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Accuracy of various human *NAT2* SNP genotyping panels to infer rapid, intermediate and slow acetylator phenotypes

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Abstract

Aim—Humans exhibit genetic polymorphism in *NAT2* resulting in rapid, intermediate and slow acetylator phenotypes. Over 65 *NAT2* variants possessing one or more SNPs in the 870-bp *NAT2* coding region have been reported. The seven most frequent SNPs are rs1801279 (191G>A), rs1041983 (282C>T), rs1801280 (341T>C), rs1799929 (481C>T), rs1799930 (590G>A), rs1208 (803A>G) and rs1799931 (857G>A). The majority of studies investigate the *NAT2* genotype assay for three SNPs: 481C>T, 590G>A and 857G>A. A tag-SNP (rs1495741) recently identified in a genome-wide association study has also been proposed as a biomarker for the *NAT2* phenotype.

Materials & methods—Sulfamethazine *N*-acetyltransferase catalytic activities were measured in cryopreserved human hepatocytes from a convenience sample of individuals in the USA with an ethnic frequency similar to the 2010 US population census. These activities were segregated by the tag-SNP rs1495741 and each of the seven SNPs described above. We assessed the accuracy of the tag-SNP and various two-, three-, four- and seven-SNP genotyping panels for their ability to accurately infer *NAT2* phenotype.

Results—The accuracy of the various *NAT2* SNP genotype panels to infer *NAT2* phenotype were as follows: seven-SNP: 98.4%; tag-SNP: 77.7%; two-SNP: 96.1%; three-SNP: 92.2%; and four-SNP: 98.4%.

Conclusion—A *NAT2* four-SNP genotype panel of rs1801279 (191G>A), rs1801280 (341T>C), rs1799930 (590G>A) and rs1799931 (857G>A) infers *NAT2* acetylator phenotype with high accuracy, and is recommended over the tag-, two-, three- and (for economy of scale) the seven-SNP genotyping panels, particularly in populations of non-European ancestry.

Keywords

acetylator genotype; acetylator phenotype; cryopreserved; human hepatocyte; *NAT2*; SNP

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Financial & competing interests disclosure

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The *N*-acetylation polymorphism was discovered over 50 years ago when individual variability in isoniazid neurotoxicity was attributed to genetic variability in *N*-acetylation capacity [1]. In addition to isoniazid, many aromatic amine drugs, such as sulfamethazine (SMZ), are subject to the acetylation polymorphism, thus affecting therapeutic efficacy and toxicity for many therapeutic drugs [2,3]. NAT2 is encoded by a 870-bp gene (*NAT2*) and genetic polymorphisms in *NAT2* is common in the human population [3–6]. *NAT2* genetic polymorphism modifies both the efficacy and toxicity of numerous arylamine and hydrazine drugs [2,3,6–8] and increases risk towards several arylamine carcinogen-related cancers [3,9,10].

The reference *NAT2**4 allele and over 65 *NAT2* allelic variants have been identified in human populations [10]. The *NAT2* variant alleles possess one or a combination of SNPs in the 870 bp *NAT2* coding region. As recently reviewed [11,12], most *NAT2* variant alleles include one or more of the following seven most frequent SNPs: rs1801279 (191G>A), rs1041983 (282C>T), rs1801280 (341T>C), rs1799929 (481C>T), rs1799930 (590G>A), rs1208 (803A>G) and rs1799931 (857G>A). The majority of studies investigating the relationship of *NAT2* genotype with drug therapy and/or disease risk use assays that detect only three SNPs (481C>T, 590G>A and 857G>A) to infer *NAT2* acetylation status. Although several *NAT2* SNPs are in linkage disequilibrium, assessment of only these three SNPs results in the misclassification of some *NAT2* alleles [9,13].

The detection of 11 SNPs (seven SNPs listed above plus 111T>C, 434A>C, 759C>T and 845A>C) as previously described [14] has been proposed as the gold standard approach for *NAT2* allele identification [13]. However, following comparisons of *NAT2* genotyping results using both the 11-SNP and seven-SNP panels, the seven-SNP assay was sufficient for *NAT2* genotyping in Caucasian and African–American populations [13].

