Enzyme Systems that Metabolise Drugs and Other Xenobiotics.
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11 Arylamine Acetyltransferases

Gerald N. Levy and Wendell W. Weber

University of Michigan Medical School, Ann Arbor, Michigan, USA

Arylamine *N*-acetyltransferase is an enzyme activity widely distributed across species and throughout many tissues. Mammalian arylamine *N*-acetyltransferase enzymes (NAT) EC 2.3.1.5 are cytosolic in location and have molecular weights of about 31 000–33 000 daltons. They catalyse three reactions involving the transfer of an acetyl moiety (reviewed in Weber and Hein 1985; Weber 1987; Hein 1988). The reaction for which the enzyme is named is the transfer of the acetyl group from acetyl CoA (AcCoA) to an arylamine to produce an arylacetamide. The reaction occurs as two half-reactions: first, the acetyl is transferred to an evolutionarily conserved cysteine residue of the NAT protein forming an acetyl-cysteinyl-NAT catalytic intermediate (Andres *et al.* 1988), and second, the acetyl is transferred to the amino nitrogen of an acceptor arylamine. The reaction mechanism is classified as ping-pong Bi Bi as the first product (CoA) is released before the second reactant (arylamine) is bound (see Figure 11.1). Amino acid sequences for NATs of many species show conservation of cysteines at positions 44, 68, and 223. In particular the sequence surrounding C68 (C69 in *Salmonella*) is highly conserved and this therefore is believed to be the active site for acetyl transfer (Watanabe *et al.* 1992).

In addition to *N*-acetylation, NAT can also catalyse the *O*-acetylation of arylhydroxylamines. This reaction is formally similar to *N*-acetylation except that the NAT enzyme, acetylated by AcCoA, donates the acetyl group to the oxygen of an arylhydroxylamine to form an acetoxyarylamine.

A third significant reaction catalysed by NAT is independent of AcCoA and involves transfer of acetyl from the nitrogen of an arylhydroxamic acid to the oxygen, producing an acetoxyarylamine. This reaction referred to as *N,O*-transacetylation or AHAT (arylhydroxamic acid acetyl transfer) produces the acetyl-enzyme intermediate

$$\mathbf{AcCoA + NAT \Leftrightarrow Ac\text{-}NAT + CoA}$$
$$\mathbf{Ac\text{-}NAT + Ar\text{-}NH_2 \Leftrightarrow Ar\text{-}NH\text{-}Ac + NAT}$$

Figure 11.1 The *N*-acetyltransfer reaction comprises two half reactions. Initially the enzyme (NAT) is acetylated by AcCoA. The second step is the transfer of the acetyl group to the acceptor arylamine, releasing the enzyme protein for further catalysis.

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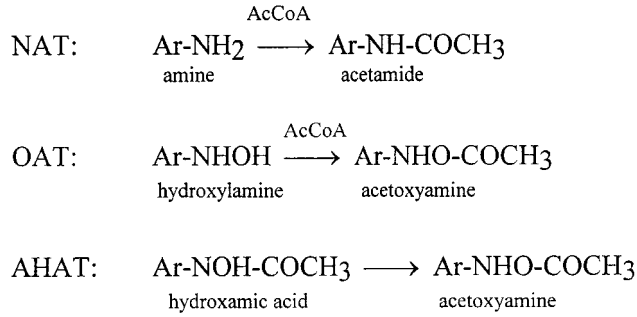

Figure 11.2 The three acetyl transfer reactions catalyzed by arylamine acetyltransferase. NAT: *N*-acetyltransfer. OAT: *O*-acetyltransfer. AHAT: arylhydroxamic acid acetyltransfer.

from the arylhydroxamic acid rather than from AcCoA. The *N* to *O* transfer of the acetyl group can be either intra- or inter-molecular depending on if the acetyl donor and acceptor are the same or different molecules. The three activities of NAT are illustrated in Figure 11.2.

Role of NAT in arylamine metabolism and toxicity

NAT, by virtue of its ability to catalyse *O*-acetylation and AHAT, in addition to *N*-acetylation, can both metabolically activate and detoxify arylamines. As a phase II conjugating enzyme, NAT produces water-soluble amides from less water-soluble, lipophilic amines, thereby hastening elimination of these potentially toxic compounds. However, further activation of hydroxylamines to acetoxyamines by the OAT and AHAT activities leads to the formation of arylnitrenium ions which combine with proteins and nucleic acids. DNA adduct formation with arylamine-derived nitrenium ions is a mechanism of DNA mutation and initiation of carcinogenesis.

