

Distribution and Concordance of *N*-Acetyltransferase Genotype and Phenotype in an American Population¹

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Abstract

Polymorphic arylamine *N*-acetyltransferase 2 (NAT2) status varies widely between individuals and ethnic groups and has been associated with susceptibility to several cancers. Few studies have reported the distribution of NAT2 status for Caucasian-American populations or evaluated the concordance between methods of assessment for cancer cases and controls. In our study, distribution of NAT2 status was classified by genotype and phenotype measurements in PANCAN, a population-based case-control study of pancreatic cancer, and concordance between measurements was evaluated for 33 cases and 222 controls. Major genotypes and alleles among controls were *5B/*6A, *5B/*5B, *4/*6A, and *5B/*4. One putative new allele was found in a single individual. Genotypes and phenotypes were classified as rapid or slow, according to a bimodal model. Presence of the *4 (wild-type) allele defined a NAT2 genotype as rapid. The NAT2 phenotype was analyzed by the caffeine assay. Ratios of 5-acetylaminofluoranthene-3-methyluracil to 1-methylxanthine were determined, and individuals with values of ≥ 0.66 were identified as having a rapid phenotype. In our population, 58.1 and 59.5% of control subjects were classified as slow acetylators by phenotype and genotype, respectively. Concordance of NAT2 genotype and phenotype classification was 97.8% in the bimodal model. A similar analysis was completed for a trimodal model. Concordance of genotype and phenotype was high in cases (90.9%) and similar to controls; genotyping alone provided an efficient, accurate method of analysis for acetylator status. A comparison with two previous reports revealed subtle differences in

genotype and allele distribution but exhibited overall similarity with other Caucasian-American populations.

Introduction

Several well-known drug-metabolizing enzymes catalyze the activation and detoxification of xenobiotics (1) and are classified as phase I or II enzymes, respectively. Members of the phase I category include cytochrome P450-related enzymes and epoxide hydrolases. Phase II enzymes are *N*-acetyltransferases, glutathione *S*-transferases, UDP-glucuronosyltransferases, and sulfotransferases. The absolute and relative amounts of phase I and II enzyme activities differ between individuals and affect biological responses to xenobiotic exposure (2). Several adverse drug reactions and cancer susceptibilities have been attributed to interindividual differences in phase I and II enzyme activities (3, 4).

The *N*-acetyltransferases catalyze acetylation of aromatic amines and hydrazines, which include carcinogenic compounds and therapeutic drugs. Many of the drugs are commonly used and include isoniazid, dapsone, procainamide, and sulfamethazine (5). Carcinogens metabolized by *N*-acetyltransferase include 2-naphthylamine, 2-aminofluorene, 4-aminobiphenyl, and benzidine (6–8). Thus, *N*-acetylation can modulate drug activities and detoxify carcinogens (8).

A wide range of *N*-acetylation activity is observed in humans and has been characterized by some authors as a bimodal and by others as a trimodal distribution (9–11). These distributions of activity have resulted in the classification of individuals as rapid and slow (bimodal model) or rapid, intermediate, and slow acetylators (trimodal model). The proportion of slow and rapid acetylators differs between ethnic populations (12, 13). The slow acetylator phenotype is most prevalent in Northern Africans (90%) and Scandinavians (75–80%) and lowest in Canadian Eskimos and Japanese (5%); in Chinese populations, the proportion is 20%, and 40–60% of African-Americans and other Caucasians are slow acetylators (12–17).

It is believed that *N*-acetyltransferase activity is primarily under genetic control, although variation in activity that is attributable to environmental factors has not been fully examined (18, 19). NAT1³ and NAT2 are encoded by two 870-bp genes, *NAT1* and *NAT2*, respectively (20). The genes are located on chromosome 8, and both are expressed in an autosomal codominant manner (20–22). NAT1 and NAT2 are both considered polymorphic. The *NAT2* locus, the focus of this report, has alleles that contain one to four single-nt substitutions. In human populations, 26 alleles have been reported for

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³ The abbreviations used are: NAT1 and NAT2, *N*-acetyltransferases 1 and 2, respectively; nt, nucleotide; HPLC, high-pressure liquid chromatography; AFMU, 5-acetylaminofluoranthene-3-methyluracil; IX, 1-methylxanthine; CI, confidence interval.

NAT2.⁴ Polymorphisms of *NAT2* are known to result in variation of acetylation activity and have been associated with the incidence of several diseases (5). Generally, single-nt substitutions in *NAT2* result in low activity, decreased expression, and enzyme instability (23). A slow acetylator phenotype is generally observed for individuals with two germ-line copies of alleles that contain any of several single-nt substitutions. Rapid acetylators generally contain two wild-type or one wild-type and one variant allele, an allele with a nt substitution, of *NAT2*. A high concordance between *NAT2* genotype and phenotype has been reported in a number of populations (24–29).

Both rapid and slow *N*-acetyltransferase phenotypes and genotypes have been associated with the risk of several cancers. Slow *N*-acetyltransferase phenotype has been associated with a higher risk of bladder cancer in arylamine-exposed workers (30). The rapid *N*-acetyltransferase phenotype and genotype have been associated with the risk of colon in some (31, 32) but not other studies (15). A positive association with rapid *NAT2* genotype has also been reported for lung cancer (33). An examination of the association between *N*-acetyltransferase genotype and breast cancer has produced conflicting results (34–36). In general, the associations of disease end points with *NAT2* phenotype are stronger for smokers than nonsmokers (34, 37, 38). These observations, together with ethnic variation in the ratio of slow and rapid acetylators, suggest that the *NAT2* genotype or a combination of environmental exposure and *N*-acetyltransferase genotype may partially explain cancer risk patterns in different populations.

Characterization of *NAT2* genotypes and phenotypes in healthy and diseased individuals allows for evaluation of both *NAT2* and cancer associations and evaluation of DNA-based methods for classifying *N*-acetylation phenotype. To date, no large studies of *NAT2* genotype distribution and concordance have been reported for the Caucasian-American population. Here, we measured *NAT2* genotype and phenotype in a mostly Caucasian Minnesota (United States) population. All major *NAT2* genotypes were characterized, and metabolic phenotyping was measured by the caffeine assay. This study was undertaken as part of a population-based case-control study of pancreatic cancer, PANCAN. Ultimately, the case-control study enrolled ~280 individuals with pancreatic cancer and 675 controls. Results from a subset of these subjects are reported here. The objectives of this study were: (a) to determine the distribution of *NAT2* genotypes in a Caucasian-American population using the control subjects and (b) to determine the concordance of *NAT2* genotype and acetylator phenotype in both cases and controls. In the course of the study, we found evidence of a new allele. Here, we describe the putative allele and propose a name for it. Also, we discuss the feasibility of applying *NAT2* genotyping and phenotyping methods in a study of pancreatic cancer.

Subjects and Methods

Subjects. Two hundred fifty-five individuals were recruited in the initial stages of a large case-control study of pancreatic cancer. These subjects included 33 cases diagnosed with pancreatic cancer (9 women and 24 men) and 222 population-based controls (103 women and 119 men). The cases were recruited from all hospitals in the seven-county metropolitan area of the Twin Cities, Minneapolis and St. Paul, Minnesota. The controls

were drawn from driver's license lists. The study was approved by the Institutional Review Board of the University of Minnesota, and informed consent was obtained from all subjects. Extensive medical, dietary, and demographic data were collected from each individual as part of the parent case-control study. The mean ages of the cases and controls were 66.0 and 67.7 years, respectively. There was one African-American among the cases. One control self-reported race as "other." The remaining 32 cases and 221 controls were self-reported as "white." Subjects who indicated they had a high sensitivity to caffeine or who had been told to avoid caffeine for medical reasons were excluded from the caffeine study. Individuals who refused blood collection or the caffeine test (for any reason) or had missing data (genotype or phenotype) were excluded from this study.