Recently, a new tag-SNP rs1495741, at location 18317161, approximately 14.5 kb 3' of the *NAT2* coding region was identified in an ongoing bladder cancer genome-wide association study [15]. Agreement between the seven-SNP *NAT2* inferred phenotype and the tag-SNP, rs1495741, was assessed in over 2000 subjects from the Spanish Bladder Cancer Study and association of rs1495741 genotypes with *N*-acetyltransferase catalytic activity was assessed in cryopreserved hepatocytes from over 150 individuals of European background [16]. Estimates for the association between *NAT2* slow phenotype and bladder cancer risk and its interaction with cigarette smoking were comparable for the seven-SNP inferred *NAT2* phenotype and rs1495741 [15]. In addition, rs1495741 genotypes were strongly related to *N*-acetyltransferase activity measured in hepatocytes derived from over 150 individuals of European descent [16].

In the current study, we investigated associations of SMZ *N*-acetyltransferase catalytic activity in cryopreserved human hepatocytes with the tag-SNP rs1495741 and each of the seven SNPs from the coding region described previously. We assessed the accuracy of the tag-SNP and various two-, three-, four- and seven-SNP genotyping panels to accurately infer *NAT2* phenotype. Based on these results, we offer recommendations for *NAT2* genotype and inferred phenotype determinations.

Materials & methods

Source & ethnicity of cryopreserved human hepatocytes

Over 250 cryopreserved human hepatocyte samples were collected for public sale and received from Celsis *in vitro* Technologies (MD, USA). They were not collected for this study. Upon receipt, hepatocytes were stored in liquid nitrogen until use. The subject identifier or any information regarding the source of the hepatocytes was not provided.

Information on gender, age, race, cause of death, substance abuse, serology and other enzyme activities for the hepatocytes was accessed at [102]. Ethnicity was available for over 99% of the samples. The ethnic frequency in our convenience sampling from 256 individuals was 79.3% Caucasian, 10.55% African-American, 7.03% Hispanic, 1.17% Asian, 1.17% Polynesian and 0.78% unknown. The convenience sample was obtained from individuals in the USA and the ethnic frequency is similar to the 2010 US population census [103].

DNA isolation & SNP determinations

Upon removal from the liquid nitrogen, the hepatocytes were thawed and centrifuged as described previously [17]. Genomic DNA was isolated from pelleted cells prepared as described above using the QIAamp® DNA Mini Kit (Qiagen, MD, USA) according to manufacturer's instructions. SNPs in the *NAT2* coding region and their corresponding alleles and haplotypes were determined with the seven-SNP panel assay as previously described [18]. Briefly, SNP-specific PCR primers and fluorogenic probes were designed using Primer Express™ (Applied Biosystems, CA, USA). The fluorogenic probes are labeled with a reporter dye (either carboxyfluorescein or VIC®, Applied Biosystems) and are specific for one of the two possible bases identified at the seven SNPs rs1801279 (191G>A), rs1041983 (282C>T), rs1801280 (341T>C), rs1799929 (481C>T), rs1799930 (590G>A), rs1208 (803A>G) and rs1799931 (857G>A) in the *NAT2* coding region.

The presence of the rs1495741 SNP was determined using a predeveloped TaqMan® SNP Genotyping Assays, Assay ID C_86841101-0 (Applied Biosystems) according to manufacturer's instructions. Briefly the 20× primer and probe mix supplied (Assay ID C_8684110_10) was used at a 1× concentration with a half volume of 2× TaqMan Universal PCR Master Mix and 12.0 ng of genomic sample per well. Assays were designed and optimized to work with TaqMan® Universal PCR Master Mix (with or without AmpErase® UNG) using the same thermal cycling conditions, which were 60°C for 30 s, 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The PCR amplification and end point reading were performed on StepOnePlus™ real-time instrument (Applied Biosystems).

Controls (no DNA template) were run to ensure that there was no amplification of contaminating DNA.

NAT2 genotype & inferred phenotype

Determination of the seven *NAT2* coding region SNPs described above, define 34 of the reported human *NAT2* alleles or haplotypes (Table 1). *NAT2* alleles possessing additional SNPs in the *NAT2* coding region are quite rare [101]. Individuals possessing two of the *NAT2* alleles associated with rapid acetylation activity (*NAT2**4, *NAT2**11, *NAT2**12 and *NAT2**13 clusters) were classified as rapid acetylators; individuals possessing one of these alleles and one allele associated with slow acetylation (*NAT2**5, *NAT2**6, *NAT2**7 and *NAT2**14 clusters) were classified as intermediate acetylators, and those individuals that possessed two slow acetylation alleles were classified as slow acetylators as previously described [18].

