Brief Original Article

The distribution of allelic and genotypic frequencies of N-Acetyltransferase-2 variants in an Argentine population

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Abstract

Introduction: Arylamine N-acetyltransferase-2 (NAT-2) is a key human enzyme in drug detoxification and elimination. Mutations in NAT-2 affect the activity of anti-tuberculosis drugs and result in three different phenotypes: rapid (RA), intermediate (IA) and slow acetylators (SA).
Methodology: The allelic, genotypic and phenotypic frequencies of NAT-2 were studied in 185 patients from Buenos Aires by restriction fragment length polymorphism.
Results: The following allele frequencies were obtained: *4 = 29.9%, *5 = 37.0, *6 = 25.6%, *7 = 8% and *14 = 1.3%. With regard to the phenotype, we observed that 53.6% of the population was SA, 35.7% was IA and 10.7% was RA.
Conclusion: A high prevalence of SA might have an impact on anti-TB drug-induced hepatotoxicity.

Key words: NAT-2; polymorphism; tuberculosis; acetylator profile; prevalence

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Introduction

Arylamine N-acetyltransferase-2 (NAT-2) is a cytosolic phase II conjugation enzyme that plays a key role in the detoxification and elimination of many commonly prescribed drugs through acetylation, and it is also involved in the metabolism of carcinogens from environmental, industrial and dietary sources [1]. NAT-2 is a highly polymorphic enzyme. In human populations, 35 variants of the NAT-2 gene have been identified and classified with respect to varying combinations of up to four out of the 17 different single nucleotide polymorphisms (SNPs) present throughout the NAT-2 coding region [2]. Some of these mutations affect the activity of the enzyme resulting in three different phenotypes: rapid acetylators (RA), intermediate acetylators (IA) and slow acetylators (SA) [3].

The major alleles groups associated with decreased enzyme activity due to amino acid changes and, therefore, a slow acetylator phenotype, are NAT-2*5, NAT-2*6, NAT-2*7 and NAT-2*14 [4]; in several surveys homozygous wild type alleles at all four loci that have no variations are called RA and are represented as NAT-2*4 [4-7].

The SA acetylation status has been associated with Isoniazid (INH)-induced hepatitis in Asian populations [1,5]. INH, a substrate of NAT-2, is a first-line drug used in tuberculosis (TB) treatment, and it is considered to be responsible for adverse drug reactions that lead to hepatotoxicity [6,8,9]. Although an association between NAT-2 acetylation polymorphism and INH-induced hepatotoxicity has been reported by several studies, considerable controversies remain as a consequence of the wide variability in the results of these studies [5-6,8-9].

Because TB is a re-emerging disease and a major public health problem, the acetylator status has become an interesting focus of study. Anti-TB drug-induced hepatotoxicity DIH causes several problems at the patient and public health levels. In patients, it can result in liver failure and death. In public health, TB is associated with a prolonged hospital treatment and the risk of developing bacterial resistance, implying an increase in health costs [5,6].

Considering that both the frequency of NAT-2 alleles and the acetylator phenotype varies with ethnic origin, it would be of interest to determine their distribution in the local population. Therefore, the aim

of the present study was to evaluate the allelic and genotypic frequencies of the four most relevant NAT-2 polymorphisms that affect the acetylation profile in a population from Buenos Aires. In addition, we want to compare these results with those obtained from other populations that have been previously published.

Methodology

All subjects gave their written informed consent prior to their enrollment in this study protocol, which was approved by the Institutional Ethics and Research Committee.

Venous blood was drawn, without stasis, from 185 Argentine healthy subjects and collected in tubes containing EDTA. Subsequently, DNA was extracted using a fully automated technique from the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany. We studied four NAT-2 polymorphisms that had been largely associated with decreased enzyme activity (*5 - C481T, *6 - G590A, *7 - G857A and *14 - G191A) by PCR-RFLP (Polymerase Chain Reaction – Restriction Fragment Length Polymorphism) and examined their acetylator profile. The allele * 5 is characterized by the presence of the T341C SNP, which cannot be easily determined by this technique. Since it is in strong linkage disequilibrium with the C481T SNP, we studied the presence of the latter [10,11].

We considered RA as being homozygous for NAT2*4 (absence of any mutation), IA as being heterozygous for NAT2*4, and SA as being without NAT2*4 alleles. Statistical analysis was performed using the SPSS analysis software for Windows (IBM, Chicago, Il, USA). Expected genotype frequencies were calculated from respective single allele frequencies and were consistent with Hardy Weinberg equilibrium.

