results were consistent and showed conclusively that (*a*) both cultured and uncultured chorionic villi were of fetal origin, (*b*) the 632C→T transition was heterozygous (Fig. 1B), and (*c*) the complex I enzyme activities were normal (Table 1). A healthy boy was born after an uncomplicated pregnancy. He is now >3 years of age and does not show any symptoms of mitochondrial disease.

This case demonstrates the difficulties that can arise from prenatal diagnosis based on biochemical results alone. When results differ between native and cultured cells, no safe prediction can be made. Unidentified culturing artifacts might influence the biochemical activity of the cultured cells. Especially if the fetal cells are complex I deficient, a few "healthy" maternal cells may overgrow the fetal cells because of their advantage in oxidative energy metabolism. When interpreting the results of biochemical tests on cultured chorionic villi, one should be aware of the possibility of maternal cell contamination and exclude it by microsatellite marker analysis. When the fetus is male, a chromosome analysis might suffice.

We are grateful to the members of the family for participation in this study, to Dr. R. Knapp for providing the patient material, to Dr. H. A. Zondervan and Vera Martens-Idenburg for providing the chorionic villi, to Christina Rovirosa and Loes van Moerkerken for technical assistance, and to Dr. C. Gerstenfeld for critical discussions about the manuscript. This study was supported by the Deutsche Forschungsgemeinschaft (SFB 577 TP B4) and the parents' support group "Helft dem muskelkranken Kind" (Hamburg).

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Complete Sequencing of a Genetic Polymorphism in *NAT2* in the Korean Population, *Soo-Youn Lee,[1] Kyung-A Lee,[1] Chang-Seok Ki,[1] O. Jung Kwon,[2] Ho Joong Kim,[2] Man Pyo Chung,[2] Gee Young Suh,[2] and Jong-Won Kim[1]** ([1] Department of Clinical Pathology and Division of Pulmonary and Critical Medicine, [2] Department of Medicine, Sungkyunkwan University School of Medicine and Samsung Medical Center, Seoul 135-710, Korea; * address correspondence to this author at: No. 50, Ilwon-dong, Kangnam-ku, Department of Clinical Pathology, Samsung Medical Center, Seoul 135-710, Korea; fax 82-2-3410-2719, e-mail jwonk@smc.samsung.co.kr)

N-Acetyltransferase 2 (NAT2) metabolizes arylamines and hydrazines. The substrates of NAT2 include many therapeutic drugs, such as isoniazid (INH), as well as chemicals and carcinogens *(1–3)*. For that reason, N-acetylation activity is associated with drug effects or toxicities and susceptibility to various cancers. The ability of NAT2 to N-acetylate arylamines is subject to a genetic polymorphism in the *NAT2* gene. The acetylation rate and *NAT2* genotype distribution are quite different among various populations. The genetic polymorphism in *NAT2* has not been studied extensively in the Korean population. Previous reports were based only on phenotyping or restriction fragment length polymorphism analysis, leading to possible misclassification of genotypes. We therefore decided to investigate *NAT2* allelic variability and genotype distributions in the Korean population by complete sequencing. We also evaluated the relationship between genotype and phenotype to understand N-acetylation pharmacogenetics.

One thousand Korean individuals who visited the health promotion center at Samsung Medical Center were anonymously studied. An additional 23 healthy volunteers and 18 patients with pulmonary tuberculosis participated in this study. DNA was extracted from peripheral blood leukocytes. We amplified a 1211-bp fragment that included an 870-bp protein-coding region of the *NAT2* gene and performed full sequencing analysis *(4)* on an ABI Prism 377 DNA Sequencer (Perkin-Elmer). We then checked nucleotide substitutions by combined use of allele-specific-PCR (AS-PCR) and restriction enzyme digestion. Specific primers *(5)* for the wild-type and mutant alleles were used in separate PCRs to detect C282T, T341C, and G590A substitutions. The nucleotides at positions 190, 481, 590, 803, and 857 were explored by digesting the 1211-bp PCR fragment or AS-PCR product carrying the wild-type or mutant allele with *Nci*I, *Kpn*I, *Taq*I, *Dde*I, and *Bam*HI, respectively. For phenotyping, the healthy volunteers and patients gave informed consent and were given 300 mg of INH orally after an overnight fast. Participants were 18–65 years of age; had no underlying liver, kidney, or gastrointestinal diseases; did not have any drug history within the previous 2 weeks; and were not chronic alcoholics. Blood samples were obtained at 6 h, and urine samples were collected up to 6 h after INH administration. We measured urine and plasma concentrations of INH and acetylisoniazid (AcINH) by

HPLC *(6)*, using a Hewlett Packard 1090 HPLC with an ultraviolet detector. The plasma concentration ratio of AcINH to INH (pAcINH/INH) and the urinary molar ratio of INH to AcINH (uINH/AcINH) were calculated. We applied the χ^2 test and the Fisher exact test for categorical variables and the Mann–Whitney test or Kruskal–Wallis test for numerical variables.