The two-SNP inferred *NAT2* acetylator phenotype was determined by analysis of two SNPs: rs1041983 (282C>T) and rs1801280 (341T>C). The three-SNP inferred *NAT2* acetylator phenotype was determined by analysis of three SNPs: rs1799929 (481C>T), rs1799930 (590G>A) and rs1799931 (857G>A). The four-SNP inferred *NAT2* acetylator phenotype was determined by an assay of four SNPs: rs1801279 (191G>A), rs1801280 (341T>C), rs1799930 (590G>A) and rs1799931 (857G>A). For each of the two-, three- and four-SNP

panel assays, samples homozygous common for all SNPs were classified as rapid acetylator phenotype, samples heterozygous for any one of the SNPs were classified as intermediate acetylator phenotype and samples homozygous variant for one or more SNPs or heterozygous for two or more SNPs were classified as slow acetylator phenotype.

NAT2 catalytic activity

Upon removal from the liquid nitrogen, the hepatocytes were thawed and centrifuged as described previously [17]. Hepatocytes were lysed in 20 mM sodium phosphate (pH 7.4), 1 mM EDTA, 1 mM dithiothreitol, 100 μ M phenylmethanesulfonyl fluoride, 1 μ g/ml aprotinin and 1 μ M pepstatin A by three rounds of freeze (-70°C)/thawing (37°C). Lysates were centrifuged at $15,000 \times g$ for 20 min at 4°C and the resulting supernatant assayed for protein and enzymatic activity as described later.

SMZ *N*-acetyltransferase assays were conducted as previously described [20]. Briefly, reactions containing the supernatant of hepatocyte lysate (<2 mg/ml protein), SMZ (300 μ M) and acetyl coenzyme A (1 mM) were incubated at 37°C . Reactions were terminated at 10 min by the addition of 1/10 volume 1 M acetic acid. The reaction tubes were centrifuged to precipitate the protein. The reaction tubes were centrifuged to precipitate the protein and supernatant was injected onto a Lichrospher[®] 100 RP-18 (5 mM) reversed phase column. Samples were eluted using a Beckman System Gold[®] HPLC system equipped with a model 126 programmable solvent module pump and a model 166 programmable UV-visible detector, and the absorbance was measured at 260 nm. The minimum level of detection of *N*-acetyl-SMZ was 5 pmol. Briefly, the substrates and acetylated products were separated with a mobile-phase of 20 mM sodium perchlorate (pH 2.5; solvent A) and acetonitrile (solvent B). A gradient of solvent B from 9 to 29%, over 5 min at a flow rate of 2 ml/min was used to separate SMZ and *N*-acetyl-SMZ. Under these conditions SMZ and *N*-acetyl-SMZ were eluted at 5.2 and 8.4 min, respectively.

Data analysis

Differences in NAT2 catalytic activity among SNPs or haplotypes were analyzed for significance by one-way analysis of variance and Tukey *post hoc* tests using GraphPad Prism[®] Software (Graphpad Software, CA, USA). NAT2 acetylator genotype/phenotype misclassification was estimated by visual inspection of the data.

Results

SMZ *N*-acetyltransferase catalytic activities in cryopreserved human hepatocytes were analyzed for each individual NAT2 SNP. Many of the SNPs (282C>T, 341T>C, 481T>C, 590G>A, 803G>A and rs1495741G>A) differed significantly ($p < 0.0001$) in SMZ *N*-acetyltransferase activities among homozygous common, heterozygote and homozygous variant samples following one way analysis of variance (Figure 1).

SMZ *N*-acetyltransferase catalytic activities were segregated by individual NAT2 SNPs and no individual SNP, including rs1495741 yielded a nonoverlapping trimodal distribution (Figure 2) of rapid, intermediate and slow acetylator phenotypes. However, when SMZ *N*-acetyltransferase activities were segregated by NAT2 genotype (Figure 3), a clear trimodal and nonoverlapping distribution of rapid, intermediate and slow acetylator phenotypes was observed and differences among NAT2 acetylator phenotypes within an NAT2 genotype group were trivial.