An arylamine faces two primary metabolic fates: it can be conjugated by various phase II enzymes to a water-soluble, excretable product or it can undergo oxidation. Oxidation can be catalysed by a number of enzymes or enzyme families including the flavin monooxygenases (chapter 3), prostaglandin H synthases (chapter 6) and other peroxidases, and cytochromes P450. In the liver, the prevalent arylamine oxidative enzymes appear to be cytochromes P450, and for arylamines CYP1A2 has been found to be a major enzyme of oxidation. Here too there is a fork in the metabolic path, for oxidation of a carbon of the aromatic ring will usually, but not always (Harris *et al.* 1989), lead to a non-toxic compound that can be conjugated and excreted. In contrast, oxidation of the amino nitrogen forms a hydroxylamine which, although often toxic itself, can be further activated by NAT leading to acetoxyamine-derived nitrenium ions. The factors that determine which path an arylamine follows, activation or detoxification, are not completely known. It is reasonable to believe that the relative expression and activities of the oxidative and conjugating enzymes, the selectivity of the competing enzymes for the specific arylamine substrate, the concentration of the

arylamine, and the intracellular environment are among the factors which influence arylamine metabolism.

NAT in drug metabolism

The role of NAT in detoxification of drugs can be illustrated by a few examples involving commonly used therapeutics. Early interest in NAT was sparked by the introduction of isoniazid (INH) as an antitubercular agent. Isoniazid is an example of an arylhydrazine (isonicotinic acid hydrazide) which is a substrate for NAT. *N*-Acetylation is the primary pathway of isoniazid metabolism. Although many metabolites are produced during INH metabolism, acetylisoniazid is the most prominent product initially formed. Acetylisoniazid is further metabolised to isonicotinic acid, which is conjugated with glycine and excreted, and to monoacetylhydrazine. Monoacetylhydrazine can follow any of three metabolic pathways: 1- hydrazone formation, 2- further acetylation to diacetylhydrazine, or 3- oxidation. The first two paths produce non-toxic excretable products while the third, probably catalysed by a P450 enzyme, leads to electrophilic reactive intermediates capable of inducing hepatotoxicity (Weber 1991).

Another example of the competition between detoxification by acetylation and activation by oxidation is seen in metabolism of the antiarrhythmic procainamide. *N*-Acetylation is the primary detoxification pathway of procainamide, producing *N*-acetylprocainamide and some *N*-acetyl-*p*-aminobenzoic acid and desethyl-*N*-acetylprocainamide (Weber 1987). Procainamide can also undergo oxidation to a hydroxylamine and subsequently to the nitroso and nitro derivatives. The catalytic activity for the oxidation is thought to be a hepatic cytochrome P450 protein (probably CYP2D6; Lessard *et al.* 1999), but more importantly for toxicities such as drug-induced lupus, myeloperoxidase catalyses the reaction in leukocytes. Activation of procainamide and similar drugs by leukocytes provides active metabolites direct access to the immune system and leads to various expressions of immunotoxicity (Uetrecht 1989).

One of the most important occurrences of the competition between detoxifying *N*-acetylation and activating oxidation is in the metabolism of sulphonamide derivatives. These antibacterial agents, such as sulphamethoxazole, are used as primary therapy of infection in transplantation and AIDS-related complications such as pneumonia caused by *Pneumocystis carinii*. For sulphonamides, as for the other examples, NAT catalyses formation of the nontoxic *N*-acetyl derivative while oxidation produces a reactive hydroxylamine. The hydroxylamine can be further oxidized to a nitroso compound leading to hypersensitivity, toxicity, and immune suppression. For sulphamethoxazole, hydroxylamine formation is catalysed primarily by CYP2C9 in the liver. The further oxidation to the nitroso compound can occur spontaneously or by CYP catalysis. The very high incidence of sulpha drug toxicity among AIDS patients is apparently due to the AIDS-induced depletion of $CD4^+$ white blood cells, leaving an excess of $CD8^+$ white cells which are very susceptible to the toxic effects of sulphamethoxazole hydroxylamine (Hess *et al.* 1999).

NAT in metabolism of carcinogens

In addition to detoxification of arylamine and arylhydrazine drugs, NAT is significant in the metabolism of carcinogenic arylamines which may be encountered in the environment. Here again there is competition between conjugation and oxidation pathways. As mentioned earlier, conjugation of arylamines by acetylation or other phase II enzymes will lead towards excretion while oxidation at the amino nitrogen will form a reactive intermediate which can be further activated by the *O*-acetylation capacity of NAT. Arylamine carcinogens can be detoxified not only by conjugation as the initial step in metabolism but also by oxidation at a ring carbon followed by glucuronide formation and excretion. It is oxidation at the amino nitrogen that truly commits the amine towards activation. Differences in the level of NAT activity can play a large part in determining the fate of arylamine carcinogens; high NAT activity will send more of the dose towards detoxification while low NAT activity will allow for more *N*-oxidation to occur. Acetylarylamines (arylacetamides) produced by NAT can also be oxidised to arylhydroxamic acids and then converted to acetoxyarylamines by AHAT, but this appears to be a less important pathway than OAT. It is also known that microsomal de-acetylases can transform the arylacetamide back to arylamine and allow the oxidative enzymes 'another chance' at activation. Genetic factors which are involved in determining the level of NAT activity will be discussed later in the section on Pharmacogenetics.

