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Arylamine N-acetyltransferase 2 genotypes in a Mexican population

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ABSTRACT. The acetylating activity of N-acetyltransferase 2 (NAT2) has critical implications for therapeutics and disease susceptibility. To date, several polymorphisms that alter the enzymatic activity and/or protein stability of NAT2 have been identified. We examined the distribution and frequency of *NAT2* genotypes in the Mexican population. Among 250 samples amplified and sequenced for the *NAT2* gene, we found seven different SNPs; the most frequent allele was 803 A>G (35.8%), followed by 282 C>T, 341 T>C, and 481 C>T. There were no differences in the distribution of SNPs between healthy subjects and cancer patients. These eight polymorphisms defined 26 diplotypes; 11.6% were wild type (*NAT2*4/NAT2*4*), while the most common diplotype was *NAT2*4/NAT2*5B*, present in 17.2%. We did not identify other common polymorphisms. The results were compared with the *NAT2* SNPs reported from other populations. All but the

Turkish population was significantly different from ours. We conclude that the mixed-race Mexican population requires special attention because *NAT2* genotype frequencies differ from those in other regions of the world.

Key words: NAT2; Acetylating phenotype; Polymorphism

INTRODUCTION

N-acetyltransferase 2 (NAT2) is a phase II metabolizing enzyme that metabolizes xenobiotic compounds containing aromatic amines and hydrazines by N- or O-acetylation, including commonly prescribed drugs such as isoniazid, dapsone, hydralazine, procainamide and sulfamethoxazole (Agundez, 2008). It also catalyzes the transformation of aromatic and heterocyclic amines (present in cigarette smoke and overcooked meat) into carcinogenic intermediates, which may entail detoxification or activation of the substrate (Hein et al., 2000; Hein, 2002).

NAT2 is encoded by the highly polymorphic, single, intronless *NAT2* gene located at 8p22 (Vatsis et al., 1995). In general, single nucleotide substitutions in *NAT2* result in low activity, decreased expression or enzyme instability (Hein et al., 1994), and as a consequence, variation in N-acetylation activity (Parkin et al., 1997; Gardiner and Begg, 2006). These differences in activity have resulted in the classification of individuals as rapid or slow acetylators (bimodal distribution) and the proportion of slow and rapid acetylators differs between ethnic populations (Lin et al., 1994; Gross et al., 1999; Fuselli et al., 2007; Garcia-Martin, 2008). Both rapid and slow phenotypes and genotypes have been associated with either an increased or a decreased risk of several types of cancer (Cascorbi et al., 1996; Costa et al., 2002; Ladero et al., 2002; Garcia-Closas et al., 2005; Agundez, 2008).

Currently, 32 nucleotide changes have been identified in the coding region of *NAT2*. The most common of which are 191 G>A, 341 T>C, 590 G>A, 803 A>G, and 857 G>A, which result in amino acid changes that lead to a reduction in acetylation activity. Changes of 282 C>T and 481 C>T are synonymous SNPs and do not modify enzyme activity (Hein et al., 2006; Zhang et al., 2007; Walraven et al., 2008). The different combinations of SNPs at *NAT2* generate the so-called *NAT2* alleles, haplotypes or diplotypes. To date, 62 alleles have been identified (http://www.louisville.edu/medschool/pharmacology/NAT.html).

Variants of *NAT2* haplotypes possessing combinations of SNPs are segregated into clusters possessing a signature SNP, either alone or in combination with others. *NAT2*4* is considered the "wild-type" or reference haplotype with no SNPs, while human *NAT2* alleles containing 341 T>C (I114T) are assigned to the *NAT2*5* cluster; alleles containing 590 G>A (R197Q) are assigned to the *NAT2*6* cluster and so on (Walraven et al., 2008).

Large interethnic and intraethnic variabilities in the *NAT2* genotypes and phenotypes have been described (Garcia-Martin, 2008). The slow acetylator phenotype is most prevalent in Northern Africans (90%) and Scandinavians (75-80%) and has its lowest prevalence in Canadian Eskimos and Japanese (5%) (Lin et al., 1994; Gross et al., 1999). To date, the prevalence of *NAT2* polymorphisms has not been extensively studied in the Mexican population, which has a high degree of admixture. The purpose of this study was to determine the distribution of *NAT2* genotypes in the Mexican population.