On the basis of genotype (Table 1), 428 (42.8%) individuals were rapid, 469 (46.9%) were intermediate, and 96 (9.6%) were slow acetylators. The major genotypes were *NAT2*4/*4*, **4/*6A*, and **4/*7B*. Genotype distribution was consistent with Hardy–Weinberg equilibrium. The frequencies of rapid and slow alleles were 66.6% and 33.1%, respectively. The major alleles that led to a reduction in NAT2 activity were **6A* and **7B*, which contained the G590A and G857A substitutions, respectively. The frequencies of T341C and G857A explain the ethnic difference in acetylator phenotypes between Caucasians and Asians *(5, 7)*. *NAT2*5B*, which contains the T341C, C481T, and A803G substitutions, is the most prevalent allele in Caucasians (40–46%), but it occurs at a very low frequency in Japanese (0.5%) *(2)* and Koreans (1.5% in this study).

Table 1. Frequencies of *NAT2* genotypes in 1000 Korean individuals.

		Observed			
Genotype	Deduced phenotype	n	%	95% confidence interval, %	Expected, %
**4/*4*	Rapid	419	41.9	38.8–45.0	43.2
**4/*12A*	Rapid	2	0.2	0.0–0.5	0.4
**4/*12C*	Rapid	7	0.7	0.2–1.2	0.7
Rapid, total		428	42.8	39.7–45.9	44.4
**4/*5B*	Intermediate	23	2.3	1.4–3.2	2.0
**4/*5C*	Intermediate	2	0.2	0.0–0.5	0.1
**4/*6A*	Intermediate	264	26.4	23.7–29.1	25.5
**4/*6B*	Intermediate	11	1.1	0.5–1.7	0.8
**4/*7A*	Intermediate	7	0.7	0.2–1.2	0.7
**4/*7B*	Intermediate	154	15.4	13.2–17.6	14.5
**6A/*12A*	Intermediate	3	0.3	0.0–0.6	0.1
**6A/*12C*	Intermediate	1	0.1	0.0–0.3	0.2
**6A/*13*	Intermediate	1	0.1	0.0–0.3	<0.1
**6C/*12C*	Intermediate	2	0.2	0.0–0.5	<0.1
**7B/*13*	Intermediate	1	0.1	0.0–0.3	<0.1
Intermediate, total		469	46.9	43.8–50.0	44.1
**5B/*6A*	Slow	4	0.4	0.0–0.8	0.6
**5B/*7B*	Slow	3	0.3	0.0–0.6	0.3
**6A/*6A*	Slow	39	3.9	2.7–5.1	3.8
**6A/*7B*	Slow	36	3.6	2.4–4.8	4.3
**7A/*7A*	Slow	1	0.1	0.0–0.3	<0.1
**7B/*7B*	Slow	13	1.3	0.6–2.0	1.2
Slow, total		96	9.6	7.8–11.4	11.0
**4/*19*	Rapid or intermediate	6	0.6	0.1–1.1	0.5
**6A/*19*	Intermediate or slow	1	0.1	0.0–0.3	0.2
Undetermined, total		7	1.0	0.2–1.2	<0.1
Total		1000	100.0		

Our results demonstrate that the G857A substitution is common (11.5%) in the Korean population, whereas it is rare in Caucasians (1–5%) *(5, 7)*. The prevalence of the slow acetylator has been reported to be 7.6–16.7% in Koreans *(8–10)*. However, because of the limited number of individuals studied, it is not clear whether these reported frequencies reflect a true genotypic distribution. Moreover, in the previous studies, there was a possibility that potential genotype and deduced phenotype misclassifications occurred because the PCR-restriction fragment length polymorphism analysis method used was designed to detect just a small subset of the alleles, including the C481T, G590A, and G857A substitutions, that are commonly found in other ethnic groups. Other issues in these studies were the exclusion of the T341C substitution, which was found to have independent functional significance, and the inclusion of the C481T substitution, which does not reduce enzyme activity *(11)*. Fortunately, the effect may be small in the Korean population because of the low prevalence of the T341C substitution. In any case, the existence of unpredictable or excluded base substitutions in genotyping analysis could cause discrepancies between genotypes and phenotypes. Additionally, a special analytical technique is required to discriminate a mutation-linkage pattern *(4, 5)*.

Considering these points, we performed full sequencing analysis in combination with AS-PCR and restriction enzyme digestion. This would allow accurate determination of all possible genotypes and allocation of nucleotide substitution to either DNA strand. In all cases with heterozygous C282T and heterozygous G590A or G857A substitutions, we found that the genotypes were **4/*6A* or **4/*7B*, not **6B/*13* or **7A/*13*. Cases with T341C, C481T, and A803G substitutions were always found to have **5B*.