The high activity of NAT is not uniformly protective against carcinogenic arylamines because the OAT activity parallels NAT activity. Some amines which are poor substrates for *N*-acetylation become, after oxidation, good substrates for *O*-acetylation to more reactive forms. This is readily illustrated by the processing of several of the carcinogenic and mutagenic heterocyclic amines produced by cooking of protein-containing food. 2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ) is not a good substrate for *N*-acetylation by NAT, but following oxidation by CYP1A2 in the liver (or prostaglandin H synthase 2 extrahepatically; (Liu and Levy 1998)), the heterocyclic hydroxylamine is *O*-acetylated by NAT producing a more reactive acetoxyheterocyclic amine that forms adducts with DNA (Probst *et al.* 1992).

Substrates

Arylamine *N*-acetyltransferase carries out the acetylation of arylamines and arylhydrazines. The latter category includes isoniazid, hydralazine and phenelzine, while the former includes procainamide, sulphamethazine, aminoglutethimide, dapsone, proguanil, and *p*-aminobenzoic and *p*-aminosalicylic acids. Substrates for NAT also include compounds which are biotransformed to arylamines by other steps in their metabolism. In this group are acebutolol, nitrazepam, sulphasalazine and caffeine (Weber 1987).

In addition to therapeutic agents, NAT is a part of the metabolic pathways for environmental arylamines such as β-naphthylamine, benzidine, 4-aminobiphenyl and 2-aminofluorene. Nitro arenes which are reduced to arylamines must also be considered potential substrates for NAT. Heterocyclic arylamines, including those produced by pyrolysis during the cooking of food, may also be NAT substrates. The structure and

properties of the specific substrate determine how likely it is for *N*-acetylation to occur. For some compounds, such as the heterocyclic arylamines, *N*-acetylation is slight to non-existent, but *O*-acetylation of the hydroxylamine is significant.

In humans, NAT is encoded by two independently expressed loci termed *NAT1* and *NAT2*, which are responsible for the corresponding cytosolic enzymes NAT1 and NAT2. The reactions catalysed by the two NATs are identical, but the enzymes show somewhat different substrate selectivity. Multiple NATs had been postulated for many years (Jenne 1965), but the actual separation of the two human NATs was not achieved until 1989 (Grant *et al.* 1989). Substrate selectivity profiles of the NATs have been studied using partially purified liver cytosolic fractions (Grant *et al.* 1991) and with enzyme expressed in bacteria (Dupret and Grant 1992; Hein *et al.* 1993), in COS cells (Grant *et al.* 1991; Minchin *et al.* 1992) and in CHO cells (Ohsako and Deguchi 1990). Results of all systems are in general agreement indicating that for *N*-acetylation NAT1 is significantly more active than NAT2 with *p*-aminobenzoic acid and *p*-aminosalicylic acid, somewhat more active with 2-aminofluorene, 4-aminobiphenyl, *β*-naphthylamine, and 5-aminosalicylic acid (5-ASA), but less active with sulphamethazine. Both enzymes are nearly equally active with procainamide. *O*-acetylation activities of the expressed enzymes were examined with the hydroxylamines of 2-aminofluorene and 4-aminobiphenyl. The activities of the two enzymes were similar with NAT1 having slightly greater activity. For the few heterocyclic hydroxylamines tested, the activity observed was very low, but *N*-OH-IQ appeared somewhat selective for NAT1 (summarised from data compiled by Vatsis and Weber 1997).

While the involvement of NAT in the metabolism of drugs and other xenobiotics has been known for decades, the function of NAT in the transformation of endogenous compounds is not clear. Since NAT is found in a wide range of organisms, from bacteria to primates, and since it is rather highly conserved throughout evolution, the expectation is that NAT must have some essential role other than protecting organisms from man-made chemicals. A possible endogenous substrate for NAT was recently demonstrated to be a metabolic product of folate metabolism, *p*-aminobenzoylglutamate (*p*-ABG) (Minchin 1995). *p*-ABG is a selective substrate for human NAT1 and mouse NAT2. Circumstantial evidence including the role of folate in protecting the developing foetus from neural tube defects and the pre-natal expression of NAT, suggests that a relationship of NAT activity and neural tube defects might be worthy of investigation. In support of a role for NAT in foetal development, Stanley *et al.* (1998) using immunochemical detection found NAT in developing neuronal tissue of embryonic mice. The NAT protein was expressed intensely in the neural tube around the time of closure. NAT was also detected in the developing heart and gut. mRNA for NAT has been found in mouse embryos during gestation and in the early postnatal period (Mitchell *et al.* 1999). NAT activity and mRNA were measured in new-born and early postnatal mouse liver and kidney by Estrada *et al.* (2000).

