LETTER TO THE EDITOR

Relationship between N-acetyltransferase 2 single-nucleotide polymorphisms and phenotype

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Dear Sir,

We read with interest a recent paper published by Agudo et al. (1) in Carcinogenesis. The investigators measured aromatic DNA adducts in white blood cells in 296 healthy adults from five regions of Spain. They analyzed functional polymorphisms in metabolic genes CYP1A1, CYP1A2, EPHX1, GSTM1, GSTT1, SULT1A1 and N-acetyltransferase 2 (NAT2) and explored potential associations between these functional polymorphisms and aromatic DNA adduct levels. As stated by the authors, the investigation is important because chemical carcinogens such as polycyclic aromatic hydrocarbons, arylamines and heterocyclic amines require metabolic activation to form metabolites capable of binding covalently to DNA in a process catalyzed by metabolism enzymes. We have some comments and questions regarding the relationships between aromatic DNA adduct levels and deduced NAT2 acetylator phenotype reported in the paper.

As is the case with many current studies, this study determined NAT2 single-nucleotide polymorphisms (SNPs) in genomic DNA in order to impute an NAT2 acetylator phenotype. Three SNPs were determined in the NAT2 coding exon: Ex2 + 487C>T (rs1799929; L161L), Ex2-580G>A (rs1799930; R197Q) and Ex2-313G>A (rs1799931; G286E). The authors utilized these SNP determinations to assign the NAT2 alleles/haplotypes NAT2*5A, NAT2*6A and NAT2*7A/B and referenced a previous review from our laboratory (2) as justification to do so. In actuality, our previous review specifically documented the potential problems encountered when assigning NAT2 alleles/haplotypes and deduced phenotypes solely from these three SNPs. Historically, these three SNPs were first identified and associated with alleles named M1, M2 and M3 (3, 5), and early studies limited their analyses to these three SNPs because other important SNPs in NAT2 had not yet been identified and characterized. In Table III of our previous review (2), we listed 18 different genotype/deduced phenotype classifications that might arise from NAT2 genotype/phenotype assignments based solely on determinations of these three SNPs. We followed this up with a more comprehensive analysis (4). Subsequently, many additional SNPs have been identified that potentially could lead to additional NAT2 genotype/phenotype misclassifications. An up to date listing of NAT2 SNPs and alleles/haplotypes is posted by the International N-acetyltransferase gene nomenclature committee at http://n-acetyltransferasenomenclature.llnl.gov.

In the Agudo et al. (1) study, aromatic DNA adduct levels were stratified by the three SNPs in NAT2. As shown in Table II of their study (1), only the NAT2 SNP that showed a significant effect on aromatic DNA adduct levels was Ex2-313G>A (rs 1799931; G286E). As shown in Table III of their study (1), only the NAT2 haplotype corresponding to 161L, 197Q and 286G significantly affected aromatic DNA adduct levels. The authors concluded that ‘regarding NAT2, when the three SNPs were analyzed together, only the allele ’7A/B was statistically significant’. They defined the allele A in NAT2*7A/B as ‘very slow’ acetylators. Furthermore, as shown in Table IV of their study (1), aromatic DNA adduct levels showed a significant decreasing trend with slower acetylation activity, stratified as (i) normal, (ii) slow, moderate and (iii) very slow. These three NAT2 phenotypes were defined in the text of the paper as follows: ‘Those with the NAT2 homozygous genotype CC for ’5A and GG for ’7A/B were considered as having normal NAT2 activity; moderately slow activity was assigned to subjects with allele T for ’5A keeping the wild-type CC for ’7A/B, and very slow acetylation was assigned to subjects with allele A for ’7A/B whatever the genotype for ’5A’. These definitions were slightly different in the footnote to Table IV.

We have several questions and comments regarding these methods and results. First, and most important, we feel that is it inappropriate to identify any NAT2 alleles or phenotypes as ‘normal’. This obviously implies that individuals who are slow acetylators (>50% of Caucasians and Africans for example) are ‘abnormal’. This is neither correct nor appropriate and this type of nomenclature must be strongly discouraged. Populations (actual frequencies dependent upon ethnic background) are classified into NAT2 acetylator phenotypes defined as rapid (not normal) or slow acetylators. As previously reviewed (6), many studies with appropriate phenotyping methods can distinguish among rapid, intermediate and slow acetylator phenotypes.

In addition, we do not understand the biological rationale for how the deduced NAT2 phenotypes were defined. The authors referenced a recent paper from our laboratory (7) as justification that individual SNPs may induce slow NAT2 acetylator phenotypes by different mechanisms and different degrees of modification of enzyme activity. Although our paper reports data suggesting heterogeneity within the slow acetylator phenotype, we feel it is important to point out that our findings showed that the Ex2-313G>A (rs 1799931; G286E) SNP conferred a lesser not a greater degree of slow acetylation activity when compared with other NAT2 SNPs particularly 341C>T (rs 1801280; I114T), the latter a common SNP that was not determined in the present paper. Thus, although our study (7) provided evidence for ‘very’ slow NAT2 phenotype, it did not suggest this was due to the Ex2-313G>A (rs 1799931; G286E) SNP. As recently reviewed (8), several laboratory-based (9,10) and population-based (11,12) studies suggest that 341C>T (rs 1801280; I114T), not Ex2-313G>A (rs 1799931; G286E), is associated with very slow NAT2 acetylator phenotype. Since the 341C>T SNP was not investigated in this study, we do not believe it is justified to further stratify within the NAT2 slow acetylator phenotype. Thus, although data are limited to three SNPs, we recommend that NAT2 phenotypes be deduced based on co-dominant expression of rapid and slow acetylator NAT2 alleles or haplotypes as described previously (6,8). Individuals homozygous for rapid NAT2 acetylator alleles are deduced as rapid acetylators, individuals homozygous for slow acetylator NAT2 alleles are deduced as slow acetylators and individuals possessing one rapid and one slow NAT2 allele are deduced as intermediate acetylators.

Finally, we do not understand how the study was able to associate human NAT2 haplotype with aromatic DNA adduct levels determined in genomic DNA from human DNA samples (Table II) since genomic DNA obtained from individuals has a NAT2 genotype (a combination of two haplotypes).

In summary, the authors have presented experimental data regarding a very important topic. Addressing the questions/comments herein may serve to improve the interpretation of the results obtained and clarify emerging subclassifications of deduced NAT2 slow acetylator phenotypes.

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