In the absence of a gold-standard reference, determinations of specificity and selectivity cannot be calculated with certainty. However, based on visual inspection of the data, NAT2

acetylator genotype/phenotype misclassification can be estimated (Figure 4). Use of the rs1495741 genotype alone resulted in an inferred phenotype accuracy of 199/256 or 77.7% of the hepatocytes sampled in this study. The misclassification included 32 rapid acetylators, 22 intermediate acetylators and only three slow acetylators. Thus, the rs1495741 SNP misclassified rapid and intermediate acetylators to a much greater extent than it did slow acetylators. Use of the historical three-SNP rs1799929 (481C>T), rs1799930 (590G>A) and rs1799931 (857G>A) genotyping protocol resulted in an inferred phenotype accuracy of 236/256 or 92.2% of the hepatocytes sampled this study. Misclassification was almost entirely intermediate acetylators (19/20; 95%). A two-SNP rs1041983 (282C>T) and rs1801280 (341T>C) genotyping protocol resulted in an inferred phenotype accuracy of 246/256 or 96.1% of the hepatocytes sampled in this study. Misclassification with the two-SNP genotype protocol was equally divided between slow (five) and intermediate (five) acetylators. A four-SNP panel of rs1801279 (191G>A), rs1801280 (341T>C), rs1799930 (590G>A) and rs1799931 (857G>A) resulted in an inferred NAT2 acetylator phenotype accuracy of 252/256 or 98.4% of the hepatocytes sampled this study. The misclassification included no rapid acetylators, three intermediate acetylators and one slow acetylator. The seven-SNP assay resulted in an identical inferred NAT2 acetylator phenotype accuracy determined by the four-SNP assay. The extent of acetylator phenotype misclassification with these NAT2 genotyping protocols is illustrated in Figure 5.

Discussion

N-acetylation capacities determined *in vivo* have primarily discriminated between rapid versus slow acetylator phenotypes. Although less commonly reported, trimodal (rapid, intermediate and slow acetylator phenotype) distributions of *N*-acetylation capacity *in vivo* have been reported in humans following administrations of SMZ [21,22], isoniazid [23–26], sulfasalazine [27] and caffeine [28–29].

Hepatic *N*-acetylation capacity *in vitro* often segregates into rapid, intermediate, and slow acetylator phenotypes as reported in humans [17,23], mice [30], Syrian hamsters [31,32] and rats [33,34]. NAT2 is highly expressed in cryopreserved liver hepatocytes [17] and SMZ is a highly selective substrate for NAT2 versus NAT1 [35,36], facilitating comparisons of NAT2 phenotype accuracy inferred from different NAT2 SNP genotype panels.

In our study, we assessed the relative accuracy of various NAT2 SNP genotyping panels to accurately distinguish among rapid, intermediate and slow acetylator phenotypes. Previous studies include calculations of sensitivity and specificity for various assays. We chose not to analyze our data in this manner because:

- There is no ‘gold’ standard for definitive assessment of rapid, intermediate and slow acetylator phenotypes
- The calculations of sensitivity and specificity are dependent upon both the method used to determine phenotype and upon the ethnic diversity of the sample population

Thus, our genotyping ‘accuracy’ comparisons are based upon the phenotype method chosen (SMZ *N*-acetyltransferase catalytic activities in cryopreserved human hepatocytes) and the ethnic composition of our sample set.

SMZ *N*-acetyltransferase catalytic activities in cryopreserved human hepatocytes were segregated by individual NAT2 SNPs and no individual SNP yielded a nonoverlapping trimodal distribution. The effects of each SNP on NAT2 activity was consistent with the effects that have been shown following recombinant expression of human NAT2 alleles in bacteria [37–39], yeast [40,41] and mammalian cells [4,42,43].

Use of the rs1495741 genotype alone resulted in an inferred phenotype accuracy of 77.7% of the hepatocytes sampled in this study. The rs1495741 is located within a large linkage disequilibrium block including each of the seven SNPs determined our study. As reported by Garcia-Closas *et al.*, linkage disequilibrium of the seven *NAT2* SNPs to the rs1495741 SNP was highest for 341T>C (pairwise $r^2 = 0.24$), 481T>C (pairwise $r^2 = 0.23$) and 803A>G (pairwise $r^2 = 0.21$) [16]. These three SNPs define the *NAT2**5B haplotype (Table 1), which is the most common slow acetylator haplotype in European populations. By contrast, linkage disequilibrium of the 857G>A SNP, common in Asian populations was dramatically less (pairwise $r^2 = 0.01$), suggesting that the rs1495741 SNP alone would be much less accurate in the inference of *NAT2* phenotype in populations of Asian ancestry. The three-SNP panel and the rs1495741 SNP should be more accurate to infer *NAT2* phenotype if restricted to populations of European ancestry.