***NAT2* Activity (Phenotype).** *In vivo* *NAT2* activity was measured with a caffeine-based assay, as described by Butler *et al.* (22), with minor modifications. Subjects ingested two tablets, each containing 100 mg of caffeine, following an overnight fast. Subjects refrained from the consumption of caffeine- and methylxanthine-containing foods and beverages from midnight until 5 h after the dose of caffeine. Four and 5 h following caffeine ingestion, subjects voided their bladders, and a urine specimen was collected at the 5-h time point. Immediately following collection, the urine samples were frozen in a home freezer and collected by study personnel within 1 week. Samples were thawed, the pH was adjusted to 3.5, aliquots were taken, and the aliquots were stored at -70°C until analysis.

Prior to HPLC analysis, frozen samples were thawed, 200 μl of urine were saturated with 125 mg of ammonium sulfate, and 6.0 ml of chloroform:isopropanol (95:5) were added. Each sample was vortexed and centrifuged, and the organic phase was removed and evaporated to dryness. The residue was resuspended in 250 μl of 0.05% acetic acid, filtered, and frozen until analysis. In an HPLC-based analysis, 50 μl of the extract were injected onto a Beckman C18 Ultrasphere octadecylsilane column (25 cm in length, 4.6-mm diameter, 5- μm particle size) and eluted with a 0.05% acetic acid-methanol solvent (flow rate, 1.2 ml/min). The analysis detects metabolites of caffeine, including AFMU and 1X.

Acetylation phenotype was assigned on the basis of a molar AFMU/1X ratio. A ratio of <0.66 identified subjects as slow acetylators, and a ratio of ≥ 0.66 identified subjects as rapid acetylators (bimodal model). Alternatively, subjects with ratios from 0.66 up to and including 3.0 were classified as intermediate acetylators, those with ratios of >3.0 were classified as rapid acetylators, and those with ratios of <0.66 were classified as slow acetylators (trimodal model).

The cutoff of 0.66 was based on a kernel density function analysis, which objectively defined the nadir of the data distribution for a bimodal model (39). The 3.0 cutoff recognizes a natural nadir in the distribution of activities among individuals carrying at least one *NAT2**4 allele. Data are presented as both bimodal and trimodal distributions.

Isolation of Genomic DNA. Thirty ml of venous blood were drawn from each patient into 10-ml EDTA tubes. Following centrifugation and removal of plasma, the buffy coat was isolated, stored at -70°C , and used for DNA isolation. DNA was isolated by a phenol-chloroform extraction method and precipitated with ethanol using an automated Applied Biosystems DNA extractor. Isolation procedures followed the manufacturer's specifications, including an RNase step. DNA was dissolved in Tris-EDTA buffer (pH 7.4) and quantified spectrophotometrically by measurement of absorbance at 260 nm.

⁴ Arylamine *N*-Acetyltransferase (EC 2.3.1.5) Nomenclature <www.louisville.edu/medschool/pharmacology/NAT.html>.

Table 1 Alleles of NAT2^{a,b}

	191	282	341	481	590	803	857
NAT2*4	G	C	T	C	G	A	G
NAT2*5A			C	T			
NAT2*5B			C	T		G	
NAT2*6A		T			A		
NAT2*6B					A		
NAT2*7A							A
NAT2*7B		T					A
NAT2*12A						G	
NAT2*13		T					
NAT2*13(B) ^{c,d}		T (C)		T			

^a nt at the sites of single-nt substitutions are given for the wild-type allele (NAT2*4) and each altered allele. nt positions are based on the NAT2 sequence published by Blum *et al.* (20).

^b Five of the seven nt substitutions result in an amino acid substitution: G¹⁹¹→A, Arg⁶⁴→Glu (*MspI*); C²⁸²→T, no change (*FokI*); T³⁴¹→C, Ile¹¹⁴→Thr (none); C⁴⁸¹→T, no change (*KpnI*); G⁵⁹⁰→A, Arg¹⁹⁷→Gln (*TaqI*); A⁸⁰³→G, Lys²⁶⁸→Arg (*DdeI*); G⁸⁵⁷→A, Gly²⁸⁶→Glu (*BamHI*). Restriction enzymes used for the detection of substitutions are in parentheses.

^c Proposed nomenclature for unique nt substitution linkage combination.

^d Due to the linkage between substitutions of nt 481 and 341, an alteration at nt 341 is anticipated for the allele.

Quality of the DNA sample was assessed by measurement of the $A_{260\text{ nm}}:A_{280\text{ nm}}$ absorbance ratio. Typical yields of DNA were $>10\ \mu\text{g/ml}$ and $A_{260\text{ nm}}:A_{280\text{ nm}}$ ratios were 1.7–1.8, indicating good yield and high-quality DNA. Samples were stored in Tris-EDTA buffer at -70°C until further analysis.

Determination of NAT2 Genotype. Known alleles of the NAT2 gene contained one to four point substitutions at nt 191, 282, 341, 481, 590, 803, and 857. Many of the substitutions were linked within single alleles. The nt position of each substitution was based on the sequence reported by Blum *et al.* (20). The standardized designation for each allele is based on the report of Vatsis *et al.* (40), and designations are given in Table 1. The most prevalent alleles of *N*-acetyltransferase are (positions of their nt substitutions are given in parentheses): NAT2*5A (341/481), NAT2*5B (341/481/803), NAT2*6A (282/590), and NAT*7B (857). Former designations for *5A, *6A, and *7B alleles were M1, M2 and M3, respectively, whereas the *4 allele was formerly referred to as wild type (41).

Polymorphisms of the NAT2 gene were determined using one of three methods. The first, based on the method of Blum *et al.* (20), involved two PCRs and use of two primer pairs for each DNA sample (reactions 1 and 2). Reaction 1 amplified a 1165-bp fragment using primer 1 (P1, CACGACGTTGTA-AAACGACAGGGGATCATGGACATTGAA), which has a sequence that is complementary to a M13 vector (first 20 bases)

and the NAT2 gene at nt -7 to 12 (last 19 bases), and primer 2 (P2, TCTAGCATGAATCACTCTGC), which is complementary to nt 1119–1138. This product was used for restriction enzyme digests that detected polymorphisms at nt 191, 481, and 857. Reaction 2 amplified a 652-bp fragment using primer 3 (P3, CACGACGTTGTA AAAACGAC ATCAACTTCTGTACT-GGGCT), which is complementary to a M13 vector (first 20 bases) and the NAT2 gene at nt 216–234, and primer 4 (P4, GGGTTGGGCACGAGATTCT), which is complementary to nt 829–848. This product was used for restriction enzyme digests, which detected a polymorphism at nt 590. Each 50- μl PCR contained 100–500 ng of genomic DNA, 0.2 mM each dNTP, 0.2 μM each primer, $1\times$ buffer [50 mM KCl and 10 mM Tris-HCl (pH 8.3)], 2.5 units of Taq DNA polymerase, and 2.0 mM (reaction 1) or 1.5 mM (reaction 2) MgCl_2 . The mixture was initially incubated for 4 min at 95°C . Thirty cycles of amplification (denaturation for 60 s at 95°C , annealing for 70 s at 58°C , and extension for 90 s at 72°C), followed by a final extension period of 4 min at 72°C , were performed using a programmable Perkin-Elmer Corp. thermocycler.

