance classes in Figures 4b and c. Taken together, these analyses suggest that geographic distances do not account for the distributions of haplotype or inferred phenotype frequencies in the American continent.

Prediction of phenotypes and pharmacogenetic implications

The proportions of inferred acetylator phenotypes based on genotype data obtained in this study are shown in Figure 3 and Table I of Supplementary Information. The highest frequencies of the *slow* acetylator phenotype are observed in Europe and the Middle East, and the lowest in East Asia.[33] On the other hand, the distribution of frequencies of acetylator phenotypes in Africa is heterogeneous.[33] A previous study on Native Americans from Panama[29] reported frequencies of *slow* acetylators of 14.7%. Here, based on a larger and more widely distributed sample of Native Americans, we observed a frequency of *slow* acetylators of ~25%, mainly owing to the common slow-function haplotypes **5B*, **6A* and **7B*. This value is similar to those observed in Central-South Asia, Oceania and in some African populations (see Figure 5 of Patin *et al*.[33]).

Discussion

A re-sequencing study of *NAT2* coding region in 15 populations of the Americas and Siberia confirmed that mutations common in other continents, known to reduce NAT2 activity (341T>C, 590G>A and 857G>A), are also

Table 1 Intra-population diversity indices in Siberians and Native Americans from this study, and in populations from the literature for which *NAT2* coding region sequences were available

Population samples	*Diversity indexes*				
	S	*Number of haplotypes*	h	π ($\times 10^3$)	θ_s/site ($\times 10^3$)
West Siberian ($n=14$) (66.08°N, 76.63°E)	6	6	0.70	1.92	1.71
Yakut ($n=22$) (62–64°N, 129–130°E)	7	7	0.82	2.72	1.79
Siberia	7	7	0.79	2.42	1.6
Chipewyan ($n=13$) (59.32°N, 107.18°W)	6	6	0.8	2.62	1.75
Cree ($n=14$) (50.38°N, 102.57°W)	6	6	0.73	2.22	1.71
Pima ($n=16$) (29°N, 108°W)	5	6	0.75	2.13	1.38
Maya ($n=16$) (19°N, 91°W)	6	5	0.71	2.43	1.66
North-Central America ($F_{st}=0.0$)	6	7	0.74	2.32	1.25
Coyaima ($n=13$) (3.8°N, 75.2°W)	8	8	0.84	2.96	2.33
Piapoco and Curripaco ($n=13$) (3°N, 68°W)	5	5	0.68	2.16	1.46
Cayapa ($n=15$) (0, 79°W)	5	4	0.6	1.61	1.40
San Martin ($n=11$) (7°S, 77°W)	5	5	0.56	1.36	1.52
Lima ($n=33$) (12°S, 77°W)	6	5	0.74	2.09	1.4
Tayacaja ($n=11$) (12.33°S, 75.83°W)	6	5	0.74	2.53	1.83
Karitiana ($n=16$) (10°S, 63°W)	5	4	0.71	2.05	1.38
Surui ($n=16$) (11°S, 62°W)	5	5	0.74	2.48	1.38
South America ($F_{st}=0.04$*)	8	9	0.75	2.21	1.48
America ($F_{st}=0.03$*)	8	9	0.75	2.27	1.37
Hispanic ($n=23$)[a]	6	6	0.73	2.62	1.52
African ancestry ($n=24$)[a]	5	5	0.79	2.45	1.25
Bakola ($n=10$)[33]	6	6	0.77	1.88	1.88
Bantu ($n=10$)[33]	9	8	0.79	2.73	2.5
Africa	9	11	0.82	2.43	1.98
European ($n=31$)[a]	6	5	0.69	2.76	1.42
Ashkenazi ($n=10$)[33]	5	3	0.61	2.85	1.57
French ($n=10$)[33]	5	4	0.72	2.61	1.57
Saami ($n=10$)[33]	6	4	0.74	2.68	1.88
Europe	6	6	0.69	2.73	1.22
Gujarati ($n=10$)[33]	6	4	0.73	2.68	1.88
Thai ($n=14$)[33]	6	5	0.73	2.20	1.71
Central-South Asia	6	5	0.72	2.41	1.50
Japanese ($n=48$)[34]	6	7	0.66	1.47	1.30
Korea ($n=1000$)[35]	7	12	0.51	1.19	0.94
Pacific Rim ($n=24$)[a]	6	4	0.70	1.92	1.50
East Asia	7	12	0.53	1.23	0.94