Interestingly, some earlier work had attributed determination of teratogen-induced and spontaneous cleft palate in mice to a specific region of chromosome 8 which included the *Nat* genes (Liu and Erickson 1986; Karolyi *et al.* 1990). At the time, it was suggested that since mice with the slow acetylator allele (now called *Nat2*9*) were more susceptible to cleft palate than mice with the *Nat2*8* rapid allele, NAT might be a marker for the gene(s) responsible for cleft palate in mice (Karolyi *et al.*

1990). However, now that a role for NAT in folate metabolism has been observed (Minchin 1995), and the expression of NAT in the area of the closing neural tube in embryonic mice detected (Stanley *et al.* 1998), it is quite conceivable that NAT may function as more than just a marker for susceptibility to cleft palate in mice.

Tissue distribution

NAT activity is found in a wide range of human tissues. Although no systematic assay of enzyme distribution has been carried out in humans as has been done for mouse NAT (Chung *et al.* 1993), numerous individual tissues have been examined. Human NAT1 and NAT2 differ in their tissue distribution in that NAT2 is primarily hepatic and NAT1 is primarily extrahepatic. Even though both NATs have been partially purified from liver (Grant *et al.* 1989), NAT1 is the primary activity in mononuclear blood cells (Cribb *et al.* 1991), colon epithelium (Rodriguez *et al.* 1993) and throughout the intestine (Hickman *et al.* 1998), placenta (Smelt *et al.* 1997), bladder urothelium (Kloth *et al.* 1994) and mammary epithelium (Sadrieh *et al.* 1996). NAT2 activity is either low or absent in these extrahepatic tissues.

The different tissue distribution of the human NATs together with their different substrate selectivity suggest that not all NAT substrates will be acetylated by first pass metabolism. Substances which are good NAT2 substrates may be expected to be metabolised by hepatic NAT2, while NAT1 selective substrates may be partially metabolised by NAT1 activity in the liver, but also by NAT1 in the various tissues. Also one should not neglect NAT1 activity in colon as a possible site of pre-hepatic xenobiotic activation and/or detoxification.

Oxidative activation of arylamines by hepatic CYP1A2 and other enzymes produces hydroxylamines, some of which are stable enough to be transported through blood to the tissues. Additionally, the hydroxylamines can be conjugated such as to *N*-glucuronides which are later hydrolysed by the low pH in bladder or by bacterial glucuronidases in the gut to release the hydroxylamine. In the various target tissues, the hydroxylamines may be further activated by the *O*-acetylation activity of NAT1 to cause local toxicity. The relative activities of NAT1 and NAT2 as well as the relative activities of oxidative enzymes are important factors in determining the fate of NAT substrates. This is particularly true for those substrates, such as the carcinogenic arylamines, that are relatively good substrates for both NATs.

Species distribution

N-Acetyltransferases are widely distributed among animal species. Enzymes with homology to human NAT occur in mammals including monkeys (Goedde *et al.* 1967), baboons (Radtke *et al.* 1979), common rodents such as mice (Tannen and Weber 1979; Levy *et al.* 1992), rats (Tannen and Weber 1979), and hamsters (Hein *et al.* 1982), rabbits (Frymoyer and Jacox 1963), and livestock animals (Watkins and Klaassen 1986). NAT is also found in quail (Watkins and Klaassen 1986), pigeons (Andres *et al.* 1983) and chickens (Deguchi *et al.* 1988). Arylamine *N*-acetyltransferase activity has been found in trout (Watkins and Klaassen 1986), frogs (Ho *et al.* 1996), and nematodes (Chung *et al.* 1996). *N*-Acetyltransferases have been reported

in insects where the enzyme is important in sclerotisation of the insect cuticle (Karlson and Sekeris 1962), puparium formation (Sekeris 1964), and tanning of oviposited eggs (Li and Nappi 1992). In insects, NAT is the major route for metabolism of biogenic amines (Dewhurst *et al.* 1972). The enzyme from housefly has been purified and found to have a molecular weight of approximately 27 000 daltons, but terminal and partial internal sequence analysis did not show any similarity to the mammalian or avian NAT enzymes (Whittaker and Goosey 1993).