Products from reaction 1 were digested separately with *MspI/HpaII*, *KpnI*, and *BamHI* to detect the loss of restriction sites at nt 191, 481, and 857, respectively. The product of reaction 2 was digested with *TaqI* to detect the loss of a restriction site at nt 590. All reactions were carried out for 2 h

at 37°C, with the exception of *TaqI* reactions, which were digested at 65°C. Five μ l of PCR product were added to 1 \times enzyme buffer [10 mM Tris-HCl (pH 7.5), 5 mM MgCl₂, 100 mM NaCl, and 1 mM 2-mercaptoethanol], 15 units of enzyme, and 0.1 mg/ml BSA and brought to a final volume of 20 μ l. All digests were analyzed on ethidium bromide-stained 1% agarose gels, except the *TaqI* digests, which were examined on 2% agarose gels.

The second method (triple-digest method) for obtaining *NAT2* genotypes was a modified procedure of Abe *et al.* (42). Briefly, 200–500 ng of genomic DNA were amplified by a PCR containing 5 pmol of each primer [TGACGGCCAG-GAATTACATTGTC (P5, nt 342–362) and ACACAAG-GGTTTATTTTGTTC (P6, nt 879–900)], 0.2 mM each dNTP, 2.5 mM MgCl₂, 1 \times buffer [50 mM KCl and 10 mM Tris-HCl (pH 8.3)], and 1 unit of *Taq* polymerase in a total volume of 50 μ l. The mixture was initially incubated for 5 min at 94°C. Thirty-five cycles of amplification (denaturation for 50 s at 94°C, annealing for 50 s at 55°C, and extension for 50 s at 65°C), followed by a final extension period of 7 min at 72°C, were performed using a programmable Perkin-Elmer thermocycler (Norwalk, CT).

Following amplification, 10 μ l of the PCR product were added to a mixture of 5 units each of *Bam*HI, *Kpn*I, and *Taq*I, 1 \times buffer [10 mM Tris-HCl (pH 7.5), 5 mM MgCl₂, 100 mM NaCl, and 1 mM 2-mercaptoethanol], and 0.01 mg/ml BSA in a total reaction volume of 20 μ l. The mixture was incubated at 37°C for 3 h and then incubated at 65°C for 1 h. Fifteen μ l of digested product were electrophoresed at 100 V for 30 min and then at 75 V for 30 min on a 3% agarose gel stained with ethidium bromide.

Although the triple-digest method was an efficient means of analysis, an unanticipated band of ~400 bp was found in DNA samples with a polymorphism at nt 590. The band was present in carriers of *NAT2**6A alleles only and did not result from nonspecific amplification of DNA samples. Because alternative methods of analysis, including the use of single enzyme digests, did not produce the fragment, it appears that the band is an incomplete digest of the PCR product from individuals with *6A alleles; an incomplete digestion of the 481 site would yield a 396-bp band. Assignments of genotypes for all analyses producing the ~400 bp band were verified by single-enzyme digests of the PCR product from the triple-digest method.

In a third method, a *Fok*I restriction digest was used to detect substitutions at nt 282, and a *Dde*I digest was used to detect substitutions at nt 803. For this analysis, an additional PCR was performed using 0.2 μ M of the first primer (P1) from the first method and P6 from the second method. The PCR was performed with 0.8 mM dNTPs, 2 mM MgCl₂, 1 \times buffer [50 mM KCl and 10 mM Tris-HCl (pH 8.3)], and 1 unit of *Taq* polymerase. Thirty cycles of amplification were performed (denaturation for 60 s at 95°C, annealing for 70 s at 57°C, and extension for 180 s at 72°C), followed by a final extension period of 4 min at 72°C. This PCR amplified a 907-bp fragment. *Fok*I digests were performed for 1 h at 37°C using 20 μ l of amplified product, 1 unit of *Fok*I, 1 \times buffer, and 0.1 mg/ml BSA. *Dde*I reactions were performed for 2 h at 37°C, using 10 μ l of amplified product, 5 units of *Dde*I, 1 \times buffer, and 0.1 mg/ml BSA. All digests were analyzed on a 3% agarose gel.

The first method of analysis was used for ~150 subjects; all other DNA samples (105 total) were analyzed by the second method. The third method was applied to all DNA samples. Analysis for the polymorphism at nt 191 was completed for

Table 2 Frequency of *NAT2* alleles in the control population

Allele	Location of nt substitution	Allele frequency
<i>NAT2</i> *4	Wild-type	103 (23.2%)
<i>NAT2</i> *5A	341/481	30 (6.8%)
<i>NAT2</i> *5B	341/481/803	165 (37.2%)
<i>NAT2</i> *6A	282/590	111 (25%)
<i>NAT2</i> *6B	590	8 (1.8%)
<i>NAT2</i> *7A	857	4 (0.9%)
<i>NAT2</i> *7B	282/857	11 (2.5%)
<i>NAT2</i> *12A	803	10 (2.3%)
<i>NAT2</i> *13	282	1 (0.2%)
<i>NAT2</i> *13(B)	341/481/282	1 (0.2%)

~140 subjects, and no polymorphism was found. This polymorphism generally has been found only in African-Americans. This study had only one African-American, who was negative for the polymorphism, and thus, we did not screen the remainder of our population.

Variant or substituted alleles were defined as alleles containing a substitution at any of following nt: 191, 282, 481, 590, 803, and 857. Individuals with two substituted alleles were classified as slow acetylators. Heterozygotes with a wild-type allele and one copy of a variant allele or individuals with no substitutions (two wild-type alleles) were classified as rapid acetylators. Alternatively, the rapid genotype was subdivided into an intermediate group, including all heterozygotes (a wild-type and substituted allele), and a rapid group, including only homozygous wild-type genotypes.

