PHARMACOGENETICS OF THE ARYLAMINE N-ACETYLTRANSFERASES: A SYMPOSIUM IN HONOR OF WENDELL W. WEBER

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ABSTRACT:

This article is a report on a symposium sponsored by the American Society for Pharmacology and Experimental Therapeutics presented at the joint meeting of the American Society for Biochemistry and Molecular Biology and the American Society for Pharmacology and Experimental Therapeutics, June 4–8, Boston, Massachusetts. The presentations focused on the pharmacogenetics of the NAT1 and NAT2 arylamine N-acetyltransferases, including developmental regulation, structure-function relationships, and their possible role in susceptibility to breast, colon, and pancreatic cancers. The symposium honored Wendell W. Weber for over 35 years of leadership and scientific advancement in pharmacogenetics and was highlighted by his overview of the historical development of the field.

N-Acetyltransferase Pharmacogenetics: From the Beginning (Wendell W. Weber)

So much has happened lately in biology and in pharmacogenetics that it is easy to lose sight of the earlier accomplishments that brought us to the present. The acetylator trait was one of the first human hereditary traits affecting human drug response to be identified, and the observations that led to its discovery contain some of the elements of a good medical detective story. Discovery of this trait followed directly on the heels of the introduction of isoniazid as an antituberculosis drug to clinical practice nearly five decades ago. At first, the identity of the drug was shrouded in secrecy, but in February 1952, hospitalized patients, hopelessly ill with tuberculosis, began to experience dramatic improvements from the drug. News of these events were recounted almost daily in the press (Kaempffert, 1952). Within a week of starting isoniazid, treated patients regained weight, strength, and appetite, and their fever disappeared. After a few weeks of treatment, tubercle bacilli could no longer be found in their saliva and within 8 months, favorable X-ray changes were seen in more than half the patients. But despite its remarkable therapeutic effectiveness, a high proportion (3.5–17%) of patients convalescing on the drug, complained of numbness and tingling in the fingers and toes, as well as other signs of progressive damage to the nervous system. As the occurrence of these devastating side effects threatened to stop the use of isoniazid, efforts to find the cause intensified, leading to discovery of this human metabolic trait (reviewed in Weber, 1987; Vatsis and Weber, 1997).

Hughes and colleagues (1954) solved the puzzle by deducing from animal studies that certain other drugs, which resembled isoniazid chemically, such as sulfonamides, underwent acetylation before the body could excrete them. They found that acetylsalicylic acid was the main urinary metabolite of humans, and that persons typically fell into either low or high excretor groups, depending on their capacity to acetylate the drug. Following this clue, they showed that patients who excreted the largest amount of unchanged isoniazid and the least amount of acetylisoniazid were most likely to suffer neurological damage from the drug. Further studies of twins and families revealed blood concentrations of the drug distributed into two (or three) genetically determined subgroups (Price Evans et al., 1960). This led to the proposal that persons with low blood levels be classified as “rapid” inactivators and those with high blood levels as “slow” inactivators of isoniazid. After biochemical studies, the genetic variability (polymorphism) in drug levels was attributed to differences in N-acetyltransferase (NAT) activity, and the term “inactivators” was supplanted by “acetylators” (reviewed in Vatsis and Weber, 1994).

Discovery of acetylation polymorphism was followed by many genetic studies of the mode of inheritance of NAT activity and the frequency of slow acetylation in different ethno-geographic populations, which now has involved more than 10,000 people. In the present...
article, I want to show how knowledge of the acetylator trait advanced from the pioneering steps taken by a few investigators in the 1950s to a much better understanding of the trait through works of many others using modern strategies of human genetics and molecular epidemiology.

In 1962, I read a description of the acetylator trait in Werner Kalow’s (1962) new book on pharmacogenetics while I was an NIH fellow in human genetics at the Galton Laboratory, University College, London. Little was known of the cause of this trait at that time, and when the opportunity arose a year or so later at New York University Medical School in Pharmacology to try to find the cause of person-to-person differences in acetylation, I jumped at it.

The time was right for such studies. The identification of the DNA double helix, the visualization and enumeration of human chromosomes, the recognition of protein polymorphism as an important biological phenomenon, and the discovery of heritable patterns of drug response that had occurred in the 1950s encouraged pharmacologists to take a more genetic approach to their research. Then too, the genetic basis of two other pharmacogenetic traits—“primaquine sensitivity” and “succinylcholine sensitivity”—had been examined. Primaquine sensitivity had been shown to be a sex-linked trait due to G6PD deficiency while succinylcholine sensitivity was found to be an autosomal recessive trait due to an atypical form of serum cholinesterase. However, these traits were both expressed and could be studied in the peripheral blood. The isoniazid acetylation polymorphism, on the other hand, is expressed mainly in the liver and gut, and the inaccessibility of these tissues necessitated an appropriate animal model before we could begin a comprehensive study of the human condition.