Bacteria have *N*- and *O*-acetylating activity. The best studied bacterial NAT is from *Salmonella typhimurium* which is used in the Ames assay for testing the mutagenicity of compounds. Many mutagenic and carcinogenic compounds activated by mammalian microsomes show increased mutagenicity in *Salmonella* strains overexpresssing NAT compared with those lacking NAT (Watanabe *et al.* 1987). The NAT gene coding for this enzyme has been cloned and the protein sequenced. The enzyme shows sequence homology to the mammalian NATs, has a similar molecular weight of about 33 000 daltons, and has a similar catalytic mechanism involving the conserved cysteine residue at position 69 (Watanabe *et al.* 1992). The *N*-acetylation substrate selectivity of the enzyme suggests it should be classified as an NAT2 and has been officially designated NAT2 10 (Vatsis *et al.* 1995).

Among mammals there are some notable exceptions to the seemingly universal distribution of NAT. While most species that have been studied have two or more isozymes of NAT, the domestic dog and other canids lack cytosolic NAT. This deficiency has been known for some time (Marshall 1954) and has more recently been shown to be caused by the absence of NAT genes (Trepanier *et al.* 1997). The only other mammal reported to lack cytosolic NAT activity is the shrew *Suncus murinus* (Nakura *et al.* 1994), for which an activity assay rather than molecular techniques were used to seek NAT.

Interestingly, although dogs lack cytosolic NAT, they are capable of acetylation, deacetylation, and transacylation of amines and their derivatives (Sone *et al.* 1991). The enzymes which catalyse these reactions include at least three microsomal activities. One of these enzymes which is responsible for most of the *N*,*O*-acyltransferase capacity has been purified to a mannose-rich glycoprotein of molecular weight 58 000 daltons. The enzyme was partially sequenced and the *N*-terminal area showed strong homology to amidase/carboxylesterase of rabbit, hamster, and rat. It was concluded that the metabolism of arylamines in dog is attributable to microsomal carboxylesterases rather than to intestinal flora (Sone *et al.* 1994).

The domestic cat and wild felids have cytosolic NAT, but only a single isoform, corresponding to NAT1, has been found. Although the activity of feline NAT for *p*-aminobenzoic acid is lower than in other species, it is present as an enzyme with sequence homology to mammalian NAT1. No NAT2 activity with the NAT2 selective substrate sulphamethazine could be demonstrated in cat hepatic cytosol (Trepanier *et al.* 1998). The lack of NAT2 activity agrees with earlier studies which could not detect *N*-acetylation of isoniazid or sulphamethazine by cat liver cytosol. Use of molecular techniques showed the presence of only the *NAT1* gene in felids and the absence of any other sequences with homology to NAT (Trepanier *et al.* 1998).

The consequences of lacking cytosolic NAT activity in canids (and shrews) and the lack of NAT2 activity in felids are not apparent. Although both dogs and cats may

develop signs of toxicity when given sulphonamides, and the toxicities are similar to those seen more commonly in humans deficient in NAT2 activity, it is not possible to relate the idiosyncratic response of the animals to their lack of NAT activity (Trepanier *et al.* 1998).

Regulation of NAT activity and expression

There are large gaps in our knowledge of the regulation of NAT expression in humans. Most of the studies which investigated variation in NAT activity occurred before the age of molecular biology, heterologous expression systems, and the appreciation of transcription elements. These early studies, for the most part, examined *in vivo* differences in drug acetylation (most often isoniazid) by administering the drug and measuring metabolites in blood or urine. The method does not give direct information about NAT activity as several possible influences on the drug, in addition to NAT expression, will affect these ratios. Absorption, distribution, metabolism and elimination will all have significant effects on the ratio of acetylated to non-acetylated drug measured. Even within the process of metabolism, we have seen that there is competition for the substrate, and *N*-acetylation rates may be altered by changes in, for example, cytochrome P450 activities. Another difficulty with the whole-body approach to determining the extent of NAT expression is that the liver is the primary site of *in vivo* drug acetylation and, therefore, liver mass has a great influence on observed acetylation. The many studies of NAT activity and age in children through the elderly, once corrected for changes in liver mass, do not convincingly demonstrate an age-related effect beyond the first year of life (Weber 1987).

Malfunction of specific organs can give the appearance of a change in NAT activity when drug/metabolite ratios are measured in blood or urine. In severe renal disease, the acetylated metabolite or the parent drug may be relatively retained resulting in an altered ratio in urine leading to the incorrect conclusion of altered NAT activity. For patients with acute or chronic liver disease, the acetylation of drugs may appear decreased. This effect is due to having fewer active hepatocytes in the affected liver and not to any intrinsic change in NAT activity.

During absorption, there are also a number of problems with the *in vivo* approach. As an example, absorption of isoniazid is reduced by high carbohydrate meals (Mannisto *et al.* 1982), antacids (Hurwitz and Schlozman 1974) and laxatives. In contrast, insulin increases isoniazid uptake (Danyz and Wisniewski 1970).