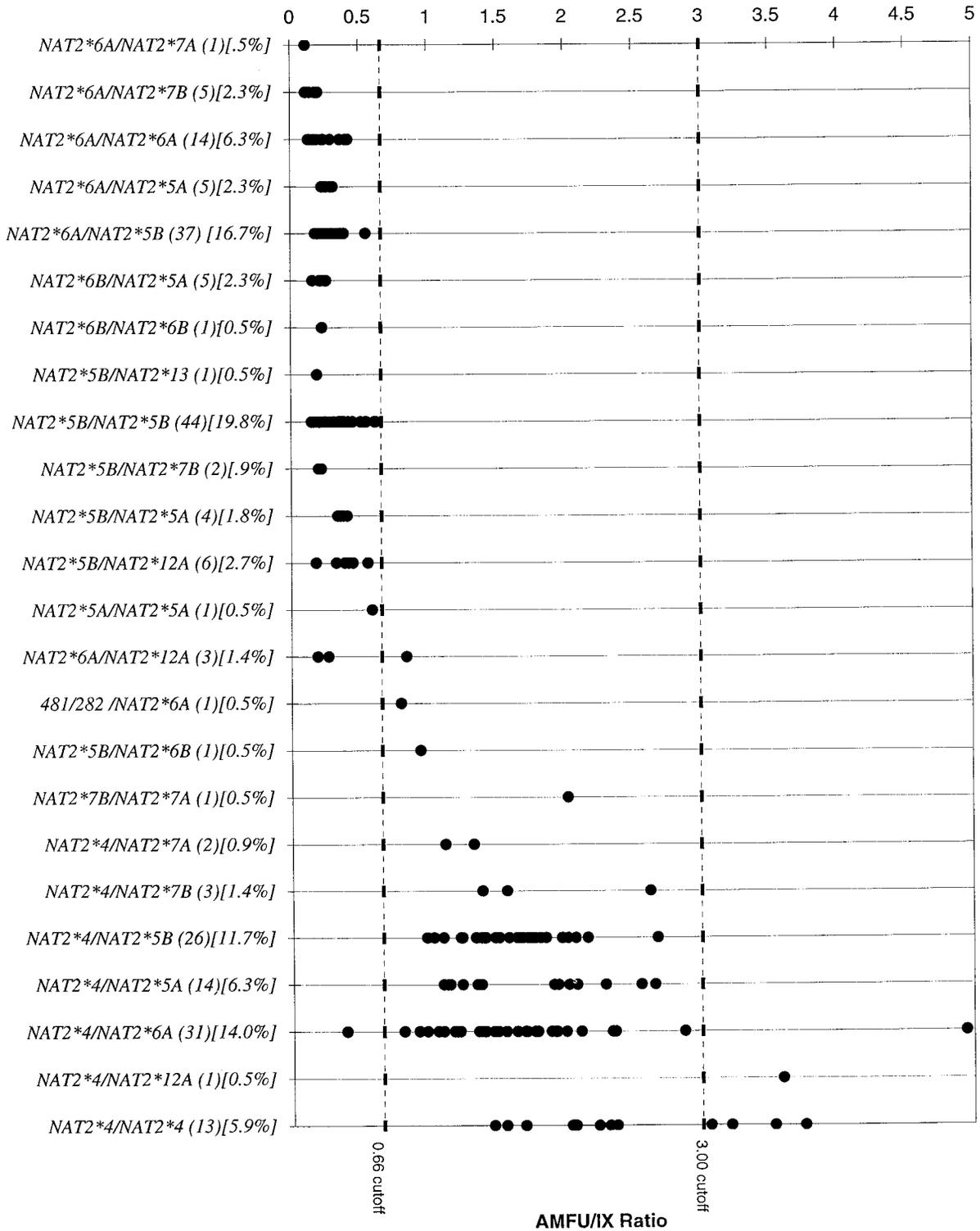
Statistical Methods. Allele and genotype frequencies are reported as percentages. The Hardy-Weinberg equation was used to calculate the expected heterozygote genotype frequency, based on frequencies of wild-type or mutant alleles. For this assessment, all mutant alleles were grouped as a single category. Cutoff points for the phenotype distributions were determined through the use of probit plots (43) and a kernel density function (39). Concordance values (percentages) and κ statistics (44) are reported as measures of agreement between genotypes and phenotypes. ANOVA was used to compare the bimodal and trimodal models for genotype and phenotype. The difference between concordance for cases and controls was compared using the χ^2 test. Mean values of the caffeine metabolite ratio (AFMU/1X) for the heterozygous and homozygous wild-type genotypes were compared using the Student's *t* test.

Results

All alleles and genotypes were identified by a combination of the first or second and third methods of analysis. For example, for the latter combination, unique bands were found at 226, 278, and 142 bp for alleles with substitutions at nt 481, 590, and 857, respectively, by the second analysis method. In the third method, mutations at nt 282 and 803 created unique bands with 717 and 101 bp, respectively.

Results are presented for the control subjects only, except where indicated. Ten *NAT2* alleles were found in our population. A list and description of the alleles are shown in Table 1; the distribution of alleles is shown in Table 2. *NAT2**5B was the major allele, accounting for 37.2% of the total. *NAT2**6A and *NAT2**4 (wild type) were the next major contributors, accounting for 25.0 and 23.2%, respectively, of the total. Each of the remaining alleles accounted for 0.2–6.8% of the total.

Table 3 Distribution of NAT2 phenotypes for each NAT2 genotype in the study population^a



^a Genotype numbers are in parentheses; genotype frequencies are in brackets. Nomenclature is from Vatsis *et al.* (40).

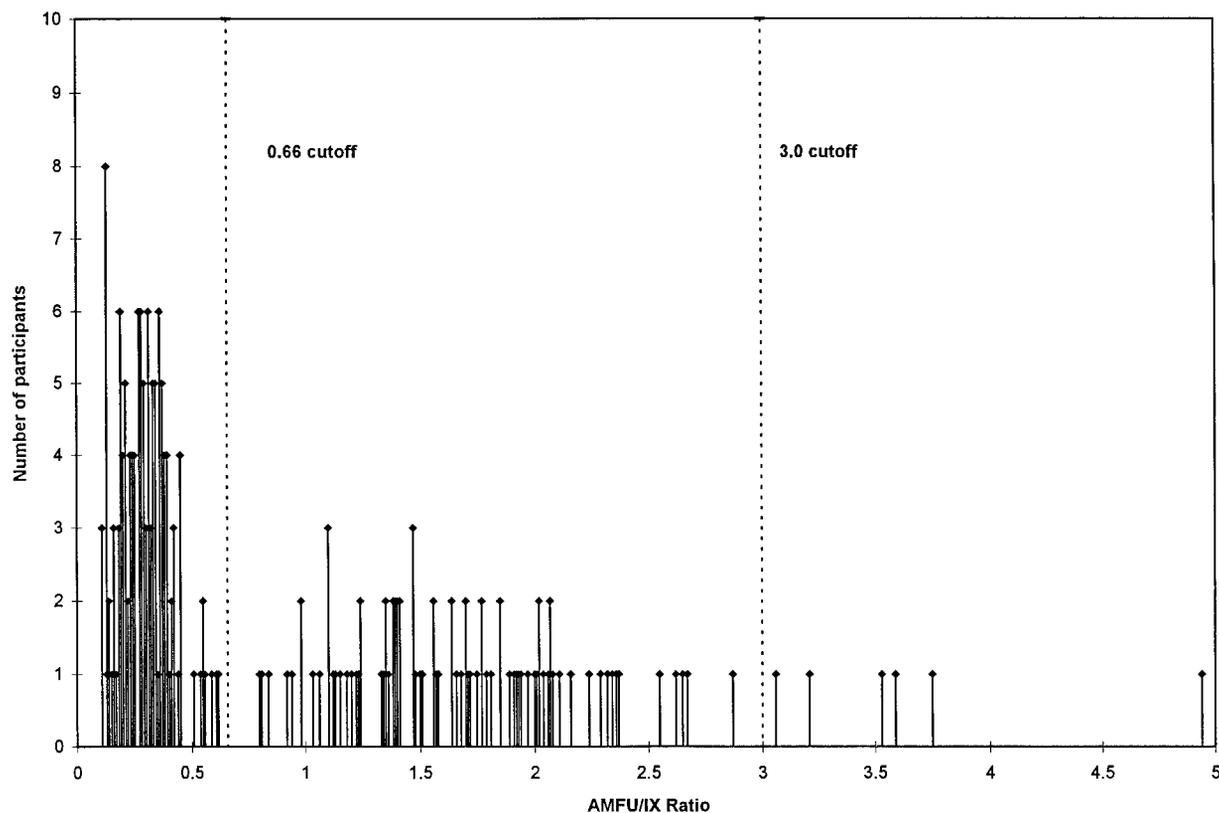


Fig. 1. Distribution of *N*-acetyltransferase activity in the study population. Results from the caffeine assay (AFMU/IX) are shown on the X axis, and the number of participants for each ratio is given on the Y axis.