Fortunately, Frymoyer and Jacox (1963a,b) reported that the variable capacity of rabbits to acetylate certain sulfonamides was genetically determined, and that “slow acetylation” was inherited in a manner like that for humans. Thus, using the rabbit model, we set out to answer the question: what causes slow acetylation? First, to be on solid enzymological grounds, we studied the enzymatic mechanism of drug acetylation. We found that this reaction proceeds according to a “ping-pong Bi-Bi” mechanism (Weber and Cohen, 1967, 1968). In this mechanism the enzyme swings back and forth between free and acetylated forms as it catalyzes the acetylation of drugs. In the course of this investigation, we also found we needed a stable source of rapid and slow acetylator rabbits and thus developed a colony of rapid and slow acetylator rabbits, which we kept for more than 15 years for use in our studies.

During the 1970s, we continued to characterize the rabbit acetylator trait and initiated studies on human acetylator polymorphism. We also began to explore other animal models for hereditary acetylation polymorphisms, including inbred strains of mice, hamsters, and rats (reviewed in Weber and Hein, 1985; Weber, 1987). During the next two decades, we gathered a great deal of fascinating information about the metabolic, genetic, toxicological, developmental, and molecular genetic aspects of these traits. In mice for example, using C57BL/6J (B6) mice as representative of rapid acetylation and A/J (A) mice as representing slow acetylation, we showed that the NAT polymorphism in liver also occurs in kidney, urinary bladder, blood, and other tissues. We also developed two congenic acetylator mouse lines derived from B6 and A mice, one on the B6 genetic background and the other on the A background (Mattano et al., 1988) A little later, the hamster was also used to develop congenic hamster lines (Hein, 1991). These congenic lines have been powerful aids to clarify the role of acetylation polymorphism in toxicity studies, particularly those involving carcinogenesis, and the mouse and hamster models are both in use today (reviewed in Levy et al., 1992; Hein et al., 1997).

As we moved through the 1980s, more than a hundred human traits of pharmacogenetic interest were discovered and characterized, but the field was shaped mainly by the study of drug-metabolizing enzyme polymorphisms like the acetylation polymorphism. The main purpose of those studies was to determine whether susceptibility of people to drugs, foods, and other exogenous chemicals, was altered by a particular polymorphism, one gene at a time. Associations between acetylator phenotype and various drug-induced disorders that were sought, revealed new insights into the causes of isoniazid hepatitis, drug-induced lupus erythematosus, sulfasalazine side effects, and toxicity from sulfonamides (reviewed in Weber, 1987). Important associations were also observed between acetylator phenotypes and occupationally induced bladder cancer, and colon cancer induced by smoking and food mutagens that occur in cooked meats. The evidence that has accumulated suggests that NAT activity in combination with other genetically determined traits is a significant risk factor for certain cancerous disorders, but this is a complex problem still under active investigation (reviewed in Weber and Hein, 1985; Hein, 1988; Kadlubar et al., 1992; Vatsis and Weber, 1997; Hein et al., 2000a; Levy and Weber, 2000).

The emergence of recombinant DNA technology during the 1980s brought the genetic analysis of protein polymorphisms within reach of many investigators, and the pace of pharmacogenetic research at all levels has increased enormously within the last 10 years. In 1987, after we reported the sequences of hepatic NAT peptides of liver NAT from homozygous rapid acetylator rabbits (Andres et al., 1987), our understanding of the molecular basis of acetylation also advanced very rapidly. In quick succession, two functional human loci, NAT1 and NAT2 were identified and characterized for humans (Blum et al., 1990), and mapped to the short arm of human chromosome 8 (reviewed in Vatsis and Weber, 1994; Grant et al., 1997; Vatsis and Weber, 1997). The human isoniazid N-acetylation polymorphism was then attributed to variation at the NAT2 locus. A systematic survey of NAT1 genotypes in Caucasians showed NAT1 to be a polymorphic locus, but the role of NAT1 in susceptibility to unwanted effects of exogenous chemicals remains to be established (Vatsis and Weber, 1994). Currently, 25 human NAT1 and 27 human NAT2 alleles have been identified (Vatsis et al., 1995; Hein et al., 2000 a,b). Most molecular genetic studies of acetylation polymorphism in humans and other species have concentrated on defects within the coding region so there are large gaps regarding effects of development, nutritional state, and hormonal factors on NAT expression. Recently, some progress has been made on these topics (Estrada-Rodriguez et al., 1998b; Mitchell et al., 1999; Estrada et al., 2000) but limitation of space precludes their consideration in this article.

During the last 40 years, we have witnessed the transformation of pharmacogenetics from a cottage industry that involved a handful of academic investigators in the 1950s to a worldwide phenomenon that has attracted the attention of clinical scientists and the pharmaceutical industry. Improved patient care through customized therapy and discovery of new drugs are now within reach of these efforts. I often think, nowadays, how the discovery of human biotransformation of exogenous chemicals by pioneering physiological chemists, of the laws of heredity by Mendel, and of the theories of the existence of drug receptors postulated by Langley and Ehrlich, created the starting point some 100 to 150 years ago for understanding the peculiarities of human drug response (Weber, 1997). From those discoveries, Archibald Garrod predicted the role of the genetic material in the chemical individuality of humans, and suggested that substances in foods, drugs, and exhalations of animals or plants produce effects in some people wholly out of proportion to any that they bring about in average individuals—effects that might vary from slight or temporary
discomfort to morbid syndromes, which amount to severe and fatal illnesses (Garrod, 1931; Scrivener and Childs, 1989). As one of the first human hereditary traits affecting drug response to be discovered, the human acetylation polymorphism occupies a position of singular importance in the history of pharmacogenetics and in the future impact of the field on the practice of medicine. There is, I believe, no better example to teach us how a broad spectrum of individual responses to exogenous chemicals, including drugs, can arise from a single, genetically determined, metabolic theme, and to demonstrate how a better understanding of such traits can guide us in devising strategies to prevent human illness of environmental origin.

Developmental Regulation of the Arylamine N-Acetyltransferases (Charlene A. McQueen)

Prenatal exposure to xenobiotics is modulated by maternal absorption, distribution, biotransformation, and excretion. The genotypes of maternal biotransformation enzymes and environmental factors will affect the chemical nature and concentration of xenobiotics reaching the placenta where additional enzymatic reactions may occur. Biotransformation enzymes expressed by the fetus can result in further activation or detoxification of xenobiotics. The capacity to acetylate aromatic amines has been associated with the likelihood of toxicity in adults. Although it is reasonable to assume that a similar relationship exists at earlier stages of life, the contribution of fetal N-acetyltransferases to the developmental toxicity of aromatic amines is less clear. During the prenatal and neonatal periods, both cigarette smoke and breast milk may serve as sources of aromatic amines. For example, babies born to mothers who smoke have higher levels of 4-aminobiphenyl (4-ABP)-hemoglobin adducts than offspring of nonsmoking mothers (Coghlin et al., 1991; Pinorini-Godly and Myers, 1996). Exposure of neonatal mice to 4-ABP one day after birth was sufficient to induce liver carcinomas at 12 months (Dooley et al., 1992), and in utero exposure of Balb/c mice to 4-ABP at gestational day (GD) 18 resulted in the formation of fetal 4-ABP DNA adducts (Lu et al., 1986). Further investigation of the formation of 4-ABP-DNA adducts was performed in C57BL/6 mice utilizing an adduct-specific antibody (Al-Atrash et al., 1995). 4-ABP-DNA adducts were present in maternal and fetal tissue from C57BL/6 mice at GD 15 and 18, 24 h after a single oral dose of 120 mg of 4-ABP/kg (McQueen et al., 2000). No interintrauterine variation was noted at either GD. Comparison of the relative fluorescent intensities of the antibody used to detect the 4-ABP-DNA adducts revealed no differences between GD 15 and 18 in either maternal or fetal tissue. Significantly higher (P > .05) average fluorescence was seen in maternal liver compared with fetal sections.

For hemoglobin or DNA adducts to be formed, 4-ABP must undergo biotransformation to genotoxic products. Detection of 4-ABP-hemoglobin and DNA adducts in fetal tissue indicates that reactive products are formed but not whether this biotransformation is maternal, placental, or fetal in origin. In adult liver, N-acetyltransferases are involved in the biotransformation of aromatic amines. N-Hydroxylation followed by NAT-catalyzed O-acetylation is considered a major route of activation of 4-ABP while N-acetylation of the parent amine is thought to be a detoxification step. Investigation of the expression of NAT1 and NAT2 in C57BL/6 mice showed that these genes were expressed before birth (Mitchell et al., 1999). Reverse transcriptase-polymerase chain reaction was used to detect NAT1 and NAT2 mRNAs at GD 10, 15 and 18. At GD 10, the middle of the second trimester, both genes were expressed in the conceptal/placental complex. At GD 15, the middle of the third trimester, and at GD 18, just before birth, placental expression of both genes was confirmed. The GD 15 fetus and GD 18 extrahepatic fetal tissue had measurable NAT1 and NAT2 mRNAs. However, only NAT2 was expressed in liver at GD 18 (Table 1). The lack of expression of NAT1 continued until neonatal day (ND) 3, the latest time analyzed. Recently, it has been shown that NAT2 mRNA was present in embryonic stem cells (Payton et al., 1999). The NAT2 protein has also been detected in CD1 mice by immunochemical analyses at GD 9.5, 11.5, and 13.5 (Stanley et al., 1998). These studies demonstrate that NAT genes are transcribed and translated in preimplantation embryonic stem cells as well as during the second and third trimesters of pregnancy. Fetal and placental N-acetyltransferase activity has been evaluated using selective and nonselective substrates. Acetylation of p-aminobenzoic acid (PABA) and sulfamethazine have been detected in human placenta while PABA NAT activity was found in fetal hepatic and extrahepatic tissue (Pacifici et al., 1986; Derewlany et al., 1994; Smelt et al., 1998). Since 4-ABP-DNA adducts were present in fetal tissue, there was particular interest in determining 4-ABP NAT activity. Fetal tissue from C57BL/6 mice at GD 10, 15, and 18 had detectable 4-ABP NAT activity (McQueen et al., 2000). This activity increased from GD 10 to GD 18 then remained constant through ND 4. Hepatic 4-ABP NAT activity was lower at ND 4 than in adult tissue. Increasing PABA NAT activity has been observed in CD1 mice in the first 25 days after birth (Estrada et al., 2000).

These studies clearly show that functional NATs are present before birth, suggesting that biotransformation of aromatic amines by the mother and the fetus can contribute to the potential fetal toxicity of aromatic amines. Additionally, the early and continued expression of murine NAT2 during gestation suggests the possibility of acetylation of endogenous substrates is required during embryonic development. The folate breakdown product, PABA glutamate (PABA-Glu) is a specific substrate for human NAT1 and murine NAT2 (Ward et al., 1998; Smelt et al., 1998). Since 4-ABP-DNA adducts were present in fetal tissue, there was particular interest in determining 4-ABP NAT activity. Fetal tissue from C57BL/6 mice at GD 10, 15, and 18 had detectable 4-ABP NAT activity (McQueen et al., 2000). This activity increased from GD 10 to GD 18 then remained constant through ND 4. Hepatic 4-ABP NAT activity was lower at ND 4 than in adult tissue. Increasing PABA NAT activity has been observed in CD1 mice in the first 25 days after birth (Estrada et al., 2000).

The overall goal of the studies described in this section is to determine the structural features of human NAT1 and NAT2 that imparts their distinct catalytic specificities for acceptor amine substrates. The human arylamine N-acetyltransferases (EC 2.3.1.5) catalyze a two-step substituted-enzyme ("ping-pong") kinetic mechanism
that involves the addition of an acetyl moiety to xenobiotics and foreign compounds bearing primary aromatic amino or hydrazino functional groups. Site-directed mutagenesis studies identified Cys<sup>68</sup> as the catalytic residue in human NAT2 that mediates the transfer of an acetyl group from the cofactor CoA to the acceptor substrate (Dupret and Grant, 1992), while studies in Salmonella typhimurium produced confirmatory results with the corresponding Cys<sup>69</sup> residue in this species (Watanabe et al., 1992). Despite the fact that there is 81% deduced amino acid sequence identity between the human NAT1 and NAT2 proteins (Blum et al., 1990), they show significant catalytic selectivity (Grant et al., 1991), so that NAT1-selective and NAT2-selective drugs such as p-aminosalicylic acid and sulfamethazine, respectively, can be effective experimental tools for the kinetic characterization of recombinant wild-type and mutant NAT proteins.

Using this approach, kinetic analyses of a panel of recombinant NAT1/NAT2 chimeric proteins identified linear amino acid segments that imparted NAT isozyme-selective kinetic characteristics (Dupret et al., 1994). Furthermore, a central region (amino acids 112–210) on each of the NAT proteins, which is distinct from the active site Cys<sup>68</sup> residue in the linear amino acid sequence, was shown to impart the respective NAT1-type or NAT2-catalytic specificity to these proteins (Dupret et al., 1994). Within this central region, a highly conserved 42-amino acid segment (amino acids 107–148) differs at only three amino acid positions (125, 127, and 129) between the two proteins. Further chimerization strategies were used to exchange all three of these amino acids between NAT1 and NAT2 to create a NAT1 protein with the NAT2 Ser residues at each of these three positions, and a corresponding NAT2 protein that contained the NAT1 Phe<sup>125</sup>, Arg<sup>127</sup>, and Tyr<sup>129</sup> residues. Kinetic analyses revealed that these three amino acids are indeed important determinants of NAT acceptor substrate selectivity (Goodfellow et al., 2000). S. typhimurium N-acetyltransferase, a homolog of human NAT1, was recently crystallized (Sinclair et al., 2000) and has provided compelling evidence that residues 125, 127, and 129 in human NAT1 are located proximal to the residues that form the active site catalytic triad.

To gain a fuller understanding of the individual contributions that these three amino acid residues make to the determination of NAT acceptor substrate specificity, we exchanged these amino acids individually and in pairs between NAT1 and NAT2 and kinetically characterized the expressed protein products with our selective probe drugs PAS and SMZ. NAT1-Phe<sup>125</sup>Ser exhibited only a 4-fold increase in <i>K<sub>m</sub></i> (PAS), <i>K<sub>m</sub></i> (SMZ) yet displayed a greater than 200-fold increase in <i>K<sub>m</sub></i> (PAS),15 SMZ. In fact, this mutant protein had a <i>K<sub>m</sub></i> (PAS) value of 20 μM that was 5-fold smaller than even that of wild-type NAT2. On the other hand, all mutant NAT1 proteins containing an Arg<sup>127</sup>Ser substitution displayed significant increases in their <i>K<sub>m</sub></i> (PAS) values. These results led us to propose the following questions: 1) what roles do the hydroxyl functional group and/or size of the amino acid side chain at position 125 play in the marked increase in SMZ affinity of NAT1-Phe<sup>125</sup>Ser? and 2) is a positive charge at amino acid position 127 a requirement for NAT1-type kinetic behavior?

We mutated the wild-type NAT1 Phe<sup>125</sup> residue to Ala, Thr, Val, and Tyr to create a set of mutant NAT1-Phe<sup>125</sup> proteins that addressed our queries about the effect of size and hydrogen bonding capabilities of residue 125 on SMZ affinity. Kinetic characterization of these recombinant mutant NAT-Phe<sup>125</sup> proteins with a panel of substrates that differed in size have revealed some very interesting results (manuscript in preparation). All of the mutant NAT1-Phe<sup>125</sup> proteins had <i>K<sub>m</sub></i> (PAS) and <i>K<sub>m</sub></i> (PABA) values comparable to wild-type NAT1 suggesting that the mutations had minimal effect on NAT1-type substrate selectivity. However, plots of the ratio of PAS and SMZ specificity constants for the wild-type and mutant NAT1-Phe<sup>125</sup> proteins versus amino acid side chain volume at position 125 displayed some unique kinetic characteristics (data not shown). For the mutants with the lowest molecular volumes (Phe<sup>125</sup>Ala and Phe<sup>125</sup>Ser) the <i>k<sub>cat</sub>/K<sub>m</sub></i> (PAS)/<i>k<sub>cat</sub>/K<sub>m</sub></i> (SMZ) ratio is close to unity and indicative of a protein with equal kinetic selectivity (Table 2). On the other hand, the NAT1 proteins with the largest amino acid side chain volumes (Phe<sup>125</sup>Tyr and wild-type NAT1) exhibited the greatest PAS:SMZ specificity constant ratios (Table 2). This suggests that the molecular volume of residue 125 in NAT1 plays a significant role in the determination of kinetic selectivity.

The proposed role of the positively charged Arg<sup>127</sup> residue was assessed by mutagenesis to negatively charged Asp and Glu residues and their amine derivatives Asn and Gln, respectively; to the long hydrophobic unbranched side chain of Met; and to Lys, producing the only other NAT1 protein in this study with a positive charge at amino acid position 127. Kinetic parameters of these mutant NAT1-Arg<sup>127</sup> proteins were determined for a panel of amine derivatives that differed in the chemical nature of the substituent para to the free amino group. Only the NAT1 proteins with a positive charge at position 127 (wild-type NAT1 and NAT1-Arg<sup>127</sup>Lys) exhibited high affinities for substrates with negatively charged para substituents such as PAS and PABA (Fig. 1). Once the positive charge on the amino acid is removed by mutagenesis, the NAT1 proteins no longer have high affinity for either PAS or PABA. This relationship was not observed for those substrates that did not have a negatively charged para substituent (data not shown).

Overall, these studies allow us to suggest that the region surrounding amino acids 125 and 127 in NAT proteins is involved in determining substrate selectivity. It appears that the size of the amino acid side chain at position 125 is a critical determinant of substrate affinity and that the positive charge of Arg<sup>127</sup> imparts NAT1-type substrate selectivity. It will be interesting to relate these functional results with structural features of the human NAT proteins now that detailed structural information is available for the homologous S. typhimurium N-acetyltransferase.

Identification of structural determinants that influence N-acetyltransferase acceptor amine specificity should also provide insight into the affinity these proteins display for the arylamine procarcinogen class of compounds. Clarification of these factors will greatly enhance our fundamental understanding of the putative roles of N-acetyltransferases in chemical carcinogenesis.

### Role of N-Acetyltransferases in Pancreatic and Colon Cancer: Evidence that the NAT1*10 Allele Is Associated with Rapid Phenotype in Vivo (Fred F. Kadlubar)

With our understanding of the structure-function aspects of the N-acetyltransferases, we now focus attention on their role in human disease. The N-acetyltransferases, NAT1 and NAT2, have been implicated in the metabolic activation or detoxification of aromatic and
Bacterial lysates containing recombinant NAT proteins were assayed for NAT enzymatic activity. Results shown are from at least three independent experiments.

heterocyclic amine carcinogens, depending on the metabolic pathway that is functionally relevant to the target tissue. While NAT2 status can be clearly assigned by genotype (reviewed in Hein, 2000) and there is excellent genotype-phenotype correlation (Gross et al., 1999), the NAT1 alleles have yielded mixed results. Some studies have reported that the common NAT1*10 variant is a rapid phenotype (Badawi et al., 1995; Yang et al., 2000) while others have not (Butcher et al., 1998; Hughes et al., 1998). To clarify this issue, we genotyped 547 normal individuals for both NAT1 and NAT2 and conducted caffeine phenotyping as described by Lang et al. (1994). Probit plots of the urinary metabolites 5-acetylaminono-6-formylmleino-3-methyluracil/1-methylxanthine (AFMU/1X) are normally used to separate rapid (homozygous/heterozygous) NAT2 from slow NAT2 individuals using a cut point of 0.6, with the remaining activity being attributed to NAT1 (Cribb et al., 1994). To clarify this issue, we genotyped 547 normal individuals for both NAT1 and NAT2 and conducted caffeine phenotyping as described by Lang et al. (1994). Probit plots of the urinary metabolites 5-acetylaminono-6-formylmleino-3-methyluracil/1-methylxanthine (AFMU/1X) are normally used to separate rapid (homozygous/heterozygous) NAT2 from slow NAT2 individuals using a cut point of 0.6, with the remaining activity being attributed to NAT1 (Cribb et al., 1994). In collaboration with Robert Delongchamp, we used this attribute to plot probits of AFMU/1X according NAT1*4 (normal variant) and NAT1*10 (putative rapid variant) homozygous and heterozygous genotypes. As shown in Fig. 2, the NAT1*10 allele shifts the values toward higher activity, clearly indicating that it is a rapid phenotype in vivo. Considering that the highest reported activity is the colon (Badawi et al., 1995), this may likely constitute the source of this phenotype, although circulating lymphocytes (Yang et al., 2000) may contribute significantly.

Aromatic amines are widespread in the environment and are major constituents of cigarette smoke. Some heterocyclic amines are also found in cigarette smoke but they are generally the major mutagenic components of high temperature cooked meat. In the pancreas, we have found that the major smoking-related DNA adduct derived from the aromatic amine, 4-aminobiphenyl (dG-C8-ABP) was strongly decreased by the presence of a rapid NAT1*10 allele. Likewise, in collaboration with Myron Gross and Kristin Anderson at the University of Minnesota and Nicholas Lang at the Central Arkansas Veterans Health Care System, we conducted a case-control study (228 cases, 547 controls) of pancreatic cancer; smoking was a significant risk factor in all subgroups (NAT1, NAT2), except in those individuals possessing an NAT1*10 allele.

In collaboration with Drs. Elena Martinez and David Alberts at the Arizona Cancer Center, we participated in a colon polyp intervention trial involving 1429 subjects. Questionnaire information was used to assess potential exposure to heterocyclic amines and individuals were genotyped for all the common NAT2 alleles. Only those in the highest tertile of red meat consumption who were rapid acetylators showed a significant increased risk and the odds ratios indicated NAT2*4 gene dose dependence. These data suggest that a one-third reduction in red meat consumption is in itself a sufficient preventive measure for colon polyp recurrence and thus should appreciably lower colon cancer risk.

The Role of Arylamine N-Acetyltransferases in Genetic Predisposition To Breast Cancer (David W. Hein)

Humans are exposed to aromatic and heterocyclic amine carcinogens through cigarette smoke (Manabe et al., 1991; Peluso et al., 1991) and consumption of well done meat (Felton et al., 1986; Layton et al., 1995). Cigarette smoking and consumption of well done meat have been shown to be risk factors for breast cancer in some human epidemiological studies, but the results have been very inconsistent (Hein et al., 2000a). Aromatic and heterocyclic amines require host-mediated metabolic activation before initiating DNA mutations (Hein, 1988), and these carcinogens have been shown to induce mammary tumors in rats (Ito et al., 1991). Two N-acetyltransferase isozymes (NAT1 and NAT2) catalyze the activation of aromatic and heterocyclic amines (via O-acetylation) to DNA binding electrophiles and both are subject to a genetic polymorphism (Hein et al., 2000a). We hypothesized that women possessing NAT1 and/or NAT2 genotypes associated with high (rapid) acetylator phenotypes should be at increased risk of breast cancer following exposure to cigarette smoke and/or consumption of well done meat.
TABLE 4

Association of breast cancer with selected NAT1 genotypes, cigarette smoking, and well done meat intake among postmenopausal women

Adapted from Zheng et al., 1999. Odds ratios (OR) adjusted for age and family history of breast cancer.

<table>
<thead>
<tr>
<th>NAT1 *4/*4, *3/*4, or *3/*3</th>
<th>NAT1 *11/*11</th>
<th>Cases/Controls</th>
<th>OR (95% CI)</th>
<th>Cases/Controls</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>54/134</td>
<td>1.0 (reference)</td>
<td>5/6</td>
<td>2.1 (0.6–7.1)</td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>28/63</td>
<td>1.1 (0.7–2.0)</td>
<td>5/1</td>
<td>13.2 (1.5–116.0)</td>
<td></td>
</tr>
<tr>
<td>Red meat intake (by tertile)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>30/73</td>
<td>1.0 (reference)</td>
<td>2/2</td>
<td>2.7 (0.4–20.6)</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>30/63</td>
<td>1.2 (0.6–2.1)</td>
<td>4/3</td>
<td>3.4 (0.7–16.2)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>23/63</td>
<td>1.9 (0.5–1.7)</td>
<td>5/2</td>
<td>6.1 (1.1–33.2)</td>
<td></td>
</tr>
<tr>
<td>Meat doneness level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not consistently well done</td>
<td>54/136</td>
<td>1.0 (reference)</td>
<td>6/4</td>
<td>3.6 (1.0–13.4)</td>
<td></td>
</tr>
<tr>
<td>Consistently well done</td>
<td>23/40</td>
<td>1.4 (0.8–2.5)</td>
<td>2/1</td>
<td>5.6 (0.5–62.7)</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 5

Joint effect of well done meat intake and NAT2 genotype on breast cancer risk among postmenopausal women

Adapted from Deitz et al., 2000.

<table>
<thead>
<tr>
<th>NAT1 (Slow)</th>
<th>NAT1 (Rapid/Intermediate)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Cases/Controls</td>
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<tr>
<td>Meat doneness score</td>
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<td>3 and 4</td>
<td>24/71</td>
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<tr>
<td>5</td>
<td>20/51</td>
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<td>6</td>
<td>26/26</td>
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<td>7</td>
<td>5/33</td>
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<td>8</td>
<td>3/4</td>
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<tr>
<td>9</td>
<td>4/3</td>
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<tr>
<td>Trend test</td>
<td></td>
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<tr>
<td>Red meat intake</td>
<td></td>
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<tr>
<td>T1 (low)</td>
<td>27/64</td>
</tr>
<tr>
<td>T2</td>
<td>36/71</td>
</tr>
<tr>
<td>T3</td>
<td>29/76</td>
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<tr>
<td>Trend test</td>
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* Adjusted for age.

To test this hypothesis, we conducted a collaborative study with Wei Zheng, Thomas Sellers, Aaron Folsom, and other investigators then and/or now at the University of Minnesota Cancer Center. Subjects were from the Iowa Women’s Health Study, a prospective cohort of over 40,000 women, virtually all Caucasian.

NAT1 (Zheng et al., 1999) and NAT2 (Deitz et al., 2000) genotypes were determined using polymerase chain reaction-restriction fragment length polymorphism assays. For NAT1 genotype, NAT1*3 and NAT1*4 were classified as “wild-type” alleles, NAT1*10 and NAT1*11 were classified as potential high activity alleles, and NAT1*14, NAT1*15, NAT1*17, and NAT1*22 were classified as low activity alleles (Hein et al., 2000a). Individuals possessing two NAT2 alleles associated with high acetylation activity (NAT2*4, *12A, *12B, *12C, and *13) were classified as rapid acetylators; individuals with one of these alleles were classified as intermediate acetylators, and individuals possessing none of these alleles were identified as slow acetylators (Hein et al., 1995). Odds ratios (OR) were calculated to measure the strength of the association between exposures and cancer risk. Meat doneness levels were assigned 1, 2, or 3 for rare/medium, and/or high NAT1 activity, NAT1*10 was slightly more common and NAT1*11 was three times more common in cases than in controls (Table 3). To evaluate potential modifying effects of NAT1 genotype on the associations of breast cancer with cigarette smoking or consumption of well done meat, adjusted ORs were calculated according to the joint distribution of selected NAT1 genotypes and exposure levels of these two variables. Cigarette smoking was not related to breast cancer risk among the combined wild-type NAT1 genotypes. The NAT1*11 frequency was uncommon, but increased risk was observed for cigarette smoking among women possessing the NAT1*11 allele (Table 4). Dose-dependent increases in breast cancer risk were observed with red meat intake and meat doneness level. Although the risks were particularly elevated among women possessing the NAT1*11 allele (Table 4), the results should be interpreted with caution due to the limited number of subjects possessing this allele.

Twenty-seven NAT2 genotypes were determined and assigned into rapid, intermediate, and slow acetylator genotypes based on recombinant enzyme data (Hein et al., 1995). NAT2 genotype alone did not associate with breast cancer risk. However, a significant dose-response relationship was observed between breast cancer risk and consumption of well done meat among women with rapid/intermediate NAT2 genotype that was not evident among women with slow NAT2 genotype (Table 5). These results suggested an interaction between NAT2 genotype and meat doneness level and a test for multiplicative interaction was marginally significant \(P = .06\). Among women with rapid/intermediate NAT2 genotype, consumption of well done meat was associated with elevated breast cancer risk compared with women consuming rare or medium done meats (Table 5). These results suggest that postmenopausal women who possess rapid NAT2 and/or high NAT1 genotypes are more susceptible to
breast cancer following consumption of well done meat. The findings implicate a role for NAT1 and/or NAT2 in breast cancer etiology but need to be confirmed in studies with larger sample sizes and more diverse ethnic populations.

Concluding Remarks (Wendell W. Weber)

This symposium comprised five presentations. The first is an historical overview of acetylation pharmacogenetics and is followed by four additional segments in diverse aspects of the field of current and topical interest. Weber’s history, somewhat personalized, covers the period from the discovery of hereditary variation in drug acetylation some 50 years ago through its identification with two closely linked genetic loci that encode two structurally similar N-acetyltransferases, NAT1 and NAT2, up to the present time.

In the next segment, McQueen describes her studies of the acetylation enzymes in the mouse model from early prenatal days to birth. Very little is known about the drug-acetylation capacity during early periods of development, and these studies represent the first attempts to define the presence and function of NAT1 and NAT2 during embryonic and fetal life.

Once it was clear that humans (and several lab animal species) possessed both NAT1 and NAT2, it was logical to ask what structural differences between them might explain their substrate selectivity differences. Grant and Goodfellow review their efforts using site-directed mutagenesis and human NAT constructs that go far toward clarifying the answer to this question.

The symposium then moved away from structure-function relationships to consider the possible role of N-acetyltransferase variation in susceptibility to cancer in two epidemiological presentations. The topic of cancer induced by exposure to aromatic amine carcinogens, which occur in cigarette smoke, or in cooked meats, or as occupational or industrial pollutants, has been of longstanding interest in acetylation pharmacogenetics. Although the results of presentations by Kadlubar and Hein suggest that acetylation is a susceptibility factor for these cancers, the precise relationship between the acetylation polymorphism and susceptibility requires further study.

References


