Polymorphism of Human Acetyltransferases

Urs A. Meyer

Biocenter of the University of Basel, Basel, Switzerland

Acetylation by arylamine N-acetyltransferases (NATs) is a major route in the metabolism of numerous drugs and carcinogens. Recent studies suggest that the same enzymes also catalyze N,O-transacetylation and O-acetylation. A genetic polymorphism of clinical relevance divides the human population into slow and rapid acetylators of arylamines. Two human NATs, NAT1 and NAT2, have recently been characterized by protein purification, cloning, and functional expression of the respective genes; both were localized to chromosome 8. NAT1 codes for a protein with ubiquitous tissue distribution and a high affinity for p-aminobenzoic acid and p-aminosalicylic acid, so-called monomorphic substrates. NAT2 codes for a protein predominantly expressed in liver with a high affinity for sulfamethazine and other polymorphically metabolized drugs. NAT2 was analyzed at the level of protein, RNA and DNA derived from phenotyped slow and rapid acetylators. Two common (M1, M2) and one rare (M3) mutant allele were identified and their mutations characterized. A simple polymerase chain reaction-based DNA test can identify >95% of mutant alleles and predict the phenotype. — Environ Health Perspect 102(Suppl 6):213–216 (1994)

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Introduction

Acetylation is an important route of bio-transformation for a large number of hydrazine and arylamine drugs and carcinogens. Several occupational and food carcinogens may be activated by acetylation (1–3). For instance, a number of food-derived heterocyclic amines are activated by drug-metabolizing enzymes, including acetyltransferases. The ultimate mutagen/carcinogen presumably is an electrophilic arylNitinrenium ion which can react with DNA and form adducts (4). Reactions involved in the metabolic activation of heterocyclic amines include N-hydroxylation by cytochrome P450 and N- and O-acetylation by cytosolic arylamine N-acetyltransferases (CoASAc; NAT, EC 2.3.1.5) (Figure 1).

Interindividual variation in the acetylation of several arylamines and hydrazine compounds in humans was shown to be genetically determined nearly 40 years ago with the antituberculosis drug isoniazid (5). Separation of individuals into either rapid or slow acetylators is determined by variation at a single autosomal locus and constitutes one of the first discovered genetic polymorphisms of drug and carcinogen metabolism (1–3). The proportions of rapid and slow acetylators vary remarkably in different ethnic and/or geographic-origin populations. For example, the percentage of slow acetylators among Canadian Eskimos is 5%; whereas it rises to over 80% among Egyptians and 90% among Moroccans. Most populations in Europe and North America have 40 to 70% slow acetylators; Asian populations, only 10 to 20% slow acetylators (2).

The association of the acetylation polymorphism with an increased risk of certain cancers (e.g., bladder cancer or colorectal cancer) has received much recent attention and has been previously reviewed (2,3,6–8). In contrast to these extensive clinical studies, until recently little was known about the molecular mechanism underlying the acetylation polymorphism.

The Molecular Mechanism of the Acetylation Polymorphism

Early studies in other laboratories established that the observed variation in acetylation of isoniazid and other arylamine and hydrazine drugs was caused by a difference in the activity of a cytosolic N-acetyltransferase (NAT) in the liver (1–3,9,10). However, some arylamines such as p-aminobenzoate and p-aminosalicylate also are N-acetylated, yet are unable to distinguish rapid and slow acetylators in vivo or in liver tissue in vitro (11). These substrates are called monomorphic, in contrast to the polymorphic substrates isoniazid, procarinamide, and others. Jenne et al. (9), during the first detailed biochemical studies of drug acetylation in man, proposed that two different N-acetyltransferase enzymes were responsible for the metabolism of monomorphic and polymorphic arylamines. Moreover, it appeared that the observed genetic variation in acetylation of polymorphic substrates was related to differences in the quantity of one of these enzymes present in human liver cytosol, rather than to altered kinetic characteristics of a structural variant. However, subsequent work using the rabbit as an animal model for the human acetylation polymorphism shed doubt on this concept, since kinetic, biochemical, and immunological experiments in rabbits suggested that a single species of enzyme mediates the acetylation of both monomorphic and polymorphic substrates (12).
Therefore, we have done experiments, both in humans and in rabbits, designed to clarify the molecular mechanism responsible for the observed genetic polymorphism and for the different handling of so-called monomorphic and polymorphic substrates. An additional goal was the development of a simple genotyping test which could predict the acetylator phenotype in genomic DNA of patients and volunteers in clinical studies.