Nine of the alleles have been described previously. We found evidence for a new allele that contained substitutions at nt 282 and 481. Analysis of fragment patterns following a series of single and multiple restriction enzyme digests were consistent with this linkage. Tentatively, we have proposed the designation *NAT2*13B* on the basis of *NAT2* nomenclature, as described by Vatsis *et al.* (40).

The control subjects contained 24 unique genotypes (Table 3). An additional genotype, *NAT2*6B/NAT2*7A*, was found only among the cases. The major genotypes were *NAT2*5B/NAT2*6A* and *NAT2*5B/NAT2*5B*, which accounted for 16.7 and 19.8% of the total genotypes, respectively. The next most frequent genotypes were *NAT2*4/NAT2*6A* and *NAT2*4/NAT2*5B*, which accounted for 14.0 and 11.1% of the total, respectively. Each remaining genotype accounted for 0.5–6.3% of the total. Thirteen individuals were homozygous for the wild-type (*NAT2*4*) allele. Seventy-seven individuals were heterozygous containing the wild-type and a substituted allele. The remaining 132 subjects had two variant alleles. The distribution of genotypes corresponded with predictions of the Hardy-Weinberg equilibrium ($p^2 + 2pq + q^2$). With p as the frequency of the wild-type allele and q as the frequency of all substituted alleles combined, the predicted number of heterozygotes was 79, which corresponds to the actual number of 77.

On the basis of a dichotomous genotype, 132 (59.5%) individuals were slow and 90 (40.5%) were rapid acetylators. With slow, intermediate, and rapid classification, these categories contained 132 (59.5%), 77 (34.6%), and 13 (5.9%) of the individuals, respectively.

The major distribution of *N*-acetylator phenotypes for control subjects was bimodal, with a nadir at 0.66 for the AFMU/IX ratio (Fig. 1). The 0.66 cutoff, determined by kernel density function analysis (Fig. 2), identified 129 (58.1%) and 93 (41.9%) of individuals as slow and rapid *N*-acetylators, respectively. Alternatively, the phenotypes included a major and minor distribution with cutoffs at 0.66 and 3.0, creating a trimodal distribution. This division split the rapid acetylators into intermediate and rapid groups. In the trimodal distribution, there were 129 (58.1%), 87 (39.2%), and 6 (2.7%) individuals with slow, intermediate, and rapid phenotypes, respectively.

The distribution of phenotype within each genotype is shown in Table 3. Individuals with two substituted alleles generally had a slow phenotype, whereas heterozygous individuals containing a wild-type and a variant allele generally had a rapid phenotype (AFMU/IX ratio > 0.66). Although individuals with two wild-type alleles also had a rapid phenotype, this population had a significantly higher mean AFMU/IX ratio than the heterozygous population; the respective means and SEs were 2.41 ± 0.21 versus 1.68 ± 0.07 ($P < 0.001$).

Concordance and κ statistics were calculated as measures of agreement for both models of *N*-acetyltransferase phenotype and genotype. Concordance values were high (97.8 and 92.8%) for the bimodal and trimodal distribution of *N*-acetyltransferase activity, respectively. The κ statistics indicated good agreement between genotype and phenotype with $\kappa = 0.95$ (95% CI, 0.913–0.994) and 0.861 (95% CI, 0.798–0.923) for the bi- and trimodal models, respectively. With a bimodal model, concordance in the cases was 90.9% ($\kappa = 0.81$; 95% CI, 0.616–1.02).

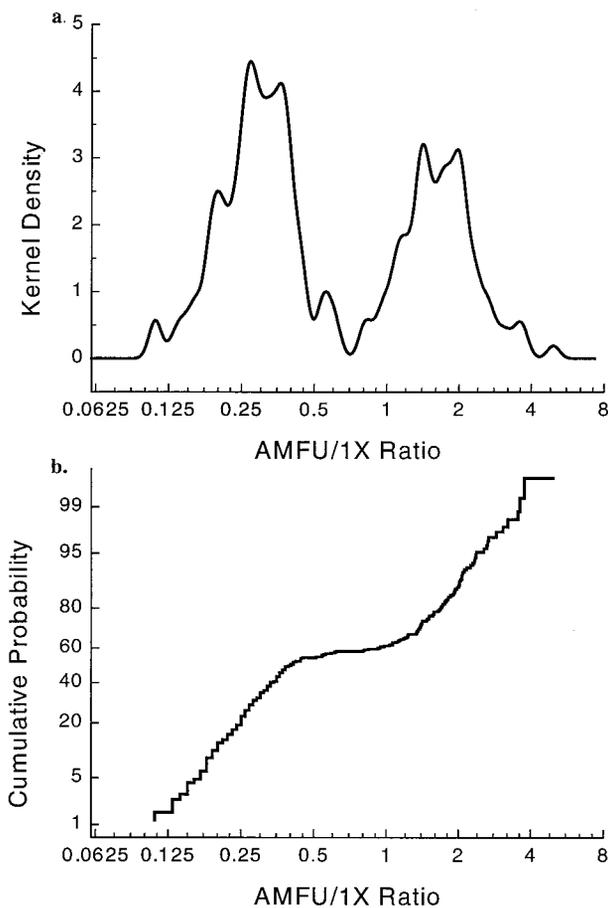


Fig. 2. A kernel density function analysis (a) and probit plot (b) of NAT2 phenotypes (AFMU/1X). a, the kernel density function analysis is a plot of the phenotype distribution for all controls (222 subjects) and indicates the relative frequency of subjects at each AFMU/1X value. b, in the probit plot, the phenotype (AFMU/1X) is given on the X axis, which has a $2\times$ scale. Cumulative probability distribution is expressed in probit values (SD units) and is given on the Y axis.

The difference in concordance values between cases and controls was not statistically significant; Fisher's exact test yielded a P of 0.07. Although this P is suggestive of a difference, both values are high (90.9 versus 97.8; $P = 0.07$), which likely reflects the precision of the data rather than a large difference in concordance.

Self-reported medication use was evaluated as a possible explanation for discordance. It did not appear to be related to genotype-phenotype discordance. These observations indicated a strong genetic influence and little, if any, effect by medication use or disease status on NAT2 phenotype.

In our study population, 128 of 132 individuals with genotypes consisting of any combination of two variant alleles had a slow acetylator phenotype. Of the four individuals who were homozygous for substituted alleles and who were not phenotypically slow, three had AFMU/1X ratios that were only slightly higher than the 0.66 cutoff. These observations indicated that all of the variant alleles in our population affected NAT2 activity, including those with single changes at nt 590, 282, and 803.

We evaluated the use of NAT2 genotypes as the primary measure for the characterization of acetylator status. Using N-acetyltransferase activity (phenotype) as the gold stand-

ard, the specificity and sensitivity of genotype analysis for subject classification were 99.2 and 95.6%, respectively, for the rapid acetylator in the bimodal model. In the trimodal model of acetylator activity, we evaluated characterization of the rapid versus intermediate phenotypes. The specificity and sensitivity of genotype analysis for the rapid acetylator were 95.4 and 66.6%, respectively. In addition, an ANOVA indicated that genotype, characterized as homozygous wild-type, heterozygous, and homozygous substituted alleles (rapid, intermediate, and slow), accounted for 82.5% of the variance in N-acetyltransferase activity ($R^2 = 0.825$). When individuals heterozygous and homozygous for the *4 allele are treated as one category, ANOVA showed that the dichotomy (rapid and slow) explained 81.6% of the variance in N-acetyltransferase activity.