Various drugs may appear to alter the rate and extent of NAT reactions. Obviously, administration of drugs which are NAT substrates will compete for the enzyme and reduce acetylation of the test drug. PAS, procainamide, PABA, chlorpromazine, and phenylramidol will all increase the concentration of co-administered isoniazid and prolong its half-life (Weber and Hein 1979). Ethanol has been observed to increase the acetylation rate and decrease the biological half-life of many NAT substrates including isoniazid (Lester 1964), sulphamethazine (Olsen and Morland, 1978) and procainamide (Olsen and Morland 1982). The exact mechanism for the ethanol effect is not proven, but experiments indicate that ethanol increases the acetyl CoA level in liver and drives the NAT reaction in the direction of acetylation (Olsen and Morland 1982).

Inhibitors of NAT include compounds which bind the essential sulphydryl group of cysteine 68: metal ions, *p*-chloromercuribenzoate, *N*-ethylmaleimide, phenylglyoxal, diethylpyrocarbonate among others (Andres *et al.* 1988). As mentioned previously, other NAT substrates are competitive inhibitors. Folate and the folate mimic, methotrexate, are inhibitors of NAT (Andres *et al.* 1983). *N*-Acetyltransferases are also inhibited by CoA-SH, which can be a problem in *in vitro* assays of NAT activity if the assay does not include an AcCoA recycling system (Andres *et al.* 1985). Two other inhibitors of NAT are pentachlorophenol and paracetamol (acetaminophen). Pentachlorophenol has been used to inhibit sulphotransferases in metabolic investigations. However, the compound is also a good inhibitor of NAT and thus can not be used to implicate sulphotransferase in a metabolic pathway as acetyltransferases will also be inhibited by this phenolic compound (Shinohara *et al.* 1986). Paracetamol is not a substrate for NAT but is a product analogue which shows inhibition of NAT *in vivo* and *in vitro*. The *in vivo* inhibition can be a problem in patients using paracetamol, as NAT inhibition by the achievable serum concentration of the drug can give the appearance of decreased acetylation activity and lead to higher and more persistent levels of NAT substrates such as sulpha drugs which may cause toxic reactions (Rothen *et al.* 1998).

Pharmacogenetics of NATs

Throughout this chapter we have referred to various effects caused by differences in NAT activity. The most common cause of altered NAT activity is the genetic polymorphism of the NAT enzymes. Discovery of the polymorphism occurred with the introduction of isoniazid. The definition of rapid and slow acetylators as well as phenotyping assays to distinguish them developed subsequent to clinical observation of interindividual variations in response to the drug (reviewed in Weber 1987). Discovery of the acetylation polymorphism was followed by many genetic studies of the mode of inheritance of NAT activity and the frequency of rapid or slow acetylation in different populations. Associations between acetylator phenotype and various disease states were investigated and several were found (Weber and Hein 1985; Weber 1987). Among the most important associations observed was that between slow acetylation and occupational bladder cancer (Cartwright *et al.* 1982). There followed much work examining the distribution of the different acetylator phenotypes in patients with various cancers as compared to healthy controls. An association was found between rapid acetylators and increased likelihood of colorectal cancer (Lang *et al.* 1986; Ilett *et al.* 1987). Other cancers were found to be more or less common in rapid or slow acetylators in some studies, but often other studies could not confirm these findings. The importance of NAT activity as a risk factor for cancers is still under active investigation.

Two events took place that dramatically changed NAT research. The first was the separation and characterisation of the second NAT isozyme (ironically called NAT1) (Grant *et al.* 1989, 1991). The second event was the blossoming of molecular biology and molecular genetics. For nearly four decades after the discovery of the isoniazid-sulphamethazine polymorphism only NAT2 was known. After the discovery of NAT1, the isozymes were often referred to as polymorphic (NAT2) and monomorphic (NAT1)

NATs. We know now that both forms are polymorphic when the proper substrates are tested, and that both enzymes are coded for by multi-allelic loci, illustrating investigators' proclivity to find what is being sought.

The human *NAT* genes are located on chromosome 8 in the region 8p22 as is a pseudogene, *NATP* (Matas *et al.* 1997). The order of the loci are (from telomeric end towards the centromere) *NAT1, NATP, NAT2* with the loci within 1000 kb of each other (Thygesen *et al.* 1999). The *NAT1* and *NAT2* loci have intronless open reading frames of 870 bases coding for proteins of molecular mass of about 33 500 daltons. More than 20 allelic variants of *NAT1* have been identified, distinguished from each other and from the 'wild type' *NAT1*4* by mutations in the coding and/or 3′-UTR (Vatsis *et al.* 1995). While many of the substitutions in the coding region cause amino acid changes in the NAT1 protein or introduce stop signals, others are silent but may influence the rate of transcription or translation or affect the stability of mRNA. Several of the mutations in the 3′-UTR are hypothesized to alter the function or efficiency of the polyadenylation signal and alter the mRNA stability (Vatsis and Weber 1993; deLeón *et al.* 2000).