S: number of polymorphic sites; h: haplotype diversity;[41] π: nucleotide diversity;[42] θ_s[43] (but on base pair basis).
*$P<0.01$.
In parentheses number of individuals and geographic coordinates.
[a]From SNP500Cancer Database (http://snp500cancer.nci.nih.gov/home.cfm).

frequent across the American continent. Notably, we did not observe novel common variants specific for the Americas or Siberia.

The similarity in haplotype structure in an *admixed* sample from the shantytown of Las Pampas (Lima) to Native Americans population is interesting. To confirm this observation, we sequenced the *NAT2* coding region in additional 95 individuals from this population. The resulting haplotype frequencies (see the Extended Lima sample in Table I of Supplementary Information) confirm that this sample resembles Native American ones. Moreover, by using 15 micro satellites, we estimated that the genetic contributions of Native Americans, Europeans and Africans to the Las Pampas (LIM) sample are 82, 12 and 6%, respectively (data not shown), both in the case of tuberculosis patients and healthy individuals. This result confirms that Las

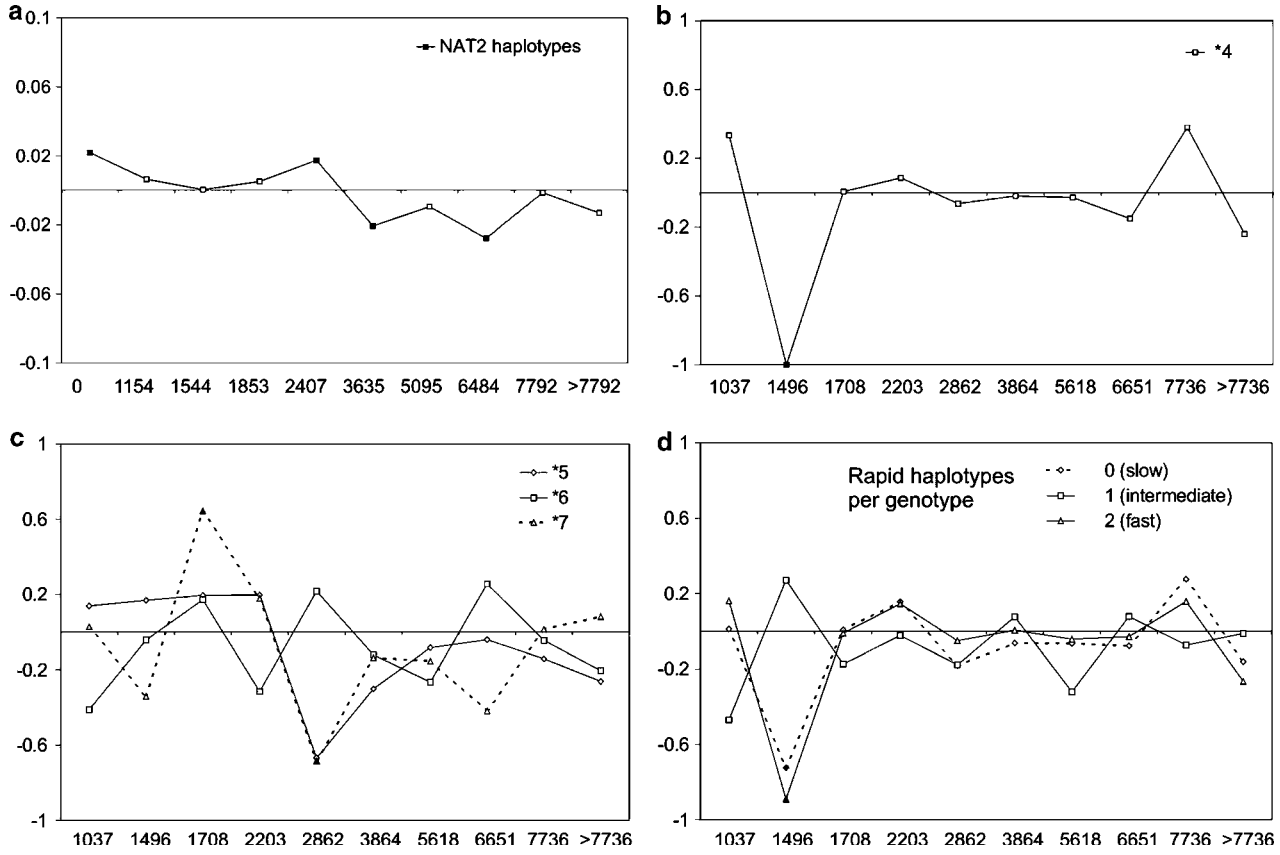


Figure 4 Spatial autocorrelation analysis in populations from the American continent of this study. *X* axis: Higher limit of geographic distance classes (in km) between localities. *Y* axis: Autocorrelation indexes II (**a**) or I (**b–d**). (**a**) Haplotypes defined as in Figure 1. (**b**) Common fast haplotype: **4*. (**c**) Common slow haplotypes **5A + B*; **6A* and **7B*. (**d**) Phenotype frequencies: two rapid haplotypes (*fast*); one rapid and one slow haplotype (*intermediate*) and two slow haplotypes (*slow*). Filled symbols indicate significant values. Classes of distance were chosen to contain the same number of comparisons.

Pampas population is predominantly Native American, with a low degree of European admixture evidenced by the presence of haplotype **6A* (*i.e.* a marker of post-Columbian admixture): 7.6% in Lima and 8.9% in the extended Lima sample. The Las Pampas sample was considered *admixed* or *mestizo* because of the population history of Lima (Peru), a city that has received contributions from European, Native American and African gene pools. In Latin-American countries, categories such as *admixed* or *mestizos* have a strong cultural and socioeconomic basis and do not necessarily reflect the genetic background of individuals or populations. Often, the same individual can be considered Native American or *admixed* at different ages, depending on cultural and socioeconomic changes. Therefore, these categories should be used with caution in genetic studies on Latin-American populations, as suggested also by other works focused on Brazilian individuals.[49,50]

Within-population diversity of *NAT2* in Native American populations was similar or higher than those characteristics of other geographic regions (Table 1). This contrasts the lower diversity usually seen among Native American populations at different loci.[51] Moreover, *NAT2* genetic variation appears evenly distributed across populations of the Americas, which do not match the results of recent studies that include part of the samples considered in our survey. On the basis of large sets of microsatellites, Serre and Paabo[52] have described the geographical pattern of diversity of the Americas as clinal, and Ramachandran *et al.*,[53] as a product of serial founder effects.