Discussion

A limited number of studies have characterized NAT2 distributions in Caucasian populations. We describe NAT2 distributions in a Caucasian-American population and compare our findings with those of similar populations. Data are presented for both bimodal and trimodal distributions of phenotypes and genotypes. The trimodal model is included because individuals classified as intermediate and rapid acetylators, on the basis of genotype, have an overlap of phenotype ranges but a statistically significant difference in the mean activities. The overlap is possibly due to the effects of unidentified genetic or environmental factors. For example, we have recently shown that hepatic NAT activity can be effectively down-regulated in rats treated with dietary kahweol and cafestol.⁵ Thus, the classification of individuals as intermediate and rapid may be important in the analysis of epidemiological data.

The nt substitutions of NAT2 occur at several positions, including 191, 282, 341, 481, 590, 803, and 857. All individuals were tested for all of these substitutions, except those at 191 and 341. Approximately 140 subjects were evaluated for the alteration at nt 191 in our study; it was not present in any of the subjects, confirming that this nt substitution is uncommon in Caucasians. We did not analyze subjects for a substitution at nt 341 because it is virtually always linked to alterations at nt 481 or 803 and, thus, it would be unlikely to affect our allele classification (28, 45).

Distribution of NAT2 alleles in our data are similar to that reported for Caucasian-American (46) and German-Caucasian (28) populations. The most prevalent alleles in our subjects were NAT2*5B, NAT2*6A, NAT2*4, NAT2*5A, and NAT2*7B, which accounted for 37.2, 25.0, 23.2, 6.8, and 2.5%, respectively (total = 94.7%), of all of the alleles. Similar frequencies (within $\leq 3\%$) were found in two other populations (46, 28). The major genotypes in our study were NAT2*5B/NAT2*6A, NAT2*5B/NAT2*5B, NAT2*4/NAT2*6A, and NAT2*4/NAT2*5B, which accounted for 16.7, 19.8, 14.0, and 11.7% of the total genotypes, respectively. These genotype frequencies are nearly identical to those reported by Bell *et al.* (46) in a Caucasian-American population. The major genotypes in a study by Cascorbi *et al.* (28) differed slightly from ours; the major genotypes are identical between the two populations, but the relative rank and percentage of the total population are different.

The proportion of rapid and slow acetylator genotypes is similar among the studies cited above, a study by Lin *et al.*

⁵ F. Kadlubar, unpublished data.

(45), and this study. In the bimodal model, the Minnesota population had 58.1% slow acetylators. Lin *et al.* (45) reported 52.6% slow acetylators among Caucasian-Americans and 60% among German-Caucasians, when screening for 6 substituted nts. Cascorbi *et al.* (28) found 58.9% slow acetylators among German-Caucasians screened for substitutions at nt 191, 282, 341, 481, 590, 803, and 857, whereas Bell *et al.* (46) detected 55% slow acetylators in an Caucasian-American population. In the trimodal model, the proportions of intermediate and rapid acetylators in our population were 34.6 and 5.9%, respectively, whereas the corresponding values reported by Bell *et al.* (46) and Cascorbi *et al.* (28) were 39.0 and 6% and 36.8 and 4.3%, respectively.

Several *NAT2* alleles such as *NAT2*12A* and *NAT2*13* are rare in human populations, and assignment of their phenotype activities has been based on few observations. These assignments require further evaluation. In our population, 10 heterozygous subjects had a *NAT*12A* allele. The second allele was *NAT2*5B* (six subjects), *NAT2*6A* (three subjects), or *NAT2*4* (one subject). The subject with a *NAT2*4/NAT2*12A* genotype and one subject with *NAT2*6A/NAT2*12A* genotype had rapid phenotypes, with the later individual having an AFMU/1X ratio only slightly above the 0.66 cutoff for slow *versus* rapid phenotype. All other subjects (8 of 10) had a slow phenotype. On the basis of these observations and recognition that an amino acid change occurs with the nt substitution of *NAT2*12A*, we have considered the *NAT2*12A* allele to have a slow phenotype in our population. This phenotype assignment is in contrast with the rapid phenotype assigned to *NAT2*12A* on the basis of a recombinant *N*-acetyltransferase protein (23) and with data from two subjects from a single family with *NAT2*12A/NAT2*5B* genotypes (47). The discrepancy between the apparent phenotypes of *NAT2*12A* may be the result of gene-gene interactions, an unmeasured dietary or environmental factor, or an additional undetected nt substitution.

The *NAT2*13* allele has a nt substitution that does not change an amino acid in the protein. Nonetheless, the apparent phenotype is slow. The slow phenotype may result from low mRNA stability or expression. A low *in vivo* activity is consistent with low-intermediate activity reported for a recombinant protein of the *NAT2*13* allele (23) and a previous report of *in vivo* activity (28). It is apparent that the definitive assignment of phenotypic activity awaits the accumulation of additional data. *NAT2*12A* and *NAT2*13* have been associated with a wide range of *in vivo* activity and may be influenced by several factors. These findings underscore the need for both phenotype and genotype measurement.

Our control population is likely to be representative of the Caucasian population in the Twin Cities area (Minneapolis and St. Paul, Minnesota). These subjects were randomly selected from the general adult population and frequency-matched by age and sex to cases (age and sex are unrelated to genotype; Ref. 5). The participation rate was 61.7% for controls. Among participants, refusal of specimens was 12% for blood and 17% for urine. The genotype distribution of subjects providing blood but refusing the urine assay was not significantly different from the genotype distribution of subjects who provided both blood and urine samples ($\chi^2 = 1.16$, $P = 0.28$), thus supporting the view that the decision to take part in the caffeine assay is unlikely to be related to *NAT2* status. There were only two individuals who provided a urine sample without a blood sample.

One of the primary objectives of this study was an analysis

of the concordance between *NAT2* genotype-predicted acetylator status and acetylator phenotype. Our results show a high correlation between the two methods of estimating acetylator status. Concordance was 97.8 and 92.8% for the bimodal and trimodal models, respectively. The data confirm results of studies in other populations. Mrozikiewicz *et al.* (25) calculated a 95.8% concordance of genotype and phenotype in 120 Polish children. Graf *et al.* (24) cited a 95% concordance for 81 European individuals, and Cascorbi *et al.* found a 93.3% concordance for 563 German subjects (28).

An important consideration in using the *NAT2* phenotype assay in case-control studies is the possibility that the disease state might alter the results. In this study, we found that concordance in cases was comparable to the value in controls; the difference was not statistically significant.

There are a number of plausible explanations for the discrepancy, albeit small, among genotype and phenotype. Biological and intraindividual variability in phenotypic expression or procedural/laboratory variability of the caffeine test may explain some of the subjects with ratios near the 0.66 cutoff point. The chemical analysis incorporates a quantitative HPLC analysis, which can have a within-day variation of up to 21% (22). Our genotyping methods analyzed for six single-nt substitutions within the coding region. It is possible that other mutations within and outside the coding region exist, and may result in reduced enzyme activity. It is also possible that recombination may have linked substitutions that are not commonly found together, and resulted in misclassification of an allele. Additional caffeine assays, DNA sequencing, and further mapping may reveal the basis for discordance.

