Short reports

A simplified assay for the arylamine N-acetyltransferase 2 polymorphism validated by phenotyping with isoniazid

C A D Smith, Mia Wadelius, Alan C Gough, D J Harrison, C Roland Wolf, Anders Rane

Abstract

Human arylamine N-acetyltransferase (NAT) activity is determined by two distinct genes, NAT1 and NAT2, and the classical acetylation polymorphism in NAT2 has been associated with a variety of disorders, including lupus erythematosus and arylamine induced cancers. Over 50% of the white population exhibit a slow acetylator phenotype. The genetic basis of the defect has been identified and several DNA based assays are available for genotyping studies.

We present here a simplified, rapid PCR based assay for the identification of the major slow acetylator genotypes and validate it using isoniazid as probe drug. This assay was 100% predictive of phenotype. The three genotypes (homozygous mutated, heterozygous, and homozygous rapid) corresponded to a trimodal distribution of Ac-INH/INH metabolic ratios (slow, intermediate, and rapid) without overlapping.

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The human acetylation polymorphism contributes to the variation in metabolism of certain drugs and xenobiotics. There is substantial evidence that this polymorphism is causally related to drug induced toxicity, disease, and cancer susceptibility.¹ Three distinct arylamine N-acetyltransferase (NAT) genes have been localised to chromosome 8p22^{2,3} and consist of two active genes (NAT1 and NAT2) and an inactive pseudogene (NATP1).⁴ The NAT2 gene is responsible for the acetylation polymorphism which determines if people are rapid, intermediate, or slow acetylators.⁴⁻⁶ In white populations, one major wild type allele (NAT2*4) and five major slow acetylator alleles have been identified.^{1,7} These mutant NAT2 alleles, termed *5A/B/C, *6, and *7, can be detected with the restriction enzymes *KpnI*/*DdeI*, *TaqI*, and *BamHI*, respectively.^{4,7-10}

A simplified PCR based assay for identification of the NAT2 genotype has been developed and validated by phenotyping with isoniazid in a group of 70 healthy, white, Swedish volunteers. The study was approved by the Ethics Committee of the Medical Faculty, Uppsala University and by the Scottish Ethical Review Board. Acetylation capacity was determined by oral administration of 300 mg of isoniazid (Tibinide®) after an overnight fast. Blood was collected after four hours. Isoniazid (INH) and its acetylated metabolite (Ac-INH) were measured in plasma by HPLC.¹¹ Subjects with an Ac-INH/INH ratio of less than 1.0 were classified as slow acetylators. The coefficient of variation of the analytical assay was 5.4% for INH and 4.7% for Ac-INH.

Genomic DNA was extracted from whole blood by a rapid lysis method,¹² and 5-10 µl was used for the genotyping assay. PCR amplification with the primer pair P1, 5'-GCTGGGTCTGGAAGCTCCTC-3' (nucleotide positions 367-386) and P2, 5'-TTGGGTGATACATACACAAGGG-3' (nucleotide positions 892-913) generates a NAT2 specific 547 bp fragment.⁵ The PCR was carried out using 2 units of *Taq* polymerase (Promega) and 100 ng of each primer with a standard mix of deoxyribonucleotides in a PCR buffer at an annealing temperature of 60°C. The mutations at positions 481, 803, 590, and 857 were distinguished by the electrophoretic banding patterns produced after separate digestions of the PCR product by the restriction enzymes *KpnI*, *DdeI*, *TaqI*, and *BamHI* (table 1, fig 1). The T341C mutation was not studied, but it is known to cosegregate

Table 1 Cleavage pattern for NAT2 alleles obtained with *KpnI*, *DdeI*, *TaqI* and *BamHI* restriction enzymes. Bold symbols indicate the changes in cleavage pattern

Alleles	Mutations	<i>KpnI</i>	<i>DdeI</i>	<i>TaqI</i>	<i>BamHI</i>
NAT2*4	No	+	-	+	+
NAT2*5A	C481T	-	-	+	+
NAT2*5B	C481T;A803G	-	+	+	+
NAT2*5C	A803G	+	+	+	+
NAT2*6	G590A	+	-	-	+
NAT2*7	G857A	+	-	+	-

+Restriction site present.
-Restriction site absent.