Subsequent to the submission of our manuscript, a recent study proposed that a two-SNP (282C>T, 341T>C) assay was more accurate than the tag-SNP and nearly as accurate as the seven-SNP assay for discriminating rapid versus slow acetylator *NAT2* phenotype as determined *in vivo* with caffeine [44]. We determined the accuracy of this two-SNP assay for discriminating rapid, intermediate or slow acetylator phenotypes as measured by SMZ *NAT2* catalytic activity in cryopreserved human hepatocytes and also found it to be more accurate than both the tag-SNP and the historical three-SNP assay.

However, we found that a four-SNP panel rs1801279 (191G>A), rs1801280 (341T>C), rs1799930 (590G>A) and rs1799931 (857G>A) was still more accurate for inferring SMZ *N*-acetyltransferase activity phenotypes in cryopreserved human hepatocytes. This four-SNP assay measures the common SNPs that directly confer reductions in *NAT2* catalytic activity. Unlike the two-SNP assay, it does not misclassify *NAT2**13A alleles as slow acetylators. *NAT2**13A allele frequency is substantially more common among populations of African than European ancestry [45–47], indicating this misclassification takes on greater significance in non-European populations.

Determination of acetylator genotype has been shown to improve efficacy and reduce toxicity in tuberculosis patients treated with isoniazid [48–50]. Indeed, *NAT2* genotyping may be superior to therapeutic drug monitoring for isoniazid dose individualization in tuberculosis therapy as specific and distinct dosing recommendations have been proposed for rapid, intermediate and slow acetylator genotypes [26]. The *NAT2* tag-SNP rs1495741, is strongly associated with urinary bladder cancer [15], plasma triglyceride levels and coronary artery disease [51] in recent genome-wide association studies. Although DNA sequencing of the entire coding exon would provide the most comprehensive haplotype and genotype information, our results suggest that use of the four-SNP panel will infer *NAT2* acetylator phenotype with high accuracy, and is recommended over the tag-, two-, three- and (for economy of scale) the seven-SNP genotyping panels, particularly in populations that include individuals of non-European ancestry.

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Executive summary

Background

- The metabolism of isoniazid and many aromatic amine drugs such as sulfamethazine (SMZ) are subject to the *NAT2* polymorphism, thus affecting therapeutic efficacy and toxicity for many therapeutic drugs.
- *NAT2* is highly expressed in cryopreserved liver hepatocytes and SMZ is a highly selective substrate for NAT2, facilitating comparisons of NAT2 phenotype accuracy inferred from different *NAT2* SNP genotype panels.

Materials & methods

- SMZ *N*-acetyltransferase catalytic activities were measured in cryopreserved human hepatocytes from a convenience sample of individuals in the USA with ethnic frequency similar to the 2010 US population census.
- SMZ *N*-acetyltransferase catalytic activities were segregated by the tag-SNP rs1495741 and each of the seven most common SNPs in the *NAT2* coding region.

Results

- 282C>T, 341T>C, 481T>C, 590G>A, 803G>A and rs1495741G>A differed significantly ($p < 0.0001$) in SMZ *N*-acetyltransferase activity among homozygous common, heterozygote and homozygous variant genotypes, but no SNP yielded a nonoverlapping trimodal distribution.
- The functional effect of each SNP on NAT2 activity in cryopreserved human hepatocytes was consistent with the effects that have been shown following recombinant expression of human *NAT2* alleles in bacteria, yeast and mammalian cells.
- The accuracy of the various *NAT2* SNP genotype panels to infer *NAT2* phenotype was as follows: tag-SNP: 77.7%; two-SNP: 96.1%; three-SNP: 92.2%; four-SNP: 98.4%; and seven-SNP: 98.4%.