The overall F_{ST} is rather low (0.03, $P<0.01$) compared with other loci,[51,54] and consistently, we did not detect any correlation between geographic distances and *NAT2* diversity. This low between-population diversity also contrasts with the identification at genomic level of regions where the geographic pattern of diversity drastically changes.[55] Our analyses suggest that overall, native populations from the Americas can be considered homogeneous with respect to the distribution of acetylator phenotypes, which may be of significance for pharmacogenetic applications.

In conclusion, we observed a peculiar apportionment of genetic diversity of *NAT2* coding region in Native Americans, although a systematic comparison with other loci is not possible owing to the scanty information about genetic

variation of these populations.[51] Patin *et al*.[33] have recently evidenced that the haplotype structure of *NAT2* in Western Eurasia is not compatible with a neutral model of evolution and suggested that positive natural selection have increased the frequency of the slow function haplotype ***5B**. In principle, their results (*i.e*. positive values for the neutrality test of Tajima[56] for three West-Eurasian populations) are also compatible with a model of balancing natural selection. Similarly, our results show that the nucleotide diversity (π) is consistently higher than θ_s (based on the number of segregating sites) across the Native American populations (Table 1), so that all the populations but one (*i.e*. San Martin) show positive, although not significant, Tajima's *D* values (data not shown). Based on these results and the low between-population differentiation ($F_{st} = 0.03$), also consistent with the signature of balancing selection, it would be tempting to speculate that natural selection could have produced the observed pattern in the Americas. However, we do not have sufficient statistical power to rigorously test this hypothesis on the basis of sequencing analysis of 900 bp, and thus, additional studies based on sequencing or SNPs genotyping across a larger region, such as that performed by Patin *et al*.,[33] are necessary in Native American populations. Moreover, caution to claim balancing selection on Native American population is required, as the founder effect associated with the peopling of the Americas can produce patterns of diversity similar to those generated by balancing selection. On the other hand, the population from the shantytown of Las Pampas in the city of Lima was found to be predominantly Native American. This result stresses the fact that in many Latin-American countries, such as those enveloping the Andes, Native Americans are not a minority group restricted to isolated settlements, but are present in large numbers in urban areas.