Human *NAT2* also has in excess of 20 alleles differing from the 'wild type' *NAT2*4*. The known mutations of *NAT2* are in the coding region and include silent mutations as well as those which cause amino acid substitutions. Currently much more information is available on the distribution and frequencies of *NAT2* alleles in different populations and about the phenotype-genotype correlations than is available for *NAT1*. Both *in vivo* and *in vitro* studies as well as several heterologous expression experiments have shown that almost all the naturally occurring mutations in *NAT2*4* lead to enzymes with reduced acetylation activity. Because of this, individuals with 2 mutated *NAT2* genes are slow acetylators, those with one mutated allele are heterozygous rapid, and those with two wild type *NAT2*4* alleles are homozygous rapid acetylators. In many phenotyping assays, the homozygous and heterozygous rapid acetylators can be distinguished resulting in a trimodal distribution of the population. The quantitative aspects of the relative 'slowness' of slow alleles have not been well studied.

The frequency of the slow NAT2 alleles shows surprising variability in various populations. Japanese populations are about 90% rapid acetylators (Vatsis and Weber 1997) while the Moroccan population was found to be 90% slow acetylators (Karim *et al.* 1981). For mixed Caucasian populations the slow and rapid phenotypes are almost equally represented. The different mutations causing the slow phenotype also show ethnic differences, as illustrated in Table 11.1. In many populations, three or four

Table 11.1 Approximate frequency (in %) of *NAT2** alleles in various populations

Allele	Caucasian	Afro-American	Hispanic-American	Asian (Japan)
*4	25	34	42	70
*5B	40	30	23	1
*6A	30	25	17	20
*14A, *14B		10		
*7A, *7B			17	7

mutations account for the vast majority of slow acetylators. Although other, much rarer slow alleles may occur, their frequencies are so small that, for most purposes, they can be ignored. A listing of reported *NAT1* and *NAT2* alleles is maintained at http://www.louisville.edu/medschool/pharmacology/NAT.html.

Our knowledge of *NAT1* allelic frequency and distribution is still sparse. The original report of structural heterogeneity of *NAT1* identified the wild type (*NAT1*4*) and two mutant alleles (*NAT1*10* and *NAT1*11*) (Vatsis and Weber 1993). This report also demonstrated Mendelian inheritance of the variants and independent expression of NAT1 and NAT2. In the small population examined, 42% of *NAT1* alleles of unrelated individuals were variants. Later reports found 28.5% of alleles from 280 subjects were variants (Hughes *et al.* 1998) and, in a large study, only a 5% mutation frequency was found in over 1800 subjects (Lin *et al.* 1998), but this study did not test for *NAT1*10*. A number of other studies of *NAT1* allelic frequency have been done, but several considered only three or four alleles. While this approach is acceptable in populations where the major alleles accounting for >95% of all alleles are known (as discussed in the previous paragraph), it is not reliable when examining new populations for a different gene. When examining an untested population it is a better strategy to genotype for as many alleles as practical. Dhaini and Levy (2000) found a combined frequency of 23.8% for the *NAT1*14A* and *14B* alleles and 6% for previously unreported alleles in a Lebanese population living in Dearborn, Michigan. This result is considerably higher than the previously reported *NAT1*14* frequencies of 1.3–3% in Caucasians of European origin (Lin *et al.* 1998; Butcher *et al.* 1998) and exemplifies that different ethnic groups may vary very greatly in *NAT1* allelic distribution. This result also demonstrates that had the authors not specifically tested for this allele, most of the *NAT1*14* individuals would have been incorrectly classified as *NAT1*4* or *NAT1*10*, giving greatly misleading information about the population.

The *NAT1*3* allele occurs with a high frequency in Asians as shown by a study of different ethnic groups living in Singapore. The population was genotyped for alleles *NAT1*3*, **4*, **10*, and **11*. Among the 122 Malay and 181 Chinese in the study, the distribution was 30–35% *NAT1*3*, 30–34% *NAT1*4*, and 30–39% *NAT1*10*. Among the 140 Indians, *NAT1*4* was increased to 51% and *NAT1*10* reduced to 17%. In all three ethnic groups *NAT1*11* occurred at a 2% frequency (Zhao *et al.* 1998).

