**Review Article**

Acetylator status, drug metabolism and disease

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**ABSTRACT**

Acetylation polymorphism, although discovered 40 years ago, still holds interest not only because many drugs and carcinogens are metabolized by acetylation in the liver but also because advances have been made in the understanding of the molecular genetics of acetylation. It is this genetic variation of drug metabolism that is one of the causes of inter-individual variation of the effect of a drug. Acetylation polymorphism relates to the metabolism of a number of arylamine and hydrazine drugs and carcinogens by cytosolic N-acetyltransferase—NAT2. In humans, 2 genes—NAT1 and NAT2—are responsible for the N-acetyltransferase activity. Studies have revealed several allelic variants of both NAT1 and NAT2. It has been suggested that some of these variants modify the individual susceptibility to disease.


**INTRODUCTION**

Several drugs (Table I) and environmental carcinogens present in cooked food and tobacco smoke are metabolized in the liver by acetylation, mediated by N-acetyltransferase isoenzymes NAT1 and NAT2. These enzymes transfer an acetyl group from acetyl coenzyme A to the amino or hydrazino group of the compound being metabolized. There is considerable variability in their activity in different subjects because of genetic polymorphism. Thus, several wild and mutant genotypes of NAT2 have been recognized. Certain genotypes are associated with high O-acetyl-transferase activity, which imparts these individuals the phenotype status of rapid or intermediate acetylators. Marked differences in the distribution of different genotypes and phenotypes in different populations have been reported from several parts of the world. It has been speculated that this may account for some of the observed variations in pharmacokinetics, drug toxicity and susceptibility to cancers in different populations. The present review is a summary of the relevant findings on this subject.

**COMPARISON OF NAT2 GENOTYPES AND PHENOTYPES**

Genes coding for N-acetyltransferase activity are expressed as NAT1 and NAT2 and located on chromosome 8 at 8p21.3–23.1. Both enzymes are capable of N-acetylation, O-acetylation, and N,O-acetylation and are involved in drug metabolism and detoxification of carcinogens. Evaluation of the NAT2 genotype is done using allele-specific polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). The gene is polymorphic and more than 25 NAT2 genotypes have been reported. They have been assigned to a rapid, intermediate or slow acetylator phenotype on the basis of characterization of recombinant NAT2 allozymes. Presence of the *4 (wild-type) allele defines a NAT2 genotype as a rapid acetylator, whereas the NAT2*5B allele is a marker for a slow acetylator. Mutant alleles M1 and M2 account for more than 90% of the slow acetylator alleles in the European population. Similarly, about 20 alleles of NAT1 have also been reported but their functional significance is less clear.

The acetylator phenotype is determined by studying the acetylation of certain drugs such as sulphadimidine, isoniazid (INH) dapsone or caffeine. Sulphadimidine and INH are typical substrates for NAT2, whereas NAT1 metabolizes para-aminobenzoic acid. Caffeine is metabolized by both enzymes. Typically, the ratio of a metabolite to the parent drug is measured in the serum, urine or saliva after a defined time following its oral administration. The frequency distribution of the ratio in a group of healthy individuals is bimodal. The antimode is used to define the cut-off between slow and rapid acetylators. Certain investigators prefer to fit their data in a trimodal distribution.

The liver is the main site for the expression of NAT2 where it regulates the metabolism and detoxification of drugs and xenobiotics. NAT1, on the other hand, is expressed in other organs of the body including the colon.

Unfortunately, factors other than the acetylator status may affect the results in disease states, so the results of the acetylator phenotype may not agree with the genotype. Thus, intestinal malabsorption, and liver or kidney diseases may influence the metabolite concentration regardless of the NAT2 genotype. However, in healthy subjects, there is good agreement between the genotype and phenotype. In a study of the acetylator status of Chinese women, it was noted that 78% and 76% had rapid acetylator phenotype and genotype, respectively. The distribution of slow acetylators in 222 Caucasian Americans was observed to be 58.1% and 59.5% as classified by the phenotype and genotype.

**TABLE I. Commonly used drugs metabolized by NAT2 enzymes**

- Isoniazid
- Dapsone
- Procainamide
- Sulphonamides
- Hydralazine
- Aminogluthethimide
- Aminosalicylate sodium

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respectively. Concordance of the classification of NAT2 genotype and phenotype was 97.8% in the bimodal model.  

In a study of patients with tuberculosis in Japan, it was observed that the metabolism of INH by NAT2 was impaired in subjects with mutations in the NAT2 gene, and genotyping for three NAT2 point mutations was adequate to predict the metabolism of INH. There was good concordance between the genotypes and phenotypes. However, in patients with AIDS, there was discordance between the acetylator genotype and phenotype as measured by a caffeine probe in 35% of the subjects. Similar observations have been reported by O’Neil et al., who found that the percentage distribution of slow:rapid acetylator phenotype seen among acutely ill AIDS patients differed with the probe substrate used; it was 70:30 with caffeine compared to 53:47 with dapsone. Phenotype assignment differed considerably between the two methods and there were numerous discrepancies between the phenotype and genotype. The NAT2 slow:rapid genotype distribution was 45:55. Control subjects, phenotyped with only caffeine had a distribution of 67:33 versus 60:40 on genotyping. We observed the proportion of slow acetylators in healthy Indian subjects to be 58% and 66%, depending upon the measurements in the urine and serum, respectively, using the sulphadimidine probe. Clearly, methodological details influence the results of phenotyping.