Conclusion

- A *NAT2* four-SNP panel of rs1801279 (191G>A), rs1801280 (341T>C), rs1799930 (590G>A) and rs1799931 (857G>A) infers *NAT2* acetylator phenotype with high accuracy, and is recommended over the tag-SNP, two-SNP, three-SNP (and for economy of scale the seven-SNP) genotyping panels, particularly in populations of non-European ancestry.

Figure 1. Sulfamethazine *N*-acetyltransferase catalytic activities (mean $\pm$ standard error of the mean) in cryopreserved human hepatocytes are plotted on the ordinate versus each individual *NAT2* SNP on the abscissa

The white bar is not shown for 191G>A and 857G>A because no homozygous variant samples were available. Sulfamethazine *N*-acetyltransferase activities for 282C>T, 341T>C, 481T>C, 590G>A, 803G>A and rs1495741G>A all differed significantly ($p < 0.0001$) among homozygous common, heterozygote and homozygous variant following one-way analysis of variance. For each of these SNPs, homozygous common was significantly higher than heterozygote ($p < 0.05$), which was significantly higher than homozygous variant samples ($p < 0.05$) following one-way analysis of variance and Tukey *post hoc* tests. Homozygous common was not significantly different than the heterozygote for 191G>A ($p = 0.6522$) or 857G>A ($p = 0.7185$).

Figure 2. Scatter plots of sulfamethazine *N*-acetyltransferase catalytic activities in cryopreserved human hepatocytes are plotted on the ordinate versus each *NAT2* single nucleotide pair on the abscissa

Each homozygous common sample is illustrated with an open square, each heterozygote is illustrated with an open circle, and each homozygous variant is illustrated with an open triangle. Mean activities are shown by the horizontal lines. Statistical analysis of these data is shown in the legend to Figure 1.

Figure 3. Sulfamethazine *N*-acetyltransferase catalytic activities (mean $\pm$ standard error of the mean) in cryopreserved human hepatocytes are plotted on the ordinate versus *NAT2* genotype(s) on the abscissa

The number of samples in each *NAT2* acetylator genotype group is shown and related *NAT2* genotypes were combined when sample sizes were less than 4.

Figure 4. Scatter plots of SMZ *N*-acetyltransferase catalytic activities plotted on the abscissa versus inferred acetylator phenotype as determined by various *NAT2* genotyping protocols Each *NAT2* rapid acetylator sample is illustrated with an open square, each *NAT2* intermediate acetylator sample is illustrated with an open circle, and each *NAT2* slow acetylator sample is illustrated with an open triangle. Mean activities are shown by the vertical lines. Since the sulfamethazine *N*-acetyltransferase catalytic activity distribution was not explicitly trimodal in our population of samples, the identification of misclassified inferred *NAT2* phenotypes was estimated by visual inspection of the data (illustrated by the inserted boxes).

Figure 5. Misclassification (percentage) of inferred acetylator phenotype as determined with various *NAT2* SNPs genotyping protocols.

Table 1Common *NAT2* single nucleotide pairs and their corresponding alleles/haplotypes.

<i>NAT2</i> allele (haplotype)	Nucleotide change(s) and rs identifiers	Amino acid change(s)
<i>NAT2</i> *4	Reference	Reference
<i>NAT2</i> *5A	341T>C (rs1801280) [†] 481C>T (rs1799929)	I114T [†] L161L (synonymous)
<i>NAT2</i> *5B	341T>C (rs1801280) [†] 481C>T (rs1799929) 803A>G (rs1208)	I114T [†] L161L (synonymous) K268R
<i>NAT2</i> *5C	341T>C (rs1801280) [†] 803A>G (rs1208)	I114T [†] K268R
<i>NAT2</i> *5D	341T>C (rs1801280) [†]	I114T [†]
<i>NAT2</i> *5E	341T>C (rs1801280) [†] 590G>A (rs1799930)	I114T [†] R197Q
<i>NAT2</i> *5G	282C>T (rs1041983) 341T>C (rs1801280) [†] 481C>T (rs1799929) 803A>G (rs1208)	Y94Y (synonymous) I114T [†] L161L (synonymous) K268R
<i>NAT2</i> *5J	282C>T (rs1041983) 341T>C (rs1801280) [†] 590G>A (rs1799930)	Y94Y (synonymous) I114T [†] R197Q
<i>NAT2</i> *5K	282C>T (rs1041983) 341T>C (rs1801280) [†]	Y94Y (synonymous) I114T [†]
<i>NAT2</i> *6A	282C>T (rs1041983) 590G>A (rs1799930) [†]	Y94Y (synonymous) R197Q [†]
<i>NAT2</i> *6B	590G>A (rs1799930) [†]	R197Q [†]
<i>NAT2</i> *6C	282C>T (rs1041983) 590G>A (rs1799930) [†] 803A>G (rs1208)	Y94Y (synonymous) R197Q [†] K268R
<i>NAT2</i> *6E	481C>T (rs1799929) 590G>A (rs1799930) [†]	L161L (synonymous) R197Q [†]
<i>NAT2</i> *6F	590G>A (rs1799930) [†] 803A>G (rs1208)	R197Q [†] K268R

