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N-acetyltransferase 2 genetic polymorphism: Effects of carcinogen and haplotype on urinary bladder cancer risk

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Abstract

A role for the *N*-acetyltransferase 2 (NAT2) genetic polymorphism in cancer risk has been the subject of numerous studies. Although comprehensive reviews of the NAT2 acetylation polymorphism have been published elsewhere, the objective of this paper is to briefly highlight some important features of the NAT2 acetylation polymorphism that are not universally accepted to better understand the role of NAT2 polymorphism in carcinogenic risk assessment. NAT2 slow acetylator phenotype(s) infer a consistent and robust increase in urinary bladder cancer risk following exposures to aromatic amine carcinogens. However, identification of specific carcinogens is important as the effect of NAT2 polymorphism on urinary bladder cancer differs dramatically between monoarylamines and aryldiamines. Misclassifications of carcinogen exposure and NAT2 genotype/phenotype confound evidence for a real biological effect. Functional understanding of the effects of NAT2 genetic polymorphisms on metabolism and genotoxicity, tissue-specific expression and the elucidation of the molecular mechanisms responsible are critical for interpretation of previous and future human molecular epidemiology investigations into the role of NAT2 polymorphism on cancer risk. Although associations have been reported for various cancers, this paper focuses on urinary bladder cancer, a cancer in which a role for *NAT2* polymorphism was first proposed and for which evidence is accumulating that the effect is biologically significant with important public health implications.

Keywords

N-acetyltransferase 2 (NAT2); urinary bladder cancer; aromatic amines; *NAT2* acetylator genotype; single nucleotide polymorphisms; *NAT2* haplotypes

Introduction

The *N*-acetyltransferase 2 (NAT2) acetylation polymorphism was discovered over fifty years ago when individual variability in isoniazid neurotoxicity was attributed to genetic variability in *N*-acetylation (Hughes *et al.*, 1954). The importance increased when it was discovered that many aromatic amine and hydrazine drugs are subject to the acetylation polymorphism thus affecting therapeutic efficacy and toxicity (Weber and Hein, 1985). It was soon apparent that many environmental and occupational aromatic amine carcinogens undergo catalysis by *N*-acetyltransferases (Hein, 1988). Thus, a role for *NAT2* acetylation polymorphism in individual risk to various cancers in which aromatic amines play an etiologic role is biologically plausible and has been the subject of numerous studies.

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N-acetyltransferase isozymes NAT1 and NAT2

Whereas the *N*-acetylation of isoniazid and sulfamethazine divided human populations by NAT2 acetylator phenotypes, the *N*-acetylation of drugs such as p-aminosalicylic acid yielded apparently unimodal distribution of individuals (Jenne, 1965). The biochemical basis relates to substrate specificity and molecular genetics of two distinct *N*-acetyltransferase isozymes, subsequently identified as *N*-acetyltransferase 1 (NAT1) and NAT2 (Vatsis *et al.*, 1995). The crystallographic structures of several prokaryotic *N*-acetyltransferases have been published (Sinclair *et al.*, 2000; Sandy *et al.*, 2002; Dupret and Rodrigues-Lima 2005). Although crystal structures of mammalian NAT1 and NAT2 have yet to be reported, molecular modeling of both human NAT1 (Rodrigues-Lima *et al.*, 2001) and NAT2 (Rodrigues-Lima *et al.*, 2002) have revealed a cysteine protease-like catalytic triad (Cys^{68}-His^{107}-Asp^{122}). The Cys^{68} residue is critical for transferring the acetyl moiety from acetyl coenzyme A cofactor to acceptor substrates (Dupret and Grant, 1992). Aromatic amines and hydrazines (*N*-acetylation), *N*-hydroxyaromatic and -heterocyclic amines (*O*-acetylation) and *N*-hydroxy-*N*-acetylaromatic amines (*N*,*O*-acetylation) are examples of acceptor substrates for both NAT1 and NAT2 (Hein, 1988). Although both human NAT1 and NAT2 catalyze these reactions, human NAT2 has a three to four-fold higher affinity than NAT1 for urinary bladder carcinogens such as 4-aminobiphenyl (ABP) and ß-naphthylamine (BNA) (Hein *et al.*, 1993b). This finding is consistent with the hypothesis that the effect of NAT2 polymorphism on urinary bladder cancer is more prevalent at low dose aromatic amine exposures (Vineis et al, 1994; 2004).

Animal models

NAT1 and *NAT2* in animal models such as rabbit, mouse, Syrian hamster, and rat are highly homologous to both human *NAT1* and *NAT2* (Hein *et al.*, 1997; Hein, 2002). Substrate specificities for Syrian hamster, mouse, and rat NAT2 may resemble human NAT1 more than they do human NAT2 (Weber and Hein, 1985). Several different mechanisms are responsible for *NAT2* polymorphisms in non-human species. The molecular basis for slow acetylator phenotype is *NAT2* gene deletion in the rabbit (Blum *et al.*, 1989), a nonsense single nucleotide polymorphism (SNP) yielding a truncated NAT2 enzyme in the Syrian hamster (Ferguson *et al.*, 1994; 1996; Nagata *et al.*, 1994), and missense SNP(s) in the mouse (Martell *et al.*, 1991) and rat (Doll and Hein, 1995). Both NAT1 and NAT2 have been identified and partially purified from Syrian hamster liver (Hein *et al.*, 1985; Smith *et al.*, 1986; Trinidad *et al.*, 1989; Ozawa *et al.*, 1990), intestine (Smith *et al.*, 1986), colon (Hein *et al.*, 1993a), prostate (Hein *et al.*, 2003), and urinary bladder (Yerokun *et al.*, 1989) cytosols. Expression of NAT1 and NAT2 isozymes also has been reported in rapid and slow acetylator mouse (Hein *et al.*, 1988) and rat (Hein *et al.*, 1991a) liver cytosols.

Phenotypic expression of the *NAT2* polymorphism

In a congenic Syrian hamster model in which all slow acetylators are homozygous for a single slow *NAT2* allele or haplotype and obligate heterozygotes all possess the same combination of rapid and slow *NAT2* allele or haplotype, the *NAT2* acetylation polymorphism clearly segregates the *N*-acetylation of aromatic amine urinary bladder carcinogens such as ABP and BNA into three phenotypes in hepatic and extrahepatic tissues (Figure 1). This trimodal distribution of rapid, intermediate and slow acetylator phenotypes in Syrian hamsters congenic at *NAT2* also is clearly evident *in vivo* (Figure 2). Although many human studies often exhibit bimodal distributions of rapid and slow acetylator NAT2 phenotypes, studies with hydrazine drugs such as isoniazid (Parkin *et al.*, 1997;Smith *et al.*, 1997), aromatic amine drugs such as sulfamethazine (Chapron *et al.*, 1980;Lee and Lee, 1982), and caffeine, a compound with a metabolite that is *N*-acetylated (Gross *et al.*, 1999;Cascorbi *et al.*, 1999;Grant *et al.*, 2004), yield rapid, intermediate, and slow acetylator phenotypes. The unequivocal detection of three

phenotypes can be confounded by various factors including catalysis by NAT1. Since isoniazid has high selectivity for catalysis via NAT2, rapid, intermediate, and slow acetylator phenotypes can be readily and unequivocally discerned as illustrated in Figure 3.

A widely held hypothesis is that human NAT2 is expressed primarily in liver and gastrointestinal tract whereas human NAT1 has widespread tissue distribution. This hypothesis derives from studies in the rabbit model where *N*-acetyltransferase activities reflected the *NAT2* genetic polymorphism in liver and gut, but not in other tissue cytosols suggesting either absence or a much smaller contribution of rabbit NAT2 in these other tissues (Hearse and Weber, 1973). Furthermore, a subsequent study reported that the rapid/slow NAT2 ratio for both sulfamethazine *N*-acetyltransferase and *N*-hydroxy-ABP *O*-acetyltransferase activities were much higher in rabbit liver than small and large intestine (Ilett *et al.*, 1991). Thus, these studies in rabbit suggested that *NAT2* genotype-dependent differences are expressed primarily in liver and to a lesser extent the gastrointestinal tract, and therefore suggest that *NAT2*-genotype dependent differences in carcinogenesis following exposures to carcinogens primarily reflect *NAT2* genotype-dependent hepatic versus extrahepatic metabolism of the carcinogen and/or its metabolites.

Studies had shown expression of both NAT1 and NAT2 in human colon (Turesky *et al.*, 1991; Kirlin *et al.*, 1991; Ilett *et al.*, 1994), intestine (Hickman *et al.*, 1998) and widespread tissue distribution of human NAT1 and NAT2 mRNA (Windmill *et al.*, 2000; Boukouvala and Sim, 2005). Although extrahepatic expression of *N*-acetyltransferase activities have been reported in humans (Pacifici *et al.*, 1986), rat (Hein *et al.*, 1991a), mouse (Chung *et al.*, 1993; Stanley *et al.*, 1997; Sugamori *et al.*, 2003), and Syrian hamster (Hein *et al.*, 1991b; 1994a) models, substrates were not selective for NAT1 and NAT2. NAT2-dependent ABP- and BNA *N*-acetyltransferase activities have been reported in human urinary bladder (Kirlin *et al.*, 1989; Frederickson *et al.*, 1992; 1994; Pink *et al.*, 1992; Badawi *et al.*, 1995). p-Aminobenzoic acid *N*-acetyltransferase (selective for NAT1) and *N*-hydroxy-ABP *O*-acetyltransferase activities (not selective for NAT1 or NAT2) in human urinary bladder cytosols did not correlate, consistent with catalysis by both NAT1 and NAT2 (Badawi *et al.*, 1995). Other studies are consistent with *N*-acetylation of ABP and *O*-acetylation of *N*-hydroxy-ABP predominantly by NAT1 in urinary bladder (Frederickson *et al.*, 1994). Recent studies with substrates selective for NAT1 versus NAT2 reported widespread distribution of both NAT1 and NAT2 catalytic activities in the rapid and slow acetylator congenic hamster (Hein et al., 2006). NAT2-dependent *N*-acetylation (Figure 1) and *O*-acetylation (Figure 4) have been reported in urinary bladder cytosol from rapid and slow acetylator Syrian hamsters congenic at the *NAT2* locus. Since both NAT1 and NAT2 catalyze the metabolism of aromatic amine carcinogens (Minchin *et al.*, 1992; Hein *et al.*, 1993b; 1994b; 1995), genetic polymorphism in NAT1 and/or NAT2 may modify cancer risk related to exposures to these carcinogens.

Molecular genetics

NAT1 and NAT2 are products of single, intronless exons containing single 870 base pair open reading frames encoding 290 amino acids (Blum *et al.*, 1991; Vatsis *et al.*, 1991). *NAT1*, *NAT2*, and a pseudogene *NATP*, are located on the short arm of human chromosome 8 (Blum *et al.*, 1990; Hickman *et al.*, 1994) in the orientation *NAT1:NATP:NAT2* (Matsas *et al.*, 1997). *NAT1* and *NAT2* share 87% nucleotide homology in the coding region, yielding 55 amino acid differences. Human *NAT2* transcripts have been identified in many human tissues and derive from the protein-coding exon and a second non-coding exon of 100 base pairs located about 8 kilobases upstream of the translation start site (Blum *et al.*, 1990; Ebisawa and Deguchi, 1991; Boukouvala and Sim, 2005).

A number of single nucleotide polymorphisms (SNPs) have been reported in the *NAT2* coding exon. Those that cause amino acid changes include 111T>C (R197Q), 190C>T (R64W), 191G>A (R64Q), 341T>C (I114T), 364G>A (D122N), 411A>T (L137F), 434A>C (Q145P), 590G>A (R197Q), 803A>G (K268R), 845A>C (K282T), 857G>A (K268R) and 859T>C (I287T). SNPs that do not change amino acids include 282C>T, 481C>T and 759C>T. 859T deletion also has been reported resulting in a frame shift at amino acid 287. Various combinations of SNPs are identified as *NAT2* alleles (Vatsis *et al.,* 1995) or haplotypes. *NAT2*4* is considered the "wild-type" allele or haplotype because of the absence of any SNPs. Variant *NAT2* alleles or haplotypes possessing combinations of SNPs are segregated into clusters possessing a signature SNP either alone or in combination with others. The more common *NAT2* alleles or haplotypes are illustrated in Table 1. The frequency of *NAT2* alleles varies widely across various ethnic groups and *NAT2*4* is not the most common in most ethnic groups, including Caucasians and Africans (Figures 5 and 6). *NAT2* alleles containing the 191G>A (R64Q), 341T>C (I114T), 590G>A (R197Q), or 857G>A (K268R) SNPs are associated with slow acetylator *NAT2* alleles (Table 1). Striking ethnic differences in the frequencies of SNPs and genotypes (http://snp500cancer.nci.nih.gov) are responsible for the corresponding ethnic differences in frequency of rapid (Figure 5) and slow (Figure 6) acetylator *NAT2* alleles or haplotypes and therefore phenotypes. For example, the 191G>A (R64Q) SNP common to the *NAT2*14* allele cluster is frequent in Africans and African-Americans, but virtually absent in Caucasian, Indian, and Korean populations (Figure 6). Similarly, the *NAT2*7* cluster possessing the 857G>A (K268R) SNP is much more frequent in South India and Korea than other populations while the *NAT2*5* cluster containing the 341T>C (I114T) SNP is much less frequent in Korea than in Europe, North America, India and Africa (Figure 6). Deduction of NAT2 phenotypes is assigned based on co-dominant expression of rapid and slow acetylator *NAT2* alleles or haplotypes as clearly documented in animals (Figures 1–2) and humans (Figure 3). 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.

Over 35 *NAT2* alleles or haplotypes have been identified in human populations. A consensus *NAT* nomenclature was first published in 1995 (Vatsis *et al.,* 1995). An international nomenclature committee publishes an internet accessible website for allele updates at www.louisville.edu/medschool/pharmacology/NAT.html.

Molecular basis for altered function of NAT2 polymorphic variants

Reductions in the amount of NAT2 protein expressed in human liver from individuals with slow acetylator phenotype have been reported (Grant *et al.,* 1990; Deguchi *et al.,* 1990; Deguchi, 1992). Slow acetylator *NAT2* alleles recombinantly expressed in COS-1 cells (Blum *et al.,* 1991; Zang *et al.,* 2004), Chinese hamster ovary cells (Abe *et al.,* 1993), and yeast (Leff *et al.,* 1999; Fretland *et al.,* 2001) show reduced levels of NAT2 protein when compared with *NAT2*4*. These data suggest that slow acetylator phenotype is conferred, at least for some *NAT2* alleles, by reduction(s) in NAT2 protein. Recent studies in COS-1 cells also show that the reduction in protein in slow acetylators is the result of increased degradation for SNPs such as 341T>C (I114T) (Zang *et al.,* 2004).

The effects of *NAT2* SNPs on catalytic activities have been investigated primarily in recombinant expression systems (reviewed in Hein *et al.,* 2000; Hein, 2002). Nucleotide substitutions identified in human *NAT2* allelic variants yield reductions in substrate affinity, catalytic activity and/or protein stability of the recombinant *N*-acetyltransferase allozymes. Recombinant human NAT2 5, NAT2 6, NAT2 7, and NAT2 14 clusters yield variable reductions in catalytic activity associated with slow acetylator phenotype, while recombinant

human NAT2 12 and NAT2 13 clusters catalyze *N*-, *O*-, and *N*,*O*-acetyltransferase activities at levels comparable to the rapid acetylator NAT2 4 (Hein *et al.,* 1995). Recently, some controversy has arisen regarding the assignment of *NAT2*12* and *NAT2*13* as rapid acetylator alleles (Bolt *et al.,* 2005). As shown in Table 1, *NAT2*12* allele clusters possess the signature 803A>G (K268R) SNP whereas the *NAT2*13* allele possesses the 282C>T SNP that does not change the amino acid. These two SNPs and identification of *NAT2*12* and *NAT2*13* alleles often are not determined in epidemiological studies since they are considered rare. However, as shown in Figure 5, their frequency is not rare in many ethnic groups and it is important both to assess their frequency and to correctly assign them as rapid or slow acetylator status in order to deduce acetylator phenotype. Previous studies have clearly shown that the 803A>G (K268R) SNP characteristic of *NAT2*12* alleles and the 282C>T SNP characteristic of *NAT2*13* alleles do not alter NAT2 catalytic activity (Hein *et al.,* 1994b; 1995; Fretland *et al.,* 2001; Zang *et al.,* 2005). Three studies using caffeine as a phenotype probe suggested that *NAT2*12* and *NAT2*13* were associated with slow acetylation phenotype (Cascorbi *et al.,* 1995; Gross *et al.,* 1999; Bolt *et al.,* 2005). Cascorbi *et al* 1995 initially reported this for *NAT2*13* but later reported that it was related to an *NAT2* genotyping artifact (Cascorbi *et al.,* 1996; Cascorbi and Roots, 1999). The other studies did not distinguish the *NAT2*12* or the *NAT2*13* allele from *NAT2* genotypes that do not possess *NAT2*12* or *NAT2*13*. Nevertheless, verification of *NAT2*12* and *NAT2*13* as rapid acetylator alleles has been provided *in vivo* (Cascorbi *et al.,* 1996; Parkin *et al.,* 1997). The latter study (Parkin *et al.,* 1997) included 5 subjects possessing the *NAT2*13* allele and 20 subjects possessing the *NAT2*12A* allele that consistently confirmed rapid acetylator status based upon measured phenotypes in several people.

Recombinant NAT2 proteins differ in heat stability (Ferguson *et al.,* 1994; Hein *et al.,* 1994b; Grant *et al.,* 1997; Leff *et al.,* 1999; Fretland *et al.,* 2001). The NAT2 7B allozyme has altered affinity for some but not other substrates (Hein *et al.,* 1994b; Hickman *et al.,* 1995) suggesting that expression of acetylator phenotype is dependent upon substrate. Some, but not all of the SNPs in human *NAT2* yield reductions in quantity of recombinant NAT2 protein in eukaryotic expression systems (Deguchi, 1992; Blum *et al.,* 1991; Abe *et al.,* 1993; Leff *et al.,* 1999; Fretland *et al.,* 2001). Clearly, more data from tissues is needed to investigate tissue-specific and other regulatory factors.

Since multiple mechanisms for reductions in NAT2 activity are associated with various combinations of SNPs that make up *NAT2* alleles, the ability to distinguish among multiple acetylator phenotypes is complex and a function of the sensitivity and specificity of the phenotyping method. Phenotype is influenced by a number of factors including diet, disease, and drug therapy. Depending upon the probe drug and analytical method used, acetylation phenotypes often exhibit overlap due to numerous genetic and/or environmental factors, including the large number and diversity of *NAT2* genotypes present in human populations. The relative specificity of the substrate for NAT2 versus NAT1 at the concentrations obtained *in vivo* will also affect acetylator phenotype. Caffeine is commonly used as a probe drug for NAT2 phenotype determinations because it is relatively non-invasive and excellent *NAT2* genotype/phenotype correlations have been reported (Cascorbi *et al.,* 1995; Grant *et al.,* 1997; 2004). Genetic and/or environmental effects on a number of enzyme systems (e.g., cytochrome P450, xanthine oxidase, NAT1) may affect metabolite levels used to assess phenotype. Other potential artifacts in the use of caffeine to determine acetylation phenotype also have been reported (Cribb *et al.,* 1994; Lorenzo and Reidenberg, 1989; O'Neil *et al.,* 2000; Svensson and Hein, 2004).

NAT2 polymorphism and urinary bladder cancer risk

Human epidemiological studies have investigated the role of the *NAT2* polymorphism in many cancers. Urinary bladder is a textbook example since individuals are frequently exposed to

aromatic amine urinary bladder carcinogens such as ABP and BNA in cigarette smoke (Luceri *et al.*, 1993; Stabbert *et al.*, 2003). These aromatic amine carcinogens require metabolic activation in order to mutate DNA and initiate carcinogenesis. Following *N*-oxidation, the *N*-hydroxyaromatic and *N*-hydroxyheterocyclic amines are further activated (via *O*-acetylation) by *N*-acetyltransferases to acetoxy intermediates which react spontaneously with DNA to form DNA adducts (Hein, 1988). Thus, biological plausability for relationships between the *NAT2* acetylation polymorphisms are strongest for cancers related to aromatic amine exposures.

The role of rapid versus slow acetylator genotype in cancer predisposition differs between organ sites as might be expected with tissue-specific expression of NAT2. Although reports of associations between *NAT2* polymorphism and a number of cancers have been reported, a focus on urinary bladder cancer is useful for illustrating the divergent effects of carcinogenic agent and *NAT2* haplotype on individual risk.

The first association between slow acetylator phenotype and urinary bladder cancer was reported over 25 years ago (Lower *et al.*, 1979). The hypothetical mechanism for this association is slow NAT2 acetylation of aromatic amine carcinogens competes poorly with metabolic activation via cytochrome P450(s) and/or prostaglandin H-synthases, thus accounting for higher risk in the slow NAT2 phenotype(s). In a landmark study, English chemical dye workers with documented exposure to aromatic amine carcinogens showed a striking association (OR = 16.7; P=0.00005) between urinary bladder cancer and slow acetylator phenotypes (Cartwright *et al.*, 1982). The population studied had documented exposures to aromatic amines and NAT2 acetylator phenotype was assessed by measurement of plasma monoacetyl-dapsone to dapsone metabolic ratios. Dapsone is an NAT2 selective substrate (more so than caffeine) and measurement of monoacetyl-dapsone to dapsone metabolic ratios in plasma (rather than urine) is a more direct assessment of NAT2 phenotype. Interestingly, the NAT2 phenotype data was not separated into two phenotypes (rapid and slow), but rather into eight ranges of metabolic ratios. Five of these ratios (0.3 and greater) correspond to rapid acetylators, and the other three (0.01 to 0.09; 0.1–0.19; and 0.2 –0.29) correspond to different levels of slow acetylator phenotype. As reviewed previously (Hein, 2002), urinary bladder cancer risk increased as NAT2 metabolic ratio (phenotype) decreased (P_{trend} = 0.0006). The risk was markedly increased in the slowest NAT2 phenotype (OR, 20.8; 95%CI, 2.63–164). Four studies found that urinary bladder cancer risk was highest in individuals possessing *NAT2*5* haplotypes (Brockmoller *et al.*, 1996; Okkels *et al.*, 1997; Filiadis *et al.*, 1999; El Desoky *et al.*, 2005). The 341T>C (I114T) SNP associated with *NAT2*5* alleles or haplotypes yields very large reductions in NAT2 protein and activity (Hein *et al.*, 1994b; 1995; Fretland *et al.*, 2001) resulting from protein degradation (Zang *et al.*, 2004). Recently, *NAT2*5* alleles also were associated with increased risk for breast cancer in women smokers (van der Hel *et al.*, 2003). These results suggest that NAT2 slow acetylator phenotype is not homogeneous, but rather that multiple slow acetylator phenotypes exist resulting from different mechanisms inferred by various SNPs and haplotypes.

Among smokers, NAT2 slow acetylators have higher levels of 4-aminobiphenyl hemoglobin adducts (Vineis *et al.*, 1994; Yu *et al.*, 1994; Probst-Hensch *et al.*, 2000). Furthermore, ABP-DNA adducts in higher grade bladder tumors are found at higher levels in smokers who are slow NAT2 acetylators (Airoldi *et al.*, 2002; Hao *et al.*, 2004). A previous review of 21 published case control studies reported that experimental evidence was not sufficient to conclude a real increase in risk for urinary bladder cancer in slow NAT2 acetylators (Green *et al.*, 2000). However, subsequent studies carried out in Europe (Vineis *et al.*, 2001), Japan (Tsukino *et al.*, 2004), the United States (Gu *et al.*, 2005), and Spain (Garcia-Closas *et al.*, 2005) each reported that NAT2 slow acetylators had a significantly increased risk of urinary bladder cancer that was stronger in smokers, particularly heavy or long term smokers. Because of the high frequency of homozygous rapid acetylators in Japan, that study also noted a higher

risk in intermediate acetylators compared to homozygous rapid acetylators (Tsukino *et al.*, 2004). Meta-analysis of these and all previous studies show that the overall association with slow *NAT2* genotype in the published literature is robust (Figure 7) providing compelling evidence for a role of *NAT2* acetylator genotype in urinary bladder cancer associated with aromatic amines in cigarette smoke.

Since ethnic differences in *NAT2* allele frequencies are quite striking (Figures 5 and 6), it has been suggested that that the role of *NAT2* polymorphism on urinary cancer risk may differ with ethnic group (Golka *et al.*, 2002). However, strong evidence has been provided by Carreon et al (2006) that this is not the case, but rather that the role of *NAT2* polymorphism on urinary bladder cancer risk differs with carcinogenic agent. Although one study found no difference in urinary bladder cancer risk between rapid and slow NAT2 acetylator Chinese workers (Ma *et al.*, 2004), another study of Chinese workers exposed to benzidine (Hayes *et al.*, 1993) subsequently confirmed in a follow-up study (Carreon *et al.*, 2006) reported that slow *NAT2* acetylator genotype was associated with *decreased* risk to urinary bladder (relative to rapid *NAT2* acetylators). As noted above and in Figure 7, the effect of *NAT2* polymorphism on urinary bladder cancer risk for smokers is not dependent upon ethnic group. Rather than an ethnic difference, these findings are explained by the observation that since benzidine is a aryldiamine, the *N*-acetylation of one aromatic amine moiety is not a deactivation step and may enhance metabolic activation and/or transport to the urinary bladder. Urinary bladder DNA adducts following benzidine exposures in humans derive from *N*-acetylated metabolite(s) (Rothman *et al.*, 1996). Support for this hypothesis also derives from the effect of *NAT2* polymorphism on hepatoxicity from the aryldiamine 4,4'-methylenedianiline in the rat (Zhang *et al.*, 2006).

NAT2 rapid acetylators potentially would be at decreased risk of urinary bladder cancer following dermal exposures to aromatic amine carcinogens if NAT2 were highly expressed in skin, since a rapid NAT2 acetylator phenotype would have higher capacity to deactivate the carcinogen prior to systemic absorption. The lack of NAT2 expression in human kerotinocytes (Reilly *et al.*, 2000) is consistent with increased risk of urinary bladder cancer in NAT2 slow acetylators following dermal exposures (Gago-Dominguez *et al.*, 2003). Thus following both inhalation and dermal exposures to aromatic monoarylamines, *N*-acetylation competes with *N*-hydroxylation conferring higher risk to the slow NAT2 acetylator phenotype(s). Furthermore, local *N*- or *O*-acetylation of arylamines or their *N*-hydroxylated metabolites resulting from dermal exposures would not be modified by *NAT2* acetylation polymorphism, except indirectly to the extent that there is linkage disequilibrium between *NAT1* and *NAT2* alleles.

The effect of *NAT2* acetylator polymorphism on urinary bladder cancer susceptibility is dependent upon accuracy of the exposure and genotype assessments (Rothman *et al.*, 1993; Deitz *et al.*, 2004). Indeed, reports suggest that ABP is present in higher yields in sidestream versus mainstream cigarette smoke and aromatic amines are present in indoor environments exposed to side stream cigarette smoke (Luceri *et al.*, 1993; Palmiotto *et al.*, 2001). Since exposure to passive smoking may also increase urinary bladder cancer risk, studies that use controls not exposed to passive cigarette smoke may yield more robust findings. *NAT2* genotyping methods resulting in misclassification may confound relationships between *NAT2* acetylator polymorphism and urinary bladder cancer risk (Deitz *et al.*, 2004). Functional understanding of the effects of *NAT2* genetic polymorphisms on metabolism and genotoxicity, tissue-specific expression, and the molecular mechanisms responsible for these effects are critical for the interpretation of previous and future human molecular epidemiology studies.

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References

Abe M, Deguchi T, Suzuki T. Biochem Biophys Res Commun 1993;191:811–6. [PubMed: 8466518]

Agundez JA, Menaya JG, Tejeda R, Lago F, Chavez M, Benitez J. Pharmacogenetics 1996;6:465–72. [PubMed: 8946479]

Airoldi L, Orsi F, Magagnotti C, Coda R, Randone D, Casetta G, Peluso M, Hautefeuille A, Malaveille C, Vineis P. Carcinogenesis 2002;23:861–6. [PubMed: 12016161]

Anitha A, Banerjee M. Int J Mol Med 2003;11:125–31. [PubMed: 12469231]

Badawi AF, Hirvonen A, Bell DA, Lang NP, Kadlubar FF. Cancer Res 1995;55:5230–7. [PubMed: 7585581]

Blum M, Demierre A, Grant DM, Heim M, Meyer UA. Proc Natl Acad Sci U S A 1991;88:5237–41. [PubMed: 1675794]

Blum M, Grant DM, Demierre A, Meyer UA. Proc Natl Acad Sci U S A 1989;86:9554–7. [PubMed: 2594784]

Blum M, Grant DM, McBride W, Heim M, Meyer UA. DNA Cell Biol 1990;9:193–203. [PubMed: 2340091]

Bolt HM, Selinski S, Dannappel D, Blaszkewicz M, Golka K. Arch Toxicol 2005;79:196–200. [PubMed: 15558239]

Boukouvala S, Sim E. Basic Clin Pharmacol Toxicol 2005;96:343–51. [PubMed: 15853926]

Brockmoller J, Cascorbi I, Kerb R, Roots I. Cancer Res 1996;56:3915–25. [PubMed: 8752158]

Carreon T, Ruder AM, Schulte PA, Hayes RB, Rothman N, Waters M, Grant DJ, Boissy R, Bell DA, Kadlubar FF, Hemstreet GP 3rd, Yin S, Lemasters GK. Int J Cancer 2006;118:161–168. [PubMed: 16003747]

Cartwright RA, Glashan RW, Rogers HJ, Ahmad RA, Barham-Hall D, Higgins E, Kahn MA. Lancet 1982;2:842–5. [PubMed: 6126711]

Cascorbi I, Brockmoller J, Bauer S, Reum T, Roots I. Pharmacogenetics 1996;6:257–9. [PubMed: 8807666]

Cascorbi I, Brockmoller J, Mrozikiewicz PM, Muller A, Roots I. Drug Metab Rev 1999;31:489–502. [PubMed: 10335449]

Cascorbi I, Drakoulis N, Brockmoller J, Maurer A, Sperling K, Roots I. Am J Hum Genet 1995;57:581–92. [PubMed: 7668286]

Cascorbi I, Roots I. Pharmacogenetics 1999;9:123–7. [PubMed: 10208651]

Chapron DJ, Kramer PA, Mercik SA. Clin Pharmacol Ther 1980;27:104–13. [PubMed: 7351110]

Chung JG, Levy GN, Weber WW. Drug Metab Dispos 1993;21:1057–63. [PubMed: 7905384]

Cribb AE, Isbrucker R, Levatte T, Tsui B, Gillespie CT, Renton KW. Pharmacogenetics 1994;4:166–70. [PubMed: 7920698]

Deguchi T. J Biol Chem 1992;267:18140–7. [PubMed: 1381364]

Deguchi T, Mashimo M, Suzuki T. J Biol Chem 1990;265:12757–60. [PubMed: 2376572]

Deitz AC, Rothman N, Rebbeck TR, Hayes RB, Chow WH, Zheng W, Hein DW, Garcia-Closas M. Cancer Epidemiol Biomarkers Prev 2004;13:1543–6. [PubMed: 15342459]

Deitz AC, Zheng W, Leff MA, Gross M, Wen WQ, Doll MA, Xiao GH, Folsom AR, Hein DW. Cancer Epidemiol Biomarkers Prev 2000;9:905–10. [PubMed: 11008907]

Delomenie C, Sica L, Grant DM, Krishnamoorthy R, Dupret JM. Pharmacogenetics 1996;6:177–85. [PubMed: 9156695]

Dewan A, Chattopadhyay P, Kulkarni PK. Indian J Cancer 1995;32:15–9. [PubMed: 7558106]

Doll MA, Hein DW. Pharmacogenetics 1995;5:247–51. [PubMed: 8528272]

Dupret JM, Grant DM. J Biol Chem 1992;267:7381–5. [PubMed: 1559981]

Dupret JM, Rodrigues-Lima F. Curr Med Chem 2005;12:311–8. [PubMed: 15723621]

Ebisawa T, Deguchi T. Biochem Biophys Res Commun 1991;177:1252–7. [PubMed: 1676262]

El Desoky ES, AbdelSalam YM, Salama RH, El Akkad MA, Atanasova S, von Ahsen N, Armstrong VW, Oellerich M. Ther Drug Monit 2005;27:297–304. [PubMed: 15905799]

Ferguson RJ, Doll MA, Rustan TD, Gray K, Hein DW. Drug Metab Dispos 1994;22:371–6. [PubMed: 7915226]

Ferguson RJ, Doll MA, Rustan TD, Hein DW. Pharmacogenetics 1996;6:55–66. [PubMed: 8845861]

Filiadis IF, Georgiou I, Alamanos Y, Kranas V, Giannakopoulos X, Lolis D. J Urol 1999;161:1672–5. [PubMed: 10210437]

Fretland AJ, Doll MA, Leff MA, Hein DW. Pharmacogenetics 2001;11:511–20. [PubMed: 11505221]

Frederickson SM, Hatcher JF, Reznikoff CA, Swaminathan S. Carcinogenesis 1992;13:955–61. [PubMed: 1600616]

Frederickson SM, Messing EM, Reznikoff CA, Swaminathan S. Cancer Epidemiol Biomarkers Prev 1994;3:25–32. [PubMed: 8118381]

Gago-Dominguez M, Bell DA, Watson MA, Yuan JM, Castelao JE, Hein DW, Chan KK, Coetzee GA, Ross RK, Yu MC. Carcinogenesis 2003;24:483–9. [PubMed: 12663508]

Garcia-Closas M, Malats N, Silverman D, Dosemeci M, Kogevinas M, Hein DW, Tardon A, Serra C, Carrato A, Garcia-Closas R, Lloreta J, Castano-Vinyals G, Yeager M, Welch R, Chanock S, Chatterjee N, Wacholder S, Samanic C, Tora M, Fernandez F, Real FX, Rothman N. Lancet 2005;366:649–59. [PubMed: 16112301]

Giannakopoulos X, Charalabopoulos K, Baltogiannis D, Chatzikiriakidou A, Alamanos Y, Georgiou I, Evangelou A, Agnantis N, Sofikitis N. Anticancer Res 2002;22:3801–4. [PubMed: 12552997]

Green J, Banks E, Berrington A, Darby S, Deo H, Newton R. Br J Cancer 2000;83:412–7. [PubMed: 10917561]

Golka K, Prior V, Blaszkewicz M, Bolt HM. Toxicol Lett 2002;128:229–41. [PubMed: 11869833]

Grant DM, Hughes NC, Janezic SA, Goodfellow GH, Chen HJ, Gaedigk A, Yu VL, Grewal R. Mutat Res 1997;376:61–70. [PubMed: 9202739]

Grant DM, Morike K, Eichelbaum M, Meyer UA. J Clin Invest 1990;85:968–72. [PubMed: 2312737]

Grant DM, Tang BK, Kalow W. Br J Clin Pharmacol 2004;58:S788–93. [PubMed: 15595973]

Gross M, Kruisselbrink T, Anderson K, Lang N, McGovern P, Delongchamp R, Kadlubar F. Cancer Epidemiol Biomarkers Prev 1999;8:683–92. [PubMed: 10744128]

Gu J, Liang D, Wang Y, Lu C, Wu X. Mutat Res 2005;581:97–104. [PubMed: 15725609]

Hanssen HP, Agarwal DP, Goedde HW, Bucher H, Huland H, Brachmann W, Ovenbeck R. Eur Urol 1985;11:263–6. [PubMed: 4043164]

Hao GY, Zhang WD, Chen YH, Zhang DX, Zhang YH. Zhonghua Zhong Liu Za Zhi 2004;26:283–6. [PubMed: 15312364]

Hayes RB, Bi W, Rothman N, Broly F, Caporaso N, Feng P, You X, Yin S, Woosley RL, Meyer UA. Carcinogenesis 1993;14:675–8. [PubMed: 8472331]

Hearse DJ, Weber WW. Biochem J 1973;132:519–26. [PubMed: 4724587]

Hein DW. Biochim Biophys Acta 1988;948:37–66. [PubMed: 3293663]

Hein DW. (2002). Mutat Res, 506–507, 65–77.

Hein DW, Doll MA, Fretland AJ, Gray K, Deitz AC, Feng Y, Jiang W, Rustan TD, Satran SL, Wilkie TR Sr. Mutat Res 1997;376:101–6. [PubMed: 9202744]

Hein DW, Doll MA, Fretland AJ, Leff MA, Webb SJ, Xiao GH, Devanaboyina US, Nangju NA, Feng Y. Cancer Epidemiol Biomarkers Prev 2000;9:29–42. [PubMed: 10667461]

Hein DW, Doll MA, Gray K, Rustan TD, Ferguson RJ. Cancer Res 1993a;53:509–14. [PubMed: 8425184]

Hein DW, Doll MA, Nerland DE, Fretland AJ. Mol Carcinog 2006;45:230–8. [PubMed: 16482518]

Hein DW, Doll MA, Rustan TD, Ferguson RJ. Cancer Res 1995;55:3531–6. [PubMed: 7627960]

Hein DW, Doll MA, Rustan TD, Gray K, Feng Y, Ferguson RJ, Grant DM. Carcinogenesis 1993b; 14:1633–8. [PubMed: 8353847]

Hein DW, Doll MA, Rustan TD, Gray K, Ferguson RJ, Feng Y. Toxicol Appl Pharmacol 1994a;124:16–24. [PubMed: 8291057]

Hein DW, Doll MA, Xiao GH, Feng Y. Pharmacogenetics 2003;13:159–67. [PubMed: 12618593]

Hein DW, Ferguson RJ, Doll MA, Rustan TD, Gray K. Hum Mol Genet 1994b;3:729–34. [PubMed: 8081359]

Hein DW, Kirlin WG, Ferguson RJ, Weber WW. J Pharmacol Exp Ther 1985;234:358–64. [PubMed: 3874952]

Hein DW, Rustan TD, Bucher KD, Martin WJ, Furman EJ. Drug Metab Dispos 1991a;19:933–7. [PubMed: 1686239]

Hein DW, Rustan TD, Bucher KD, Miller LS. J Pharmacol Exp Ther 1991b;259:699–704. [PubMed: 1941618]

Hein DW, Trinidad A, Yerokun T, Ferguson RJ, Kirlin WG, Weber WW. Drug Metab Dispos 1988;16:341–7. [PubMed: 2900723]

Hickman D, Palamanda JR, Unadkat JD, Sim E. Biochem Pharmacol 1995;50:697–703. [PubMed: 7669073]

Hickman D, Pope J, Patil SD, Fakis G, Smelt V, Stanley LA, Payton M, Unadkat JD, Sim E. Gut 1998;42:402–9. [PubMed: 9577349]

Hickman D, Risch A, Buckle V, Spurr NK, Jeremiah SJ, McCarthy A, Sim E. Biochem J 1994;297 (Pt 3):441–5. [PubMed: 8110178]

Horai Y, Fujita K, Ishizaki T. Eur J Clin Pharmacol 1989;37:581–7. [PubMed: 2612554]

Hsieh FI, Pu YS, Chern HD, Hsu LI, Chiou HY, Chen CJ. Br J Cancer 1999;81:537–41. [PubMed: 10507782]

Hung RJ, Boffetta P, Brennan P, Malaveille C, Hautefeuille A, Donato F, Gelatti U, Spaliviero M, Placidi D, Carta A, Scotto di Carlo A, Porru S. Int J Cancer 2004;110:598–604. [PubMed: 15122594]

Hughes HB, Biehl JP, Jones AP, Scmidt LH. Am Rev Res Dis 1954;70:266–73.

Ilett KF, Ingram DM, Carpenter DS, Teitel CH, Lang NP, Kadlubar FF, Minchin RF. Biochem Pharmacol 1994;47:914–7. [PubMed: 8135868]

Ilett KF, Reeves PT, Minchin RF, Kinnear BF, Watson HF, Kadlubar FF. Carcinogenesis 1991;12:1465–9. [PubMed: 1860167]

Ishizu S, Hashida C, Hanaoka T, Maeda K, Ohishi Y. Jpn J Cancer Res 1995;86:1179–81. [PubMed: 8636007]

Jaskula-Sztul R, Sokolowski W, Gajecka M, Szyfter K. J Appl Genet 2001;42:223–31. [PubMed: 14564055]

Jenne JW. J Clin Invest 1965;44:1992–2002. [PubMed: 5851956]

Kaisary A, Smith P, Jaczq E, McAllister CB, Wilkinson GR, Ray WA, Branch RA. Cancer Res 1987;47:5488–93. [PubMed: 3652049]

Karakaya AE, Cok I, Sardas S, Gogus O, Sardas OS. Hum Toxicol 1986;5:333–5. [PubMed: 3770769]

Kim WJ, Lee HL, Lee SC, Kim YT, Kim H. J Urol 2000;164:209–13. [PubMed: 10840461]

Kirlin WG, Ogolla F, Andrews AF, Trinidad A, Ferguson RJ, Yerokun T, Mpezo M, Hein DW. Cancer Res 1991;51:549–55. [PubMed: 1985773]

Kirlin WG, Trinidad A, Yerokun T, Ogolla F, Ferguson RJ, Andrews AF, Brady PK, Hein DW. Cancer Res 1989;49:2448–54. [PubMed: 2784998]

Ladero JM, Kwok CK, Jara C, Fernandez L, Silmi AM, Tapia D, Uson AC. Ann Clin Res 1985;17:96–9. [PubMed: 4051447]

Lan Q, Rothman N, Chow WH, Lissowska J, Doll MA, Xiao GH, Zatonski W, Hein DW. Cancer Epidemiol Biomarkers Prev 2003;12:384–6. [PubMed: 12692115]

Lee EJ, Lee LK. Br J Clin Pharmacol 1982;13:375–8. [PubMed: 7059437]

Lee SY, Lee KA, Ki CS, Kwon OJ, Kim HJ, Chung MP, Suh GY, Kim JW. Clin Chem 2002;48:775–7. [PubMed: 11978608]

Leff MA, Fretland AJ, Doll MA, Hein DW. J Biol Chem 1999;274:34519–22. [PubMed: 10574910]

Lorenzo B, Reidenberg MM. Br J Clin Pharmacol 1989;28:207–8. [PubMed: 2775628]

Loktionov A, Moore W, Spencer SP, Vorster H, Nell T, O'Neill IK, Bingham SA, Cummings JH. Cancer Detect Prev 2002;26:15–22. [PubMed: 12088198]

Lower GM Jr, Nilsson T, Nelson CE, Wolf H, Gamsky TE, Bryan GT. Environ Health Perspect 1979;29:71–9. [PubMed: 510245]

Luceri F, Pieraccini G, Moneti G, Dolara P. Toxicol Ind Health 1993;9:405–13. [PubMed: 8367883]

Ma QW, Lin GF, Chen JG, Xiang CQ, Guo WC, Golka K, Shen JH. Biomed Environ Sci 2004;17:291–8. [PubMed: 15602826]

Martell KJ, Vatsis KP, Weber WW. Mol Pharmacol 1991;40:218–27. [PubMed: 1875909]

Matas N, Thygesen P, Stacey M, Risch A, Sim E. Cytogenet Cell Genet 1997;77:290–5. [PubMed: 9284941]

Miller ME, Cosgriff JM. J Urol 1983;130:65–6. [PubMed: 6864917]

Minchin RF, Reeves PT, Teitel CH, McManus ME, Mojarrabi B, Ilett KF, Kadlubar FF. Biochem Biophys Res Commun 1992;185:839–44. [PubMed: 1627140]

Mittal RD, Srivastava DS, Mandhani A. Int Braz J Urol 2004;30:279–85. [PubMed: 15679955]discussion 285–8.

Mommsen S, Wolf H. Scand J Urol Nephrol 1985;19:203–4. [PubMed: 4070990]

Nagata K, Ozawa S, Miyata M, Shimada M, Yamazoe Y, Kato R. Pharmacogenetics 1994;4:91–100. [PubMed: 7915937]

Okkels H, Sigsgaard T, Wolf H, Autrup H. Cancer Epidemiol Biomarkers Prev 1997;6:225–31. [PubMed: 9107426]

O'Neil WM, Drobitch RK, MacArthur RD, Farrough MJ, Doll MA, Fretland AJ, Hein DW, Crane LR, Svensson CK. Pharmacogenetics 2000;10:171–82. [PubMed: 10762005]

Ozawa S, Abu-Zeid M, Kawakubo Y, Toyama S, Yamazoe Y, Kato R. Carcinogenesis 1990;11:2137–44. [PubMed: 2265466]

Pacifici GM, Bencini C, Rane A. Pharmacology 1986;32:283–91. [PubMed: 3487092]

Palmiotto G, Pieraccini G, Moneti G, Dolara P. Chemosphere 2001;43:355–61. [PubMed: 11302581]

Parkin DP, Vandenplas S, Botha FJ, Vandenplas ML, Seifart HI, van Helden PD, van der Walt BJ, Donald PR, van Jaarsveld PP. Am J Respir Crit Care Med 1997;155:1717–22. [PubMed: 9154882]

Peluso M, Airoldi L, Armelle M, Martone T, Coda R, Malaveille C, Giacomelli G, Terrone C, Casetta G, Vineis P. Cancer Epidemiol Biomarkers Prev 1998;7:341–6. [PubMed: 9568791]

Pink JC, Messing EM, Reznikoff CA, Bryan GT, Swaminathan S. Drug Metab Dispos 1992;20