The activity of the various *NAT1* alleles, that is, the phenotype-genotype correlation for NAT1, is still being discovered. Before the identification of NAT1 was achieved, it was noted that large variation in PAS and PABA acetylation occurred between individuals. An 80-fold variation in PAS acetylation in 131 human blood samples (Motulsky and Steinman 1962; Evans 1963), a 4-fold variation in PABA acetylation by seven human liver biopsy samples (Glowinski *et al.* 1978), a 90-fold variation in V_{max} values for PABA acetylation by 39 (Grant *et al.* 1991) and 8 (Cribb *et al.* 1993) liver samples, as well as several other reports, illustrated the variability in human NAT1 activity.

Further evidence for an NAT1 activity polymorphism has come from a study of the urinary N-Ac-PAS to PAS ratio of 130 subjects given PAS (Grant *et al.* 1992) and a measurement of whole blood lysate PABA acetylation activity from 200 subjects (Weber and Vatsis 1993). Both studies showed a significant variability in activity and a strong tendency towards a bimodal distribution. More recently, Butcher *et al.* (1998)

Table 11.2 Frequency of NAT1 alleles in 314 Germans

NAT1 Allele	%
*3	3.0
*4	70.9
*10	20.1
*11	3.3
*14	2.2
*15	0.5

Data from Bruhn *et al.* (1999)

reported that lysed white cells from 85 individuals showed a bimodal distribution of PABA-NAT activity with roughly 8% of the subjects being slow (NAT1) acetylators. Genotyping showed that the seven slow individuals had either $G^{560}A$ (*NAT1*14*) or $C^{190}T$ (*NAT1*17*) mutations together with an allele lacking these mutations. Both mutations resulted in a V_{max} approximately half that of the wild type.

Hughes *et al.* (1998) examined the ratio of N-Ac-PAS to PAS in urine and plasma of 144 subjects given PAS. A greater than 65-fold variation in the urinary ratio and a greater than 5.6-fold variation in the plasma ratio was found. *In vitro* determination of PAS acetylation (whole blood lysates), cloning, sequencing, and expression in *E. coli* were carried out for a subset of eight individuals. No significant differences were found between *NAT1*4* and *NAT1*11* in terms of activity, apparent K_m or V_{max}. However, *NAT1*14* ($G^{560}A$: $Arg^{187}Gln$) had a 15-fold higher apparent K_m and 4-fold lower V_{max} than *NAT1*4*. *NAT1*15* ($C^{559}T$: $Arg^{187}stop$) had no detectable activity and thus non-measureable K_m and V_{max} values. A single individual in the study with the *NAT1*14/*15* genotype had an extremely low N-Ac-PAS to PAS ratio, while subjects with *NAT1*4/*14*, **10/*14*, **4/*15*, and **10/*15* had urinary ratios not markedly different from the wild type or group means. This suggests that presence of a single allele with normal activity (*NAT1*4* or *NAT1*10*) can compensate for the low or absent activity of the *NAT1*14* or *NAT1*15* alleles, at least *in vivo*.

A group of 314 German volunteers were genotyped for *NAT1*3*, **4*, **10*, **11*, **14*, and **15* by Bruhn *et al.* (1999) and a subset of 105 were phenotyped *in vitro* (whole blood lysate) with PABA. Allelic frequencies are shown in Table 11.2. There was no functional difference between alleles *NAT1*3*, **4*, or **10*. Carriers of *NAT1*11*, an allele with mutations in the 5′ and 3′ regions as well as in the coding region ($Val^{149}Ile$, $Ser^{214}Ala$), had reduced enzyme activity, those with *NAT1*14* had a further reduction in activity, and an individual with the *NAT1*15/*15* genotype had no detectable PABA-NAT activity. These results demonstrate the variable effects of *NAT1** mutations on NAT1 activity: a range of activity is possible depending on the specific allele(s) present. However, none of the studies has yet dealt with the question of variability in gene expression and the factors which may influence this important determinant of activity.

Summary

N-Acetyltransferase activity, along with *O*-acetyltransferase and *N,O*-acyltransferase, are important determinants of the fate of numerous environmental and pharmaceutical

arylmines. Disposition of these aromatic nitrogen compounds has very significant effects on their interactions with organisms. The NATs influence activation and detoxification of arylamine carcinogens and the efficacy and toxicity of therapeutic agents. It is also possible that NAT is important in normal foetal development.

Any compound with an arylamine moiety, or that can be biotransformed to expose such moeity, must be considered a potential substrate for acetyl transferases. The very significant differences in human NAT activity resulting from genetic polymorphisms in both NAT1 and NAT2 can render substrates toxic in certain individuals while non-toxic in others. The combination of oxidative and acetyltransfer activities of an individual needs to be considered in determining the safety and efficacy of arylamine drugs, both in drug trials and in prescribing doses of approved pharmaceutical agents. Knowledge of the frequency and activity of the major NAT alleles in the ethnic group of the individual given these agents can help to guide the selection of a safe and effective dosage.

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