NAT2 allele (haplotype)	Nucleotide change(s) and rs identifiers	Amino acid change(s)
<i>NAT2*6J</i>	282C>T (rs1041983)	Y94Y (synonymous)
	590G>A (rs1799930) [†]	R197Q [†]
	857G>A (rs1799931)	G286E
<i>NAT2*6N</i>	282C>T (rs1041983)	Y94Y (synonymous)
	481C>T (rs1799929)	L161L (synonymous)
	590G>A (rs1799930) [†]	R197Q [†]
<i>NAT2*7A</i>	857G>A (rs1799931) [†]	G286E [†]
<i>NAT2*7B</i>	282C>T (rs1041983)	Y94Y (synonymous)
	857G>A (rs1799931) [†]	G286E [†]
<i>NAT2*7C</i>	282C>T (rs1041983)	Y94Y (synonymous)
	803A>G (rs1208)	K268R
	857G>A (rs1799931) [†]	G286E [†]
<i>NAT2*7D</i>	191G>A (rs1801279)	R64Q
	282C>T (rs1041983)	Y94Y (synonymous)
	857G>A (rs1799931) [†]	G286E [†]
<i>NAT2*7E</i>	282C>T (rs1041983)	Y94Y (synonymous)
	481C>T (rs1799929)	L161L (synonymous)
	857G>A (rs1799931) [†]	G286E [†]
<i>NAT2*11A</i>	481C>T (rs1799929) [†]	L161L (synonymous) [†]
<i>NAT2*12A</i>	803A>G (rs1208) [†]	K268R [†]
<i>NAT2*12B</i>	282C>T (rs1041983)	Y94Y (synonymous)
	803A>G (rs1208) [†]	K268R [†]
<i>NAT2*12C</i>	481C>T (rs1799929)	L161L (synonymous)
	803A>G (rs1208) [†]	K268R [†]
<i>NAT2*13A</i>	282C>T (rs1041983) [†]	Y94Y (synonymous) [†]
<i>NAT2*14A</i>	191G>A (rs1801279) [†]	R64Q [†]
<i>NAT2*14B</i>	191G>A (rs1801279) [†]	R64Q [†]
	282C>T (rs1041983)	Y94Y (synonymous)
<i>NAT2*14C</i>	191G>A (rs1801279) [†]	R64Q [†]
	341T>C (rs1801280)	I114T
	481C>T (rs1799929)	L161L (synonymous)
	803A>G (rs1208)	K268R

<i>NAT2</i> allele (haplotype)	Nucleotide change(s) and rs identifiers	Amino acid change(s)
<i>NAT2*14D</i>	191G>A (rs1801279) [†]	R64Q [†]
	282C>T (rs1041983)	Y94Y (synonymous)
	590G>A (rs1799930)	R197Q
<i>NAT2*14E</i>	191G>A (rs1801279) [†]	R64Q [†]
	803A>G (rs1208)	K268R
<i>NAT2*14F</i>	191G>A (rs1801279) [†]	R64Q [†]
	341T>C (rs1801280)	I114T
	803A>G (rs1208)	K268R
<i>NAT2*14G</i>	191G>A (rs1801279) [†]	R64Q [†]
	282C>T (rs1041983)	Y94Y (synonymous)
	803A>G (rs1208)	K268R
<i>NAT2*14I</i>	191G>A (rs1801279) [†]	R64Q [†]
	481C>T (rs1799929)	L161L (synonymous)
	803A>G (rs1208)	K268R

[†]Signature SNP for each haplotype cluster.

Data from [101].