Results and discussion

Table 1 describes the allelic distribution of the four variants of NAT-2 that had the strongest impact on the acetylation profile in the Buenos Aires population and in different populations from various regions of Europe [1], Africa [1,12], Asia [1,6,9] and America [1,7 ,9, 13]. Our study group consisted of an admixture of ethnic groups with European (mainly from Spain, Italy and, to a lesser extent, from Germany and other countries of Eastern Europe), and Native American ancestries. Thus we selected from the published data two European populations (Germany and Spain), a Native American group, other admixed populations such as Brazilians, and other ethnic groups, to compare with our results.

In the Buenos Aires population, the allelic frequencies of variants *4, *5, *6, *7 and *14 were 29.9%, 37.0%, 25.6%, 8.0% and 1.3%, respectively. Table 2 shows genotypic and phenotypic distributions of NAT-2 in the Buenos Aires population. With regard to phenotype, the results show that 53.6% of the study population was SA, 35.7% was IA and 10.7% was RA.

As shown in Table 1, in the Buenos Aires population, the allelic frequencies of variants *4, *5, *6 and *14 were similar to those found in European populations. These results could be explained by the European ancestry in the Buenos Aires population. Conversely, the allele *7 variant had a frequency that reached intermediate values (8%) between the Southern Amerindian populations (20.1%) and the European population, where it is considered a rare allele. Thus the observed differences between European and Buenos Aires populations might be due to the penetrance of Southern Amerindian alleles. The frequency of the allele *7 variant reached intermediate values in the admixed populations with Amerindian and European ascendancy, such as the Argentinean and Brazilian populations. However, there were observed differences in other NAT-2 alleles between the Argentineans and Brazilians that could be explained by the African influence in Brazil. Nevertheless, it is worth noting that the sample size used in another study of the native populations of South America was too small for the study of polymorphisms; however, it is the only current source of reported data [7].

The prevalence of allele *14 that was observed in our study was very low (1.3%). There is evidence that this allele has an African origin [14], which might explain the low frequency observed in our studies and in the other analyzed populations.

Finally, the allelic frequencies of the Korean population showed great differences as compared with the Buenos Aires population, which might reflect the diverse ethnic origin of both populations.

As shown in Table 2, more than half of the researched population had a slow acetylator phenotype. The slow acetylation of some drugs, such as Isoniazid, retards their elimination from the body and can also result in the accumulation of precursors in the liver, such as hydrazine and acetyl hydrazine, leading to hepatotoxicity. Therefore, we believe that it is of great interest to examine the possible relationship

Table 1. Comparison of the frequency distribution of the four NAT-2 variants between the Buenos Aires population and others

	NAT-2 Allele (%)					
	***4**	***5**	***6**	***7**	***14**	**n**
Buenos Aires	**29.9**	**37.0**	**25.6**	**8.0**	**1.3**	185
Southern Brazil [7]	13.8	28.9	10.4	2.1	1.4	254
Central Brazil [12]	20.0	35.0	27.0	4.4	4.1	404
Germany [1]	22.7	42.5	27.8	1.3	0.1	844
Korea [1]	61.3	1.0	22.4	13.2	0.0	288
Southern Amerindians [6]	51.2	25.0	6.1	20.1	-	90
USA [1]	24.2	43.7	26.6	1.9	0.1	387
Spain [1]	25.8	47.0	25.0	0.6	0.4	258
Senegal [1]	40.6	32.2	18.8	0.0	8.4	101
South Africa [1]	29.9	36.1	17.0	6.7	10.3	97
Morocco [1]	20.5	51.1	25.0	3.4	0.0	44

NAT-2: N-acetyltransferase-2, n: number of individuals

Table 2. The genotype and phenotype distribution of the NAT2 gene

Genotype	Genotypic Frequency	Phenotype
NAT-2*4/4	10.7	RA
NAT-2*4/5	19.0	IA
NAT-2*4/6	12.5	IA
NAT-2*4/7	4.2	IA
NAT-2*4/14	0.0	IA
NAT-2*5/5	11.9	SA
NAT-2*5/6	19.6	SA
NAT-2*5/7	8.3	SA
NAT-2*5/14	0.0	SA
NAT-2*6/6	8.3	SA
NAT-2*6/7	3.0	SA
NAT-2*6/14	6.0	SA
NAT-2*7/7	0.6	SA
NAT-2*7/14	0.0	SA
NAT-2*14/14	1.2	SA
SA	53.6	
IA	35.7	
RA	10.7	

NAT-2: N-acetyltransferase-2; SA: slow acetylators; IA: intermediate acetylators; RA: rapid acetylators

between the acetylator profile and anti-TB drug-induced hepatotoxicity in the near future.