SUBJECTS AND METHODS

Population

The sample consisted of 250 unrelated subjects from Mexico's central region: 118 healthy donors and 132 cancer patients (cervical, ovarian, breast cancer, and myelodysplastic syndrome), including 140 women and 110 men. All samples were taken after informed written consent, and the protocol was approved by the Ethics and Scientific Committee of the Instituto Nacional de Cancerología, México.

DNA extraction

DNA was obtained from 10 mL peripheral blood. Genomic DNA was extracted with phenol:chloroform and ethanol-precipitated. DNA samples were resuspended in nuclease-free water, spectrophotometrically quantified and stored at -20°C.

PCR

Oligonucleotides were designed to amplify the *NAT2* gene sequence, divided into two amplicons called NAT2-5' and NAT2-3'. The primer sequences were: NAT2-5'-S 5'-cct taacatgcattgtgggcaagc-3'; NAT2-5'-AS 5'-ctgatccttcccagaaattaattct-3', and NAT2-3'-S 5'-gcttga cagaagagagaggaatctgg-3', NAT2-3'-AS 5'-gtgagttgggtgatacatacacaagg-3'. The size of the product was 313 bp for NAT2-5' and 467 bp for NAT2-3'. PCR was performed in a total volume of 20 µL containing 100 ng DNA, 1 µM of each primer (forward and reverse), 200 µM dNTPs (Applied Biosystems, Foster City, CA, USA), 2 mM $MgCl_2$, 0.25 U TaqPol (Applied Biosystems) and 1X buffer provided by the manufacturer. PCR was performed in a 2700 Thermalcycler (Applied Biosystems). Amplification reactions consisted of an initial denaturation at 95°C for 5 min, followed by 15 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 30 s, 20 cycles in which annealing was carried out at 61°C, and a final extension at 72°C for 5 min. Amplification was verified by agarose gel electrophoresis.

NAT2 genotyping

PCR amplicons were purified by isopropanol precipitation and then sequenced in both forward and reverse directions for at least two independent amplification products. Purified PCR products were diluted and cycle-sequenced using the ABI BigDye Terminator kit v3.1 (ABI, Foster City, CA, USA) according to manufacturer instructions. Sequencing reactions were electrophoresed on an ABI3100 genetic analyzer. Electropherograms were analyzed in both sense and antisense directions for the presence of polymorphisms. The sequences obtained were compared with the reference *NAT2* sequence (GenBank accession No. X14672).

Haplotype reconstruction

Haplotype reconstruction was performed with the PHASE v2.1.1 program using the default model for recombination rate variation (Stephens et al., 2001; Stephens and Donnelly,

2003; Stephens and Sheet, 2005). This reconstruction is needed as commonly used genotyping methods for *NAT2* simultaneously analyze the two alleles (i.e., the paternal and the maternal alleles). This characteristic is a major source of uncertainty in the case of *NAT2* analysis, because 2 or more enzyme-inactivating polymorphisms can be located in one allele, leaving the other allele intact, or the polymorphisms can be distributed between the two alleles, leading to a lack of functional *NAT2* alleles. In fact, rather than indicating real genotypes, most genotyping method results indicate the sum of two haplotypes (diplotypes), and many such diplotypes can represent different genotypes with different functional consequences. Thus, the PHASE algorithm uses a Bayesian approach for the reconstruction of haplotypes to obtain unambiguous genotypes, which determines the most likely genotype pair (Agundez et al., 2008).

Statistical analysis

Expected genotype frequencies were calculated using the Hardy-Weinberg equation from the allele frequencies ($p2 + 2pq + q2 = 1$). Data were analyzed statistically using the two-tailed χ^2 test with Yates' correction.