The caffeine metabolite assay is currently the gold standard for assigning acetylator status. However, a number of potential limitations of the assay warrant discussion. Many large-scale studies such as our own obtain only one phenotypic measurement. In theory, a single phenotypic measurement may be influenced by short-term dietary changes, medications, or the disease process itself, although we found no evidence for the latter two in the study; we did not address dietary influences in these analyses. The five-hour time commitment and medical status of the subject hinders compliance. In our study participants, there was a higher refusal rate for the caffeine test than for the blood draw.

In contrast, the genotyping method requires a single blood draw, minimizing participant time commitment. DNA could also be obtained from buccal cells or archived samples, allowing for a retrospective analysis. DNA is stable for long periods of time, is not influenced by short-term environmental factors, and is less expensive than phenotyping. As a consequence, genotyping may be preferable to phenotyping in large epidemiological studies.

The modified method of Abe *et al.* (42) is an efficient method for genotyping the most common nt substitutions of positions 481, 590, and 857, requiring only one PCR amplification and one restriction digest. One additional PCR and three additional digests are needed to screen for most restriction sites. This study demonstrates that the determination of the five mutations at nt 282, 481, 590, 803, and 857 is sufficient for the identification of the major slow alleles in this population. The reduced number of steps minimizes supply cost, sample handling time, and risk of contamination.

In conclusion, genotyping is an accurate and efficient means to determine acetylator status that can be used either alone or as a supplement to phenotyping data in large epidemiological studies.