ICRF Molecular Pharmacology Unit, Biomedical Research Centre, Ninewells Hospital, Dundee, UK
C A D Smith
C R Wolf

Department of Clinical Pharmacology, University Hospital, S-751 85 Uppsala, Sweden
M Wadelius
A Rane

University Surgical Unit and GenoVar Diagnostics, Southampton General Hospital, Southampton, UK
A C Gough
C A D Smith

Department of Pathology, University of Edinburgh, Edinburgh, UK
C A D Smith
D J Harrison

Correspondence to: Professor Rane.

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Table 2 Distribution of NAT2 alleles in a Scottish and a Swedish population. Correlation between isoniazid phenotype and NAT2 genotype in 70 Swedish subjects

Genotype	Scots (%) (n=96)	Swedes (%) (n=70)	Phenotype	Metabolic ratio (mean) Swedes
*4/*4	5 (5.2)	6 (8.6)	Fast	7.89-13.50 (10.83)
*4/*5	16 (16.7)	7 (10.0)	"	1.40-4.92 (3.14)
*4/*6	12 (12.5)	7 (10.0)	"	2.89-5.94 (4.19)
*4/*7	1 (1.0)	1 (1.4)	"	2.73
*5/*5	24 (25.0)	19 (27.1)	Slow	0.26-0.64 (0.42)
*6/*6	6 (6.2)	4 (5.7)	"	0.28-0.33 (0.30)
*5/*6	26 (27.1)	24 (34.3)	"	0.24-0.50 (0.36)
*5/*7	4 (4.1)	2 (2.9)	"	0.27-0.34 (0.30)
*6/*7	2 (2.1)	0 (—)	"	— (—)

Figure 1 The PCR primers, P1 and P2, span the polymorphic region containing the KpnI, DdeI, TaqI, and BamHI restriction enzyme sites which are diagnostic for mutations at nucleotide positions 481, 803, 590, and 857, respectively. The diagnostic bands for analysis of the mutation sites are shown. The restriction pattern is visualised after two hours of electrophoresis at 150-170 V in 8% non-denaturing polyacrylamide gel, ethidium bromide staining, and UV transillumination.

Figure 2 Genotypes for the NAT2 gene in 70 subjects phenotyped with isoniazid (INH) as substrate for acetylation. The acetyl-INH/INH ratio was measured in plasma after a single oral dose of the drug. An antimode of 1.0 was used to distinguish between rapid and slow acetylators.

Table 3 NAT2 allele frequencies in white Scots and Swedes

	No	*4	*5	*6	*7	MAF†
Scots	192	0.203	0.490	0.271	0.036	0.797
Swedes	140	0.193	0.507	0.278	0.021	0.807

†Mutant allele frequency, the total frequency of mutant alleles in the population studied.

with the A803G mutation to approximately 89% and with the silent C481T mutation to approximately 98%.^{1 13} Furthermore, subjects genotyped *4/*5B are not possible to distinguish from slow acetylators with the *5A/*5C genotype, but according to allele frequencies the latter genotype occurs only in about 3 in 1000 subjects.¹³

Using the isoniazid phenotyping assay, 21 (30%) of the Swedish volunteers were identified as fast acetylators (table 2). Forty-nine (70%) were phenotyped as slow acetylators. The phenotyped subjects were all correctly identified by genotyping (fig 2). The 15 heterozygous fast acetylators have a discrete intermediate acetylation capacity, and it is clear that they had significantly lower rates of isoniazid acetylation than the six homozygous fast acetylators (95% non-parametric confidence interval (5.06; 9.84)) (table 2, fig 2). Other investigators have not observed such a clear distinction between slow, intermediate, and fast acetylators using caffeine and dapsone.^{14 17}

In addition, 96 random white Scottish subjects were genotyped for NAT2 (table 2). Thirty-four (35%) were fast acetylators and 62 (65%) were slow acetylators. There was no significant difference in the distribution of genotypes between the Swedish and Scottish populations ($\chi^2=0.350$, $p<0.7$). When mutant allele frequencies were compared, the two populations were almost identical (table 3, $\chi^2=0.766$, $p<0.5$).

Several genetic assays are available for genotyping of NAT2, but they are complicated by the use of different primers for allele specific PCR.^{1 12 17} The simplified assay presented here requires only one PCR amplification and was 100% predictive of phenotype. Furthermore, use of isoniazid for phenotyping allows total segregation between the three groups of acetylators identified by our genotyping method.

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