RESULTS

A total of 250 samples (118 healthy donors and 132 cancer patients) were amplified and sequenced. We found seven different SNPs: 191 G>A (rs1801279), 282 C>T (rs1041983), 341 T>C (rs1801280), 481 C>T (rs1799929), 590 G>A (rs1799930), 803 A>G (rs1208), and 857 G>T (rs1799931). Figure 1 shows the frequency of the SNPs found in the total population. The most frequent allele was 803 A>G (35.8%), followed by 282 C>T, 341 T>C, and 481 C>T, where these three were found in 29.2 to 31.6% of cases. SNP 191 G>A occurred in less than 1% of cases.

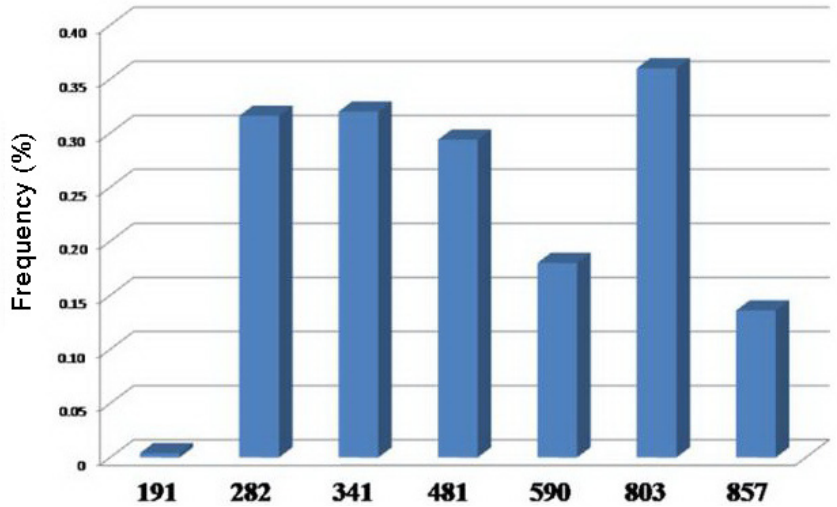

Figure 1. SNP frequencies. The most frequent allele was 803 A>G (35.8%), followed by 282 C>T, 341 T>C, and 481 C>T, where these three were between 29.2 and 31.6%. SNP 191 G>A occurred in less than 1% of cases.

As expected, Table 1 shows that there were no differences in the distribution of SNPs between healthy subjects and cancer patients.

Table 1. Comparison of the wild-type (wt) and normal alleles found for the different SNPs in healthy (N = 118, 236 alleles) and cancer (N = 132, 264 alleles) individuals.

Status	SNP	wt alleles	Mutated alleles	P value
Healthy	191	235	1	0.945
Cancer		263	1	
Healthy	282	163	73	0.891
Cancer		179	85	
Healthy	341	160	76	0.951
Cancer		180	84	
Healthy	481	165	71	0.843
Cancer		188	76	
Healthy	590	188	48	0.237
Cancer		222	42	
Healthy	803	147	89	0.538
Cancer		173	91	
Healthy	857	208	28	0.312
Cancer		224	40	

No statistically significant differences between cancer patients and healthy individuals were found.