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References

- Raunio, H., Husgafvel-Pursiainen, K., Anttila, S., Hietanen, E., Hirvonen, A., and Pelkonen, O. Diagnosis of polymorphisms in carcinogen-activating and inactivating enzymes and cancer susceptibility: a review. *Gene*, *159*: 113–121, 1995.
- Kadlubar, F. F., Butler, M. A., Kaderlik, K. R., Chou, H.-C., and Lang, N. P. Polymorphisms for aromatic amine metabolism in humans: relevance for human carcinogenesis. *Environ. Health Perspect.*, *98*: 69–74, 1992.
- Ayesh, R., and Smith, R. L. Genetic polymorphism in human toxicology. In: P. Turner and G. N. Volans (eds.), *Recent Advances in Clinical Pharmacology and Toxicology*, pp. 137–157. Edinburgh: Churchill Livingstone, 1989.
- Hein, D. W. Acetylator genotype and arylamine-induced carcinogenesis. *Biochim. Biophys. Acta*, *948*: 37–66, 1988.
- Weber, W. W. The acetylator genes and drug response. New York: Oxford University Press, 1987.
- Grant, D. M., Blum, M., Beer, M., and Myer, U. A. Monomorphic and polymorphic human arylamine *N*-acetyltransferases: a comparison of liver isozymes and expressed products of two cloned genes. *Mol. Pharmacol.*, *39*: 184–191, 1991.
- Grant, D. M., Josephy, P. D., Lord, H. L., and Morrison, L. D. *Salmonella typhimurium* strains expressing human arylamine *N*-acetyltransferases: metabolism and mutagenic activation of aromatic amines. *Cancer Res.*, *52*: 3961–3964, 1992.
- Hein, D. W., Rustan, T. D., and Grant, D. M. Human liver polymorphic (NAT2) and monomorphic (NAT1) *N*-acetyltransferase isozymes catalyze metabolic activation of *N*-hydroxyarylamines and *N*-hydroxy-*N*-acetyl-arylamines proximate carcinogens. *FASEB J.*, *6*: A1274, 1992.
- Kilbane, A. J., Silbart, L. K., Manis, M., Beitins, I. Z., and Weber, W. W. Human *N*-acetyltransferase genotype determination with urinary caffeine metabolites. *Clin. Pharmacol. Ther.*, *47*: 470–477, 1990.
- Parkin, D. P., Vandenplas, S., Botha, F. J., Vandenplas, M. L., Seifart, H. I., vanHelden, P. D., van der Walt, B. J., Donald, P. R., and van Jaarsveld, P. P. Trimodality of isoniazid elimination: phenotype and genotype in patients with tuberculosis. *Am. J. Respir. Crit. Care Med.*, *155*: 1717–1722, 1997.
- Mitchell, R. S., and Bell, J. C. Clinical implications of isoniazid, PAS and streptomycin blood levels in pulmonary tuberculosis. *Trans. Am. Clin. Chem. Assoc.*, *69*: 98–105, 1957.
- Ellard, G. A. Variations between individuals and populations in the acetylation of isoniazid and its significance for the treatment of pulmonary tuberculosis. *Clin. Pharmacol. Ther.*, *19*: 610–625, 1976.
- Evans, D. A. P. Therapy. In: W. Kalow, H. W. Goedde, and D. P. Agarwa (eds.), *Ethnic Differences in Reactions to Drugs and Xenobiotics*, pp. 491–526. New York: Alan R. Liss, Inc., 1986.
- Aynacioglu, A. S., Cascorbi, I., Mrozikiewicz, P. M., and Roots, I. Arylamine *N*-acetyltransferase (NAT2) genotypes in a Turkish population. *Pharmacogenetics*, *7*: 327–331, 1997.
- Slattery, M. L., Potter, J. D., Samowitz, W., Bigler, J., Caan, B., and Leppert, M. NAT2, GSTM-1, cigarette smoking, and risk of colon cancer. *Cancer Epidemiol. Biomark. Prev.*, *7*: 1079–1084, 1998.
- Chen, J., Stampfer, M. J., Hough, H. L., Garcia-Closas, M., Willett, W. C., Hennekens, C. H., Kelsey, K. T., and Hunter, D. J. A prospective study of *N*-acetyltransferase genotype, red meat intake and risk of colorectal cancer. *Cancer Res.*, *58*: 3307–3311, 1998.
- Taylor, J. A., Umbach, D. M., Stephens, E., Castranio, T., Paulson, D., Robertson, C., Mohler, J. L., and Bell, D. A. The role of *N*-acetylation polymorphisms in smoking-associated bladder cancer: evidence of a gene-gene-exposure three-way interaction. *Cancer Res.*, *58*: 3603–3610, 1998.
- Le Marchand, L., Sivaraman, L., Franke, A. A., Custer, L. J., Wilkens, L. R., Lau, A. F., and Cooney, R. V. Predictors of *N*-acetyltransferase activity: should caffeine phenotyping and NAT2 genotyping be used interchangeably in epidemiological studies? *Cancer Epidemiol. Biomark. Prev.*, *5*: 449–455, 1996.
- Sinha, R., and Caporaso, N. Heterocyclic amines, cytochrome P450 1A2 and *N*-acetyltransferase: issues involved in incorporating putative genetic susceptibility markers into epidemiological studies. *Ann. Epidemiol.*, *7*: 350–356, 1997.
- Blum, M., Grant, D. M., McBride, W., Heim, M., and Meyer, U. A. Human arylamine *N*-acetyltransferase genes: isolation, chromosomal localization, and functional expression. *DNA Cell Biol.*, *9*: 193–203, 1990.
- Vincent-Viry, M., Pontes, Z. B., Gueguen, R., Galteau, M.-M., and Siest, G. Segregation analyses of four urinary caffeine metabolic ratios implicated in the determination of human acetylation phenotypes. *Genet. Epidemiol.*, *11*: 115–129, 1994.
- Butler, M. A., Lang, N. P., Young, J. F., Caporaso, N. E., Vineis, P., Hayes, R. B., Teitel, C. H., Massengill, J. P., Lawsen, M. F., and Kadlubar, F. F. Determination of CYP1A2 and NAT2 phenotypes in human populations by analysis of caffeine urinary metabolites. *Pharmacogenetics*, *2*: 116–127, 1992.
- Hein, D. W., Ferguson, R. J., Doll, M. A., Rustan, T. D., and Gray, K. Molecular genetics of human polymorphic *N*-acetyltransferase: enzymatic analysis of 15 recombinant wild-type, mutant, and chimeric NAT2 allozymes. *Hum. Mol. Genet.*, *3*: 729–734, 1994.
- Graf, T., Broly, F., Hoffmann, F., Probst, M., Meyer, U. A., and Howald, H. Prediction of phenotype for acetylation and for debrisoquine hydroxylation by DNA-tests in healthy human volunteers. *Eur. J. Clin. Pharmacol.*, *43*: 399–403, 1992.
- Mrozikiewicz, P. M., Drakoulis, N., and Roots, I. Polymorphic arylamine *N*-acetyltransferase (NAT2) genes in children with insulin-dependent diabetes mellitus. *Clin. Pharmacol. Ther.*, *56*: 626–634, 1994.
- Hickman, D., and Sim, E. *N*-Acetyltransferase polymorphism. Comparison of phenotype and genotype in humans. *Biochem. Pharmacol.*, *42*: 1007–1014, 1991.
- Rothman, N., Stewart, W. F., Caporaso, N. E., and Hayes, R. B. Misclassification of genetic susceptibility biomarkers: implications for case-control studies and cross-population comparisons. *Cancer Epidemiol. Biomark. Prev.*, *2*: 299–303, 1993.
- Cascorbi, I., Drakoulis, N., Brockmüller, J., Maurer, A., Sperling, K., and Roots, I. Arylamine *N*-acetyltransferase (NAT2) mutations and their allelic linkage in unrelated Caucasian individuals: correlation with phenotypic activity. *Am. J. Hum. Genet.*, *57*: 581–592, 1995.
- Ilett, K. F., Chiswell, G. M., Spargo, R. M., Platt, E., and Minchin, R. F. Acetylation phenotype and genotype in aboriginal leprosy patients from the northwest region of Western Australia. *Pharmacogenetics*, *3*: 264–269, 1993.
- Caporaso, N., Landi, M. T., and Vineis, P. Relevance of metabolic polymorphisms to human carcinogenesis: evaluation of epidemiologic evidence. *Pharmacogenetics*, *1*: 4–19, 1991.
- Ilett, K. F., David, B. M., Detchon, P., Castleden, W. M., and Kwa, R. Acetylation phenotype in colorectal carcinoma. *Cancer Res.*, *47*: 1466–1469, 1987.
- LeMarchand, L., and Kolonel, L. N. Cancer in Japanese migrants to Hawaii: interaction between genes and environment (in French). *Rev. Epidemiol. Santé Publique*, *40*: 425–430, 1992.
- Cascorbi, I., Brockmüller, J., Mrozikiewicz, P. M., Bauer, S., Loddenkemper, R., and Roots, I. Homozygous rapid arylamine *N*-acetyltransferase (NAT2) genotype as a susceptibility factor for lung cancer. *Cancer Res.*, *56*: 3961–3966, 1996.
- Ambrosone, C. B., Freudenheim, J. L., Graham, S., Marshall, J. R., Vena, J. E., Brasure, J. R., Michalek, A. M., Laughlin, R., Nemoto, T., Gillenwater, K. A., and Shields, P. G. Cigarette smoking, *N*-acetyltransferase 2 genetic polymorphisms, and breast cancer risk. *J. Am. Med. Assoc.*, *276*: 1494–1501, 1996.
- Millikan, R. C., Pittman, G. S., Newman, B., Tse, C.-K. J., Selmin, O., Rockhill, B., Savitz, D., Moorman, P. G., and Bell, D. A. Cigarette smoking, *N*-acetyltransferases 1 and 2, and breast cancer risk. *Cancer Epidemiol. Biomark. Prev.*, *7*: 371–378, 1998.
- Gertig, D. M., Hankinson, S. E., Hough, H., Spiegelman, D., Colditz, G. A., Willett, W. C., Kelsey, K. T., and Hunter, D. J. *N*-acetyltransferase 2 genotypes, meat intake and breast cancer risk. *Int. J. Cancer*, *80*: 13–17, 1999.
- Vineis, P., Caporaso, N., Tannenbaum, S. R., Skipper, P. L., Glogowski, J., Bartsch, H., Coda, M., Talaska, G., and Kadlubar, F. F. Acetylation phenotype, carcinogen-hemoglobin adducts and cigarette smoking. *Cancer Res.*, *50*: 3002–3004, 1990.
- Okkels, H., Sigsgaard, T., Wolf, H., and Autrup, H. Arylamine *N*-acetyltransferase 1 (NAT1) and 2 (NAT2) polymorphisms in susceptibility to bladder cancer: the influence of smoking. *Cancer Epidemiol. Biomark. Prev.*, *6*: 225–231, 1997.
- Silverman, B. W. *Density Estimation for Statistics and Data Analysis*, pp. 13–19. New York: Chapman and Hall, 1986.
- Vatsis, K. P., Weber, W. W., Bell, D. A., Dupret, J. M., Evans, D. A., Grant, D. M., Hein, D. W., Lin H. J., Meyer, U. A., Relling, M. V., Sim, E., Suzuki, T., and Yamazoe, Y. Nomenclature for *N*-acetyltransferases. *Pharmacogenetics*, *5*: 1–17, 1995.
- Grant, D. M. Molecular genetics of the *N*-acetyltransferases. *Pharmacogenetics*, *3*: 45–50, 1993.

42. Abe, M., Suzuki, T., and Deguchi, T. An improved method for genotyping of *N*-acetyltransferase polymorphism by polymerase chain reaction. *Jpn. J. Hum. Genet.*, 38: 163–168, 1993.
43. Jackson, P. R., Tucker, G. T., and Woods, H. F. Testing for bimodality in frequency distribution of data suggesting polymorphisms of drug metabolism: histograms and probit plots. *Br. J. Clin. Pharm.*, 28: 647–653, 1989.
44. Cohen, J. A coefficient of agreement for nominal scales. *Ed. Psychol. Meas.* 20: 37–46, 1960.
45. Lin, H. J., Han, C. Y., Lin, B. K., and Hardy, S. 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, 1993.
46. Bell, D. A., Taylor, J. A., Butler, M. A., Stephens, E. A., Wiest, J., Brubaker, L. H., Kadlubar, F. F., and Lucier, G. W. Genotype/phenotype discordance for human arylamine *N*-acetyltransferase (*NAT2*) reveals a new slow-acetylator allele common in African-Americans. *Carcinogenesis (Lond.)*, 14: 1689–1692, 1993.
47. Cascorbi, I., Brockmöller, J., Bauer, S., Reum, T., and Roots, I. *NAT2*12A* (803A→G) codes for rapid arylamine *N*-acetylation in humans. *Pharmacogenetics*, 6: 257–259, 1996.

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