Results and Discussion

Studies with Purified N-Acetyltransferases

Two closely related but kinetically distinct NAT activities, NAT1 and NAT2, were purified from frozen human liver and the polyclonal rabbit antiserum, which recognizes these proteins on Western blots, was developed (13,14). Microsequencing of tryptic peptides and comparison with sequence data from the purified rabbit enzyme (15) established the identity of the purified enzyme. NAT2 was shown to be separable on anion exchange resins into two closely related acetylating enzymes, NAT2A and NAT2B, which apparently are translational products derived from the same gene (11,14). Using a polyclonal antibody, we saw a close correlation between the in vitro NAT activity measured with sulfamethazine and the amount of immunoreactive protein of both NAT2 fractions in cytosols from 50 human livers (14). Thus, the immunoreactive protein was markedly decreased or absent in livers with low N-acetyltransferase activity. Apparently, as later studies (11) indicated, NAT1 is unstable and was not detected. Furthermore, we observed an excellent correlation between in vitro NAT2 activity, with immunoreactive protein and acetylator phenotype determined in vivo using caffeine as a probe drug (14). These experiments unequivocally established that the acetylation polymorphism is due to the decreased amount of a N-acetyltransferase enzyme in the liver.

Cloning of Human and Rabbit NAT Genes

We first cloned a rabbit NAT cDNA (16,17) and used it to isolate DNA clones from a λEMBL3 library constructed with genomic DNA from an individual characterized in a family study as obligate heterozygous rapid acetylator (18). The 18 recombinant clones obtained defined three different human NAT genes, designated NAT1, NAT2, and NATP. Both NAT1 and NAT2 have single coding exons of 870 base pairs and high homology (81% for amino acids, 87% for nucleotides) between them. These two genes were localized to human chromosome 8; NATP represents a pseudogene (18). To determine which NAT gene codes for the polymorphic enzyme, NAT1 or NAT2 (i.e., the EcoRI fragments of 1.3 and 1.9 kilobase pairs containing these genes) were inserted into the expression vector p91023 and transiently expressed in COS-1 cells, with analysis of the protein product by Western blotting and sulfamethazine acetylation activity (18). We could demonstrate that NAT2 encodes a protein with identical mobility on SDS-PAGE and identical affinity for arylamine substrates as that exhibited by the NAT2 proteins purified from human liver (11,13). The NAT1 gene product had an apparently lower mobility and much lower affinity for the polymorphic substrate sulfamethazine.

The discovery of two separate genes encoding NAT1 and NAT2 also resolved the puzzle of monomorphic and polymorphic substrates. NAT2 has a high affinity for polymorphic substrates such as sulfamethazine and procainamide, whereas NAT1 has higher affinities for the monomorphic substrates p-aminobenzoate and p-aminosalicylate and is not affected by the polymorphism. NAT1 apparently was missed in our earlier purifications from frozen human liver because it is unstable and rapidly degrades upon thawing. The present data thus support the concept that the two activities separated on anion exchange resins NAT2A and NAT2B (13,14) represent two forms of polymorphic NAT2 and are derived from a single gene (11).

Mutations of the NAT2 Gene Causing Slow Acetylation

Mutant alleles were initially defined by restriction fragment analysis of either genomic DNA samples from 25 healthy individuals whose acetylator phenotype had been established by measuring the acetylated metabolite of caffeine in urine, or of DNA from 33 human liver samples with known NAT2 enzyme activity measured with the substrate sulfamethazine (19). Restriction fragment length polymorphisms (RFLPs) generated by KpnI, TagI, and BamHI segregate with acetylator phenotype and define three mutant alleles of NAT2, M1, M2, and M3. Two of these RFLPs were also described by Deguchi et al. (20) in a Japanese population. To explore the molecular mechanism of slow acetylation, we cloned M1 and M2 genes from genomic λEMBL3 and Agt10 libraries, respectively, and compared their sequence to the wild-type gene (19) (Table 1). Both M1 and M2 point mutations causing amino acid changes in the deduced protein sequence were combined with silent base substitutions. The silent mutation in M1 (481 C→T) alters the recognition sequence for the restriction enzyme KpnI, explaining the observed RFLP. In M2, the point mutation (590 G→A) causes the change in (Arg197→Glu197) and eliminates a TagI restriction site. Studies in additional samples revealed that M1 very frequently has an additional 3d point mutation at position 803 (A803→G) (21,22). A third mutant allele, M3, apparently rare in the Caucasian population first examined by us, has been characterized as cDNA and sequenced by Ohsako et al. in a Japanese population (23). M3 has one point mutation (857 G→A) causing an amino acid change (Gly286→Glu286), a loss of a BamHI restriction site, and a silent mutation (C282→T). We also have observed this mutant to occur relatively frequently in the Chinese population (Table 2). Determination of the mutations at position 481, 590, and 857 is sufficient to define alleles M1, M2, and M3. The nucleotide substitutions at position 341 and 803 are virtually always associated with the 481 T mutation (22).