GEOGRAPHICAL VARIATION OF SLOW AND RAPID ACETYLATOR PHENOTYPES

Significant variation has been noted in the distribution of various phenotypes among subjects from different parts of the world. The Eskimos and Japanese have the lowest rates for slow acetylators (about 10%) with the Chinese having a rate of about 20%. A very low proportion (<10%) of slow acetylators has also been reported in Papua New Guinea. Slow acetylators are less common among the populations of Hong Kong, Malaysia and Singapore. In one study from Berlin, 62% of the Caucasian subjects were classified as slow acetylators while in France about 53% were slow acetylators. Using INH plasma half-life, 41% of 106 black South African men with tuberculosis were phenotyped as slow acetylators. There is considerable variation in the proportion of slow acetylators reported from India in different studies, but on the whole it is high, at about 60%. Similar results have been reported from south India. Slow acetylators predominate in countries in the middle East as well. Population frequencies of NAT2 genotypes and phenotypes have been recently reviewed. Some of the variation in the reported distribution of phenotypes is probably related to methodological differences, both in relation to the selection of subjects, as well as the technical aspects of measurement of metabolites. However, genetic polymorphism is substantial and it may have important implications for the metabolism and detoxification of drugs and xenobiotics.

In summary, there is considerable variation in the proportion of slow acetylators in different ethnic groups. Almost 60% of Indians are slow acetylators as compared to a much smaller proportion of Caucasians, Chinese and Japanese (Table II).

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<tr>
<th>Table II. Geographical variation in acetylator phenotype distribution</th>
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Drug-induced lupus

Hydralazine, procainamide, INH and sulphasalazine are known to predispose to the development of drug-induced lupus (SLE). In sulphasalazine-induced SLE, slow acetylator genotype and HLA haplotypes associated with idiopathic SLE are recognized risk factors. In a study of 106 patients with rheumatoid arthritis treated with sulphasalazine, a significant increase of the hepatic enzyme aspartate transaminase was noted mainly in slow acetylators, but this was not associated with clinical disease. It appears that acetylator status does not relate significantly to either the efficacy or toxicity of sulphasalazine in rheumatoid arthritis. Slow acetylators with ulcerative colitis on sulphasalazine therapy require a lower daily dose of the drug than fast acetylators to maintain remission of the disease without major side-effects. Slow acetylator phenotype along with female gender and the presence of predisposing HLA-DR antigens appear to be risk factors in the development of hydralazine-induced SLE.

Idiosyncratic adverse drug reactions

Slow acetylator status has been linked to idiosyncratic adverse drug reactions during therapy with sulphonamides. Differences in metabolic handling of the drug can increase the likelihood of covalent binding of reactive metabolites with cellular antigens resulting in cytotoxicity and immune response to neoantigens. Considering the rarity of these reactions even in slow acetylators, other factors such as a difference in the rate of production and detoxification of hydroxylamine metabolites may be important.

In summary, a slow acetylator status is associated with an increased risk of hepatotoxicity and peripheral neuropathy among patients receiving INH. There is also an enhanced risk of drug-induced lupus among slow acetylators who receive INH, hydralazine or procainamide. Idiosyncratic reactions to sulphonamides also occur more frequently in these subjects.
ACETYLATOR STATUS AND CANCER

Acetylation of several potential carcinogens and xenobiotics in food, environment or tobacco smoke may be influenced by the acetylator genotype and may thus influence the risk for certain human cancers. A large volume of literature is available on this subject, but definite evidence of NAT2 gene–environment interaction in carcinogenesis is limited. This is because of methodological limitations of genotyping/phenotyping, case selection and small number of subjects in individual studies. This subject has been recently reviewed.23

A meta-analysis of 21 published case–control studies in patients with cancer of the urinary bladder gave an odds ratio of 1.31 (95% CI 1.11–1.55) for slow compared to fast acetylators.24 The risk may be higher in smokers compared to non-smokers but stratified analysis was possible in only 5 of 21 studies due to lack of information on smoking status in others.25

An association between fast acetylator genotype and colorectal cancer has been suggested and investigated by several investigators. No association between the NAT2 genotype and invasive colorectal cancer was found in 10 of the 11 studies.24 It has been hypothesized that the rapid acetylator phenotype may confer susceptibility to colorectal cancer because of greater activation of dietary heterocyclic amines present in well-done meat. Heterocyclic amines are activated by O-acetylation, suggesting that NAT2 genotypes with high O-acetyltransferase activity (rapid acetylator phenotype) may increase the risk of cancers induced by these compounds. There appears to be an interaction between the fast acetylator genotype and consumption of fried or well-done meat as risk factors for colorectal cancer.26 A similar association has been reported for breast cancer in postmenopausal women.27 The NAT2 slow acetylator genotype was found to be more frequent in non-smoking Chinese women with lung cancer in a case–control study from Singapore.28 Others have reported a lack of association with NAT2 genotypes but some association with NAT1 genotypes.29 Interactions between NAT2 polymorphism, p53 gene mutation and tobacco smoking (active or passive) as risk factors for lung cancer and cancer of the breast have been investigated by several workers but in different studies have remained inconsistent and inconclusive. Similarly, no consistent association between acetylator status and cancers of the oral cavity or prostate have been found.30

PROSPECTS FOR THE FUTURE

With the mapping of the entire genome, there are high expectations that pharmacogenetics will advance rapidly in the coming years and it would be possible to individualize drug treatment and reduce the risk of toxicity. An understanding of the interactions between NAT genotypes, genes identified in familial cancers (colon, breast) and a variety of environmental factors would permit identification of individuals at high risk for specific cancers. These advances are likely to have a major impact in the practice of medicine in the next few decades.

REFERENCES