Because the phenotyping tests are known to be influenced by environmental factors, including disease status and concurrent drug therapy *(1)*, we also studied tuberculous patients receiving quadruple therapy as well as healthy individuals and used INH as an indicator drug. Contrary to our expectations, there was no significant difference in INH metabolism between the two groups. Acetylator typing is known to be helpful in patients on a once-daily INH-containing regimen *(12)*. Our patients responded equally well with standard daily INH treatment for tuberculosis. It is unfortunate that we could not find any potential therapeutic significance of acetylator types in patients with tuberculosis because of the small number of participants.

The three groups categorized by genotypes showed significant differences in their acetylation activity ($P < 0.05$). However, there was an overlap between rapid and intermediate types and a wide range in the rates of acetylation in both types. Although there was a good correlation between pAcINH/INH and uINH/AcINH, pAcINH/INH provided better discrimination between rapid and intermediate types than did uINH/AcINH. The concordance rate of genotyping and phenotyping by pAcINH/INH was 90.2%. Four discordant cases had genotypes of **4/*4* with intermediate acetylation activity.

Genotyping seems more relevant than phenotyping because three of four discrepant cases showed conflicting results with different phenotyping methods. We believe that the discrepancies between phenotypes and genotypes suggest possible problems in phenotyping or the presence of another factor that contributes to the variability in NAT2 activity, such as the *NAT1* gene.

The correlation between pAcINH/INH and nine genotypes is shown in Fig. 1. Wide variations in acetylation activities were noted in individuals with the same genotypes. Homozygous rapid acetylators with *NAT2*4/*4* exhibited mostly higher activities than did heterozygotes such as **4/*6A* or **4/*7B*. Individuals with two **6A* alleles showed significantly decreased activities compared with intermediate acetylators with **4/*6A*, suggesting a gene dosage effect.

Metabolic activity data were trimodally distributed with some overlap between rapid and intermediate acetylators. It was difficult to discern intermediate from rapid acetylators by phenotyping alone. Because rapid and intermediate acetylators account for most of the general population and the two groups have different metabolic activities in spite of overlaps, it is very important to correctly discriminate the two. Accordingly, genotype analysis is indispensable to determine acetylator status rather than, or as a supplement to phenotyping. Moreover, there are differences in NAT2 activity within each genotypic group. Several studies have demonstrated significant quantitative or qualitative differences in in vivo acetylation capacities, even among slow alleles *(4, 13)*. Our aim was to determine whether mutation linkage patterns and allelic composition, as well as the presence of nucleotide changes at the *NAT2* locus, may account for the diverse acetylation activities, but we lacked sufficient numbers of alleles and individuals with each allele in this study to achieve this aim. We expect that, in the near future, quantitative estimation of NAT2 activity may be possible with knowledge of the function of each variant allele that may cause subtle differences in metabolism.

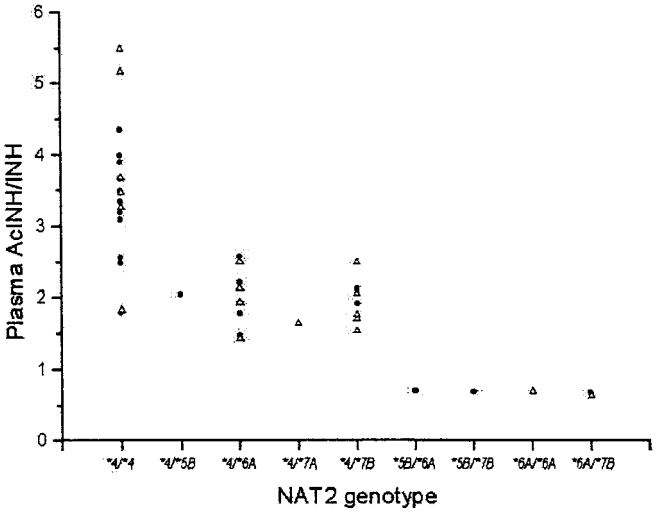

Fig. 1. Correlation between the plasma AcINH/INH ratio and nine *NAT2* genotypes in 23 healthy individuals (●) and 18 patients with tuberculosis (△).

The results of this study will be helpful for future epidemiologic or clinical studies and for understanding the genetic basis of acetylation polymorphisms in the Korean population. The design of a cost-effective genotyping method to search for targeted mutations and prediction or determination of ambiguous alleles and genotypes will be facilitated. We confirmed that N-acetylation activity is significantly affected by a genetic polymorphism in *NAT2*. However, further studies are required to define the influence of *NAT2* allelic variants and the effects of other genetic factors on the phenotypic outcome.

This work was supported by the fund from The Center for Functional Analysis of Human Genome, Korea.

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