To dissect the contribution of single mutations to decreased enzyme protein chimeric gene constructs between wild-type alleles, M1 and M2 were generated and transiently expressed in COS-1 cells (19). Constructs bearing only one of the two mutations of allele M1 on expression both behaved like the normal enzyme, demonstrating that two nucleotide substitutions of M1 (341 T→C and 481 C→T) are required to decrease enzyme protein and activity. Analysis of the M2-derived constructs showed that the silent mutation (282 C→T) does not affect the enzyme. The mutation (590 G→A) causing the amino acid change (Arg197→Glu197) alone causes a reduction of enzyme protein and activity. When stabilities of expressed proteins were compared, the half-lives at 37°C of wild-type and M1-derived activities were identical to wild-type; whereas, that of M2 appeared markedly reduced (although there was considerable variation between experiments in the half-lives). Thus, the (Arg197→Glu197) mutation of M2 presumably produces a less stable protein, and the combination of the two nucleotide substitutions in M1 causes defective translation, possibly due to alterations in transcript sec-
wild-type allele (R), and M2 (slow), which has a C → T transition at position 341, causing a silent change in amino acid codon 114 from serine to phenylalanine. M3 (slow), with a single nucleotide polymorphism (SNP) at position 481, is the most frequent allele at 26.2% in Europeans/Caucasians. It is associated with a silent change at codon 160 from serine to arginine.

Table 1. Alleles of NAT2 and their mutations.

| Designation of allele | 282 | 341 | 481 | 590 | 803 | 857 |
|-----------------------|-----|-----|-----|-----|-----|-----|
| Wild-type (rapid, R)  | C   | T   | C   | G   | A   | G   |
| M1 (slow, S, t1)     | C   | C0  | T   | G   | G6  | G   |
| M2 (slow, S, t2)     | T   | T   | C   | A6  | A   | G   |
| M3 (slow, S, t3)     | T   | T   | C   | G   | A   | A6  |

*According to Blum et al. (18) *Mutation causes a change in an amino acid. *Mutation generates or eliminates a restriction site. *Silent mutation, no change in amino acid.

Table 2. Frequencies of wild-type and three mutant alleles of NAT2 in different populations.

| Allele designation | European/Caucasian, % | Japanese, % | Chinese, % |
|--------------------|-----------------------|-------------|------------|
| Wild-type          | 29.8                  | 68.8        | 60.2       |
| M1                 | 40.1                  | 0           | 7.4        |
| M2                 | 27.9                  | 24.4        | 26.2       |
| M3                 | 2.2                   | 7.0         | 8.3        |

Slow acetylator phenotype, % 40–70 10–20 10–20

*Analysis by polymerase chain reaction-amplification of 322 alleles of Caucasians from Graf et al. (24) and additional unpublished studies in the author's laboratory. *Data from Deguchi et al. (18). Analysis by restriction fragment length polymorphism of 172 alleles. *Data from Hayes et al. (25).

Development of a Genotyping Assay

This knowledge on the mutations of M1, M2, and M3 was used to develop a set of mutation-specific primers for allele-specific amplification of small amounts of DNA by the polymerase chain reaction (19). Our present data are summarized in Table 2. Allele M1 had the highest frequency (40%) and together with M2 (27.9%) accounted for 68% of all alleles in the Caucasian population. Of the slow alleles, 54.8% were M1, 38.6% M2, and 2.2% M3. The phenotype of 78 of the 81 individuals was correctly predicted (96.3%, 24). Some apparent wild-type alleles in slow acetylators probably carry unknown mutations and account for approximately 5% of slow alleles. Thus, M3 indeed is quite rare in Caucasians. On the other hand, in a Japanese population analyzed by RFLP by Deguchi et al. (18), M1 was not observed, but M3 was represented at a somewhat higher level of 7%. Of all slow alleles, M3 accounted for 22% in the study of Deguchi et al. (18). These data suggest that most of the observed interethnic difference in the occurrence of the slow acetylator phenotype between Oriental and Caucasian populations is due to the almost unique presence at high frequency of M1 in Caucasians, where M3 shows a relatively higher incidence in Oriental populations. Moreover, because of the high incidence of the slow acetylator allele in the Caucasian population, only 10% of fast acetylators are homozygous for the wild-type gene and cannot easily be differentiated by the usual phenotyping procedure from the predicted approximately 40% heterozygous carriers of a slow NAT2 allele. These interethnic comparisons were confirmed in a recent study in a Chinese population and are summarized in Table 2.

Conclusion

These studies at the protein, RNA, and DNA level provide a molecular basis for the clinical and experimental data on slow acetylation in man. The described DNA amplification assay for NAT2 mutations allows the prediction of the acetylator phenotype in over 95% of individuals tested with a small sample of DNA, which may be derived from leucocytes, single hair roots, buccal epithelia, or any other tissue. This will allow the genotyping of patients and volunteers in clinical studies or of workers at high risk for toxicity upon exposure to arylamine chemicals. The heterologous expression of NAT genes (4, 11, 17–19) will allow the efficient testing of new substrates for these enzymes and of the activation of carcinogens to mutagenic products.

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