Because we sequenced the complete open reading frame of the *NAT2* gene, we could exclude from our population the occurrence of several NAT2 SNPs that have been described to occur in human populations, such as rs111750824, rs72466457, rs78003756, rs72466458, rs72466459, rs4986996, rs12720065, rs72554617, rs79050330, rs72466461, rs45618543, rs45607939, rs56387565, rs45518335, rs56011192, rs55700793, rs56054745, or rs72554618. Apart from the seven SNPs shown in Table 1, so far, several other SNPs have been described within the coding region of the *NAT2* gene, although their frequencies remain unknown. Our findings indicate that the frequencies for these alleles are, at best, extremely low. Table 2 shows the additional *NAT2* SNPs tested, which were not identified in the population study. No new polymorphisms were identified in 500 alleles, thus indicating that, besides the already known common *NAT2* polymorphisms, the *NAT2* gene has no other SNPs in the population under study. Regarding SNP association, SNP 282 was highly associated with 590 and 857, SNP 341 was highly associated with 481 and 803, SNP 481 was strongly associated with SNP 341 but poorly with SNP 191, and so on (Figure 2). Haplotype assignment was done with the PHASE 2.1.1 program. Genotypic and allelic frequencies are shown in Table 3. All variant alleles were in Hardy-Weinberg equilibrium. Genotyping for these seven variants allowed for the detection of 26 different haplotypes, the most common of which was *NAT2*4/NAT2*5B*, present in 17.2% of participants, followed by the homozygous wild-type diplotype *NAT2*4/NAT2*4* (11.6%), *NAT*6A/NAT2*5B* (11.2%), *NAT2*5B/NAT2*7B* (8.8%), *NAT2*5B/NAT2*5B* and *NAT2*4/NAT2*7B* (7.6% each), and *NAT2*4/NAT2*6A* (6.8%), where the remaining haplotypes were present in less than 5% (Figure 3). The analysis of allele frequencies indicates that the most common allele was *NAT2*4* (30.4%), followed by *NAT2*5B* (29%) and so on (Figure 4). To gain further insight into how our population stands in comparison to 41 worldwide samples that were analyzed for their genotype at the seven common SNPs of the *NAT2* gene, we compared each one of these with our sample. Table 4 shows that all but the Turkish population was statistically different compared to ours.

Table 2. *NAT2* SNPs that were analyzed and not identified in the present study.

SNP	Effect	Base exchange	Amino acid exchange	Present in allele
rs111750824	Missense	148 T/A	50 Leu/Met	-
rs72466457	Missense	152 G/T	51 Gly/Val	*NAT2*6M*
rs78003756	Missense	169 G/T	57 Asp/Tyr	-
rs72466458	Missense	203 G/A	68 Cys/Tyr	*NAT2*12G*
rs72466459	Synonymous	228 C/T	76 Tyr/Tyr	*NAT2*12I*
-	Synonymous	345 C/T	115 Asp/Asp	*NAT2*6L*
rs4986996	Missense	364 G/A	122 Asp/Asn	*NAT2*12D*
rs12720065	Missense	403 C/G	135 Leu/Val	*NAT2*12H*
rs72554617	Missense	499 G/A	167 Glu/Lys	*NAT2*10*
-	Missense	518 A/G	173 Lys/Arg	*NAT2*6G*
rs79050330	Missense	578 C/T	193 Thr/Met	*NAT2*12E*, *NAT2*13B*, *NAT2*5P*
rs72466461	Synonymous	600 A/G	200 Glu/Glu	*NAT2*20*
rs45618543	Missense	609 G/T	203 Glu/Asp	*NAT2*12G*
rs45607939	Missense	613 A/T	205 Met/Leu	-
rs56387565	Missense	622 T/C	208 Tyr/His	*NAT2*12F*
-	Missense	638 C/T	213 Pro/Leu	*NAT2*6K*
rs45518335	Missense	683 C/T	228 Pro/Leu	NAT2*14H
rs56011192	Synonymous	759 C/T	253 Val/Val	NAT2*5F
rs55700793	Missense	766 A/G	256 Lys/Glu	*NAT2*6H*
rs56054745	Missense	845 A/C	282 Lys/Thr	*NAT2*18*
rs72554618	Missense	859 T/C	287 Ser/Pro	-
-	Missense	859 del	287 Frameshift	*NAT2*5H*

These *NAT2* SNPs are described in detail at the websites [http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?chooseRs=coding&go=Go&locusId=10] and [http://louisville.edu/medschool/pharmacology/consensus-human-arylamine-n-acetyltransferase-gene-nomenclature/nat_pdf_files/Human_NAT2_alleles.pdf].

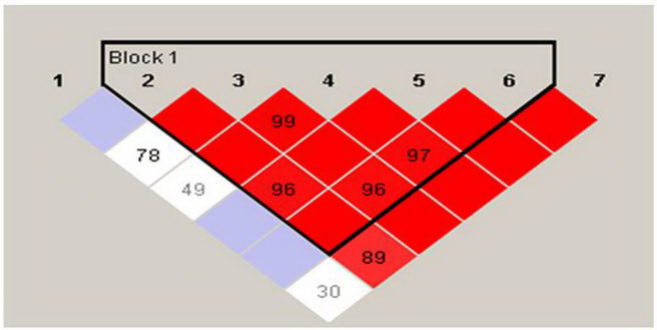

Figure 2. SNP association. Association of the most common SNPs found in the population studied: 1 = 191, 2 = 282, 3 = 341, 4 = 481, 5 = 590, 6 = 803, 7 = 857. The number within each cell corresponds to the D' value divided by 100. The red color indicates higher association.

Table 3. Genotypic and allelic frequencies.

SNP	Genotypes			Alleles		χ^2 test P value
	wt/wt	wt/mut	mut/mut	wt	mut	
rs1801279	G/G (0.992)	G/A (0.008)	A/A (0.000)	0.996	0.004	0.949
rs1041983	C/C (0.472)	C/T (0.424)	T/T (0.104)	0.684	0.316	0.762
rs1801280	T/T (0.444)	T/C (0.472)	C/C (0.084)	0.680	0.320	0.181
rs1799929	C/C (0.492)	C/T (0.428)	T/T (0.080)	0.706	0.294	0.623
rs1799930	G/G (0.688)	G/A (0.264)	A/A (0.048)	0.820	0.180	0.095
rs1208	A/A (0.380)	A/G (0.520)	G/G (0.100)	0.640	0.360	0.052
rs1799931	G/G (0.748)	G/A (0.232)	A/A (0.020)	0.864	0.136	0.839

This table shows the genotypic and allelic frequencies found for each SNP found in the population study. It also shows that all genetic changes found were in Hardy-Weinberg equilibrium (HWE). If $P < 0.05$ - not consistent with HWE. wt = wild type; mut = mutated.

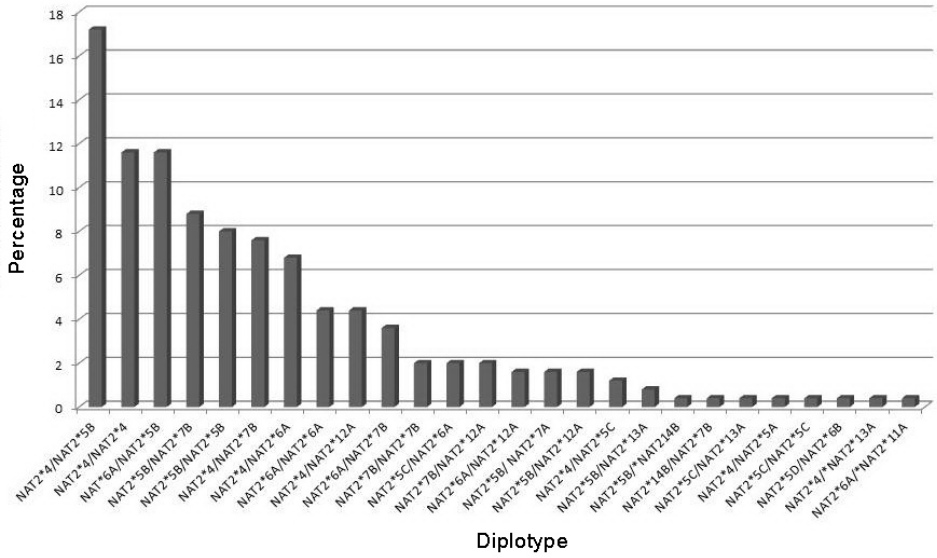

Figure 3. NAT2 haplotypes. There were 26 haplotypes; the most common was *NAT2*4/NAT2*5B* (17.2%), followed by the homozygous wild-type haplotype *NAT2*4/NAT2*4* (11.6%), *NAT*6A/NAT2*5B* (11.2%), *NAT2*5B/NAT2*7B* (8.8%), *NAT2*5B/NAT2*5B* and *NAT2*4/NAT2*7B* (7.6% each), *NAT2*4/NAT2*6A* (6.8%). The others were present at less than 5%.

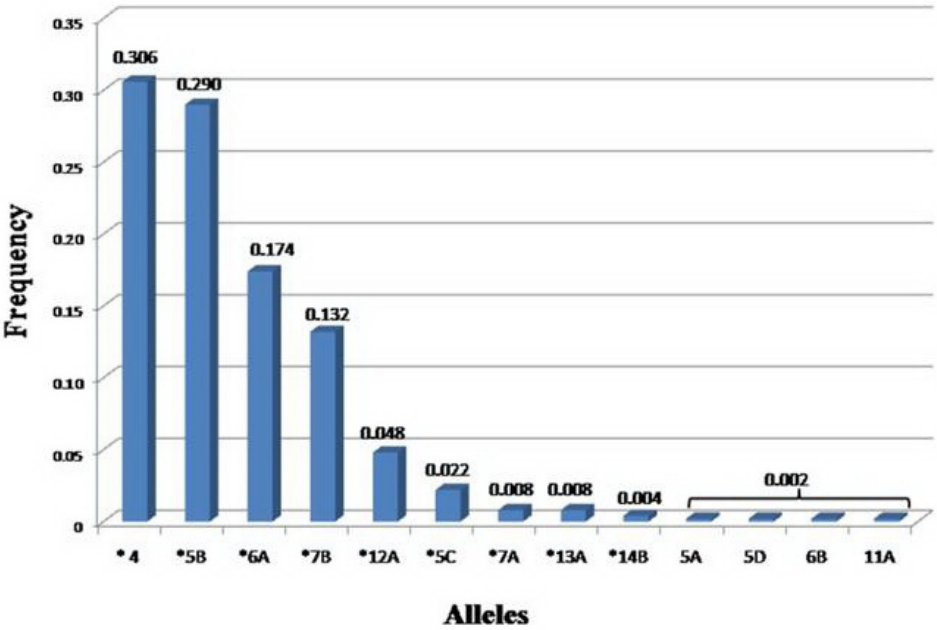

Figure 4. Allele frequency. The determination of allele frequencies showed that the most common allele was *NAT2*4* (30.4%), followed by *NAT2*5B* (29%), *NAT2*6A* (17.4%), and *NAT2*7B* (13.2%).

Table 4. Comparison by the χ^2 test (two-tailed) of population reported here with each of the others and the total.

	*4	*5A	*5B	*5C	*5D	*5E	*5M	*6A	*6B	*6C	*7A	*7B	*11A	*12A	*12B	*12C	*13	*13B	*14A	*14B	*6J[f]	Global (2N[g])
SNP position																						
191 (G)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**A**	**A**	.	
282 (C)	.	.	.	.	.	.	T	T	.	T	.	T	.	.	T	.	T	T	.	T	T	
341 (T)	.	**C**	**C**	**C**	**C**	**C**	**C**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
481 (C)	.	T	T	.	.	.	.	.	.	.	.	.	T	.	.	T	.	T	.	.	.	
590 (G)	.	.	.	.	.	**A**	.	**A**	**A**	**A**	.	.	.	.	.	.	.	.	.	.	**A**	
803 (A)	.	.	G	G	.	.	.	.	.	G	.	.	.	G	G	G	.	.	.	.	.	
857 (G)	.	.	.	.	.	.	.	.	.	.	**A**	**A**	.	.	.	.	.	.	.	.	**A**	
Population code[a]																						
1	27	1	47	17	0	0	0	34	4	0	0	0	0	34	7	1	13	0	6	11	0	**202**
2	10	1	37	3	0	0	1	16	0	0	0	2	0	9	2	0	6	0	3	10	0	**100**
3	8	0	4	0	0	0	0	11	0	0	0	0	0	39	1	0	13	0	0	4	0	**80**
4	4	0	13	5	0	0	0	8	0	0	0	0	0	19	3	0	6	0	0	2	0	**60**
6	18	2	65	3	0	0	0	32	0	0	0	13	0	30	0	0	10	0	17	3	1	**194**
7	13	0	22	8	0	0	0	32	5	0	2	1	0	7	1	0	4	0	2	3	0	**100**
8	3	0	19	0	0	0	0	17	0	0	0	1	0	7	0	0	0	0	1	0	0	**48**
9	13	0	45	0	0	0	0	22	0	0	0	3	0	4	0	0	1	0	0	0	0	**88**
10	133	1	242	0	0	0	0	127	2	0	0	3	0	6	0	0	0	0	2	0	0	**516**
11	20	1	52	1	0	0	0	24	0	0	0	0	0	0	0	0	0	0	0	0	0	**98**
12	22	6	54	2	0	0	0	30	0	0	0	1	0	0	0	0	5	0	0	0	0	**120**
14	44	8	104	6	0	0	0	54	1	0	0	5	0	1	0	0	1	0	0	0	0	**224**
15	187	20	318	17	0	0	0	206	0	0	0	15	0	3	0	0	7	0	0	1	0	**774**
17	11	6	50	3	0	0	0	28	0	0	0	2	0	0	0	0	0	0	0	0	0	**100**
18	23	3	42	0	0	0	0	15	0	0	0	13	0	0	0	0	0	0	0	0	0	**96**
19	383	70	647	68	0	0	0	470	0	0	0	22	0	0	0	0	26	0	0	2	0	**1688**
21	109	26	164	30	0	0	0	149	0	0	0	17	0	1	0	0	0	0	0	0	0	**496**
23	9	0	41	0	0	0	0	29	0	0	0	1	0	0	0	0	0	0	0	0	0	**80**
25	134	12	213	17	0	0	0	184	0	0	0	17	0	3	0	0	0	0	0	0	0	**580**
26	140	8	216	29	0	0	0	185	0	0	0	27	0	1	0	0	0	0	0	0	0	**606**
27	13	1	29	4	0	0	0	43	0	1	0	6	0	3	0	0	0	0	0	0	0	**100**
28	**31**	**0**	**23**	**3**	**0**	**0**	**0**	**30**	**0**	**0**	**0**	**12**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**100**
30	132	0	4	0	1	0	0	46	0	0	0	38	0	0	0	0	3	0	0	0	0	**224**
32	46	0	6	0	0	0	0	22	0	0	0	13	0	0	0	0	1	0	0	0	0	**88**
33	208	0	1	0	0	1	0	55	0	0	0	22	0	0	0	0	1	0	0	0	0	**288**
36	353	1	5	0	1	0	0	126	3	0	6	70	0	4	0	1	6	0	0	0	0	**576**
38	26	0	10	0	0	0	0	34	0	0	0	17	0	0	0	0	1	0	0	0	0	**88**
41	114	7	86	5	0	0	0	46	1	1	0	0	0	8	0	2	3	0	1	0	0	**274**
Here	**154**	1	145	11	1	0	0	86	1	0	4	66	1	24	0	0	4	0	0	2	0	**500**
Global	**2234**	174	2559	221	2	1	1	2075	16	2	8	321	0	180	14	4	107	0	32	36	1	**7988**

Underlined *NAT2* haplotypes were not taken into account for analysis due to the small numbers. Population 28 (in bold; Turkish) was not significantly different (P = 0.095); the P value for Saami population (18) was 0.001; P values for the remaining populations including the comparison with the global number were P = 0.000.
[a]Samples are numbered according to their population code (1) 101 Tswana, (2) 50 Ateke Bantus, (3) 40 Bakola Pygmies, (4) 30 Baka Pygmies, (6) 97 Mandenka, (7) 50 Dogons, (8) 24 Somali, (9) 44 Moroccans, (10) 258 Spanish, (11) 49 Sardinians, (12) 60 French, (14) 112 UK Caucasians, (15) 387 US Caucasians, (17) 50 Swedes, (18) 48 Saami, (19) 844 Germans, (21) 248 Polish, (23) 40 Ashkenazi Jews, (25) 290 Russians, (26) 303 Turks, (27) 50 Gujarati, (28) 50 Turkmen, (30) 112 Han Chinese, (32) 44 Chinese, (33) 144 Japanese, (36) 288 Koreans, (38) 44 Thai, and (41) 137 Nicaraguan (taken from Sabbagh et al., 2008).

DISCUSSION

Large interethnic and intraethnic variabilities in the *NAT2* genotypes and phenotypes have been described in populations around the world. There is little information in this regard for the Mexican Mestizo population. In this study, by sequencing the *NAT2* gene in a population of 250 individuals, we found that the most frequent SNP was 803 A>G, with 35.8% of cases, followed by an almost equal proportion of 282 C>T, 341 T>C, and 481 C>T. The SNP at position 282 was highly associated with those located at 590 and 857, whereas the SNP at 341 was associated with those at 481 and 803. All variant alleles were in Hardy-Weinberg equilibrium, and there were no differences in the distribution of SNPs between healthy subjects and cancer patients. This is in agreement with the lack of association between *NAT2* genotypes and the cancer types of the patients enrolled in our previous study (Agundez, 2008).

We detected 26 different haplotypes, the most common being *NAT2*4/NAT2*5B*, present in 17.2% of individuals. Besides the identification and the determination of common *NAT2* SNP frequencies and the haplotype structure in the population study, this study analyzed the occurrence of rare SNPs within the coding region of the *NAT2* gene. Our findings indicate that in the population study, in addition to the already well-known seven major *NAT2* SNPs, several other SNPs that have been described in other populations are absent in the 500 alleles studied here.

It has been established that variations in acetylator phenotype may lead to an increased risk of adverse drug reactions or a lack of therapeutic efficacy (Meisel et al., 2001; Meisel, 2002), as well as being likely associated with a variety of complex human disorders, such as several malignancies, atopic diseases, diabetes, Parkinson's disease, and many others (Hein et al., 2000; Butcher et al., 2002). These facts underscore the need to investigate *NAT2* gene variations in different populations. Lin et al. (1993, 1994) reported that US-Hispanics (from Mexico, Colombia, Guatemala, Nicaragua, and El Salvador) contain an admixture of white acetylator alleles, because of the higher frequency of the 481 C>T polymorphism and the lower frequency of the 857 G>A polymorphism, compared with the Harbor Hispanic population. These data are in agreement with our results, which also show a similar proportion in the occurrence of these two changes. Likewise, Martinez et al. (1998) reported for a mixed Indian-white population of Nicaragua, that the most common alleles were *NAT2*5B* (31.4% alleles) and *NAT2*6A* (16.8% alleles), and that the frequencies of alleles among Nicaraguans were in all cases intermediate between those of pure Central American Indians and white Spanish subjects, indicating that genetic admixture has played an important role in the frequency of *NAT2* alleles in this mixed population. A recent report for a Northern Mexican population identified an almost identical frequency of polymorphisms at T341C, C481T, G590A, A803G, and G857A, although this study did not analyze the 282 polymorphism (Ramos et al., 2011), which clearly suggests that the changes found here are common to the Mexican Mestizo population, as no bias was introduced by limiting the analyses to common SNPs and as the whole *NAT2* gene was sequenced.

In an attempt to better characterize the worldwide haplotype diversity and structure of *NAT2*, Sabbagh et al. (2008) reported the results of 41 population samples (including 6727 individuals) from four continental regions (Africa, Europe, Asia, America). Our results were compared with data for each of these populations and showed statistically significant differences with all but the Turkish population. Methodological issues were not taken into account

in this comparison. However, it can be hypothesized that the simplified view of the interethnic differences in *NAT2*, according to which every "major" race has similar characteristics and that mixed racial ancestry populations are in-between, may not totally explain intraethnic and interethnic differences between populations.

In conclusion, the results of this study add support to the genetic admixture of the Mexican Mestizo population and underscore the need for further testing in a larger number of individuals for NAT2 genotype frequencies to improve our understanding of the role of NAT2 acetylation polymorphisms in our population.

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