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References

1. Sabbagh A, Langaney A, Darlu P, Gerard N, Krishnamoorthy R, Poloni E (2008) Worldwide distribution of NAT2 diversity: Implications for NAT2 evolutionary history. BMC Genetics 9: 21.
2. Yuliwulandari R, Sachrowardi Q, Nishida N, Takasu M, Batubara L, Susmiarsih TP, Rochani JT, Wikaningrum R, Miyashita R, Miyagawa T, Sofro AS, Tokunaga K (2008) Polymorphisms of promoter and coding regions of the arylamine N-acetyltransferase 2 (NAT2) gene in the Indonesian population: proposal for a new nomenclature. J Hum Genet 53: 201-209.
3. Ohno M, Yamaguchi I, Yamamoto I, Fukuda T, Yokota S, Maekura R, Ito M, Yamamoto Y, Ogura T, Maeda K, Komuta K, Igarashi T, Azuma J (2000) Slow N-acetyltransferase 2 genotype affects the incidence of isoniazid and rifampicin-induced hepatotoxicity. Int J Tuberc Lung Dis 4: 256-261.
4. Walker K, Ginsberg G, Hattis D, Johns DO, Guyton KZ, Sonawane B (2009) Genetic polymorphism in N-Acetyltransferase (NAT): Population distribution of NAT1 and NAT2 activity. J Toxicol Environ Health B Crit Rev 12: 440-472.
5. Yamada S, Tang M, Richardson K, Halaschek-Wiener J, Chan M, Cook VJ, Fitzgerald JM, Elwood RK, Brooks-Wilson A, Marra F (2009) Genetic variations of NAT2 and CYP2E1 and isoniazid hepatotoxicity in a diverse population. Pharmacogenomics 10: 1433-1445.
6. Higuchi N, Tahara N, Yanagihara K, Fukushima K, Suyama N, Inoue Y, Miyazaki Y, Kobayashi T, Yoshiura K, Niikawa N, Wen CY, Isomoto H, Shikuwa S, Omagari K, Mizuta Y, Kohno S, Tsukamoto K (2007) NAT2 6A, a haplotype of the N-acetyltransferase 2 gene, is an important biomarker for risk of anti-tuberculosis drug-induced hepatotoxicity in Japanese patients with tuberculosis. World J Gastroenterol 13: 6003-6008.
7. Bailliet G, Santos MR, Alfaro EL, Dipierri JE, Demarchi DA, Carnese FR, Bianchi NO (2007) Allele and genotype frequencies of metabolic genes in Native Americans from Argentina and Paraguay. Mutat Res 627: 171-177.
8. Possuelo LG, Castelan JA, de Brito TC, Ribeiro AW, Cafrune PI, Picon PD, Santos AR, Teixeira RL, Gregianini TS, Hutz MH, Rossetti ML, Zaha A (2008) Association of slow N-acetyltransferase 2 profile and anti-TB drug-induced hepatotoxicity in patients from Southern Brazil. Eur J Clin Pharmacol 64: 673-681.
9. Cho HJ, Koh WJ, Ryu YJ, Ki CS, Nam MH, Kim JW, Lee SY (2007) Genetic polymorphisms of NAT2 and CYP2E1 associated with antituberculosis drug-induced hepatotoxicity in Korean patients with pulmonary tuberculosis Tuberculosis (Edinb) 87: 551-556.
10. Batra J, Sharma SK, Ghosh B (2006) Arylamine N-acetyltransferase gene polymorphisms: markers for atopic asthma, serum IgE and blood eosinophil counts. Pharmacogenomics 7: 673-682.
11. Lin HJ, Han CY, Lin BK. Hardy S (1993) Slow acetylator mutations in the human polymorphic N-acetyltransferase gene in 786 Asians, blacks, Hispanics, and whites: application to metabolic epidemiology Am J Hum Genet 52: 827-834.
12. Bell DA, Taylor JA, Butler MA, Stephens EA, Wiest J, Brubaker LH, Kadlubar FF, Lucier GW (1993) Genotype/phenotype discordance for human arylamine N-acetyltransferase (NAT2) reveals a new slow-acetylator allele common in African-Americans. Carcinogenesis 14: 1689-1692.
13. Teixeira RL, Miranda AB, Pacheco AG, Lopes MQ, Fonseca-Costa J, Rabahi MF, Melo HM, Kritski AL, Mello FC, Suffys PN, Santos, AR (2007) Genetic profile of the arylamine N-acetyltransferase 2 coding gene among individuals from two different regions of Brazil. Mutat Res 624: 31-40.
14. Cavaco I, Reis R, Gil JP, Ribeiro V (2003) CYP3A4*1B and NAT2*14 alleles in a native African population. Clin Chem Lab Med 41: 606-609.

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