Materials and methods

Samples and populations genotyped in this study

We re-sequenced the coding region and part of the 3′ UTR (900 bp) of *NAT2* gene from the ATG starting codon of exon 2 (positions 108–1007 GenBank NM_000015), using standard polymerase chain reaction (PCR) and sequencing methods. PCR and sequencing primers and conditions used can be requested from the authors. Our sample includes 456 chromosomes from the following 14 native populations from Siberia and the American continent (codes used in Figure 3 and previous studies on these samples are in parentheses): Western Siberians (WSI), Yakut Siberians (YAK), Chipewyan and Cree from Canada (CHI and CRE), Mexican Pima and Maya (PIM, MAY), Colombian Piapoco and Curripaco (P&C) and Coyaima (COY), Cayapa from Ecuador (CAY),[57] San Martín and Tayacaja from Peru (SMA, TAY),[31,40,58] and Karitiana (KAR), Surui (SUR), Xavante (XAV) from Brazil. YAK, PIM, MAY, P&C, KAR and SUR individuals are from the HGDP-CEPH Human Genome Diversity Cell Line Panel.[59] Figure 3 shows the geographic distributions of the samples and their sizes. Additionally, we have studied 66 chromosomes from tuberculosis patients from the admixed population of Las Pampas, a shantytown in Lima, Peru (LIM). All samples were collected with informed consent of the donors and have been anonymized. We also sequenced the same genomic region in one chimpanzee provided by the European Collection of Cell Cultures (Ecacc, ref. EB176 (JC) 89072704) to identify ancestral states of alleles.

For the admixture analyses of the Las Pampas sample, we used 15 microsatellites included in the AmpFLSTR Identifiler Kit (Applied Biosystems, Foster City, CA, USA).

Statistical analyses

Haplotypes were inferred using the software PHASE version 2.1.[60] Phylogenetic relationships between different haplotypes were explored by a network implemented in the software TCS.[61]

The within-population variability was estimated by haplotype diversity (h),[41] and by calculating two estimators of the parameter $\theta = 4N_e\mu$, (where N_e is the effective population size and μ the mutation rate per generation): (1) nucleotide diversity (π), which is the per-site mean number of pair-wise differences between sequences[42] and (2) θ_s, based on the number of segregating sites (*S*).[43] Xavante from Brazil were excluded from this analysis because of the small sample size. Native Americans were grouped into North-Central America and South America, and standard F_{ST} values were calculated to estimate the between-population component of genetic variance for each of these groups, using the software Arlequin 2.0.[62]

We applied two kinds of spatial autocorrelation analyses. In the first case, we considered the whole set of populations and haplotypes and calculated the similarity index II by means of the AIDA software (Autocorrelation Index for DNA Analysis[47] http://web.unife.it/progetti/genetica/Giorgio/giorgio_soft.html). In the second case, a classic spatial autocorrelation analysis was performed on haplotype and phenotype frequencies using the software PASSAGE.[63]

The admixture was estimated using a sample of 58 tuberculosis patients and 65 healthy individuals from Las Pampas (LIM). To represent the parental populations, we included 42 natives from TAY and SAM, and 31 Europeans and 24 African ancestry individuals from *SNP500Cancer*. The estimation of admixture was performed using the software *Structure*,[64] assuming that individuals are admixed, and that the allele frequencies of parental populations are correlated.

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Duality of interest

The authors declare that do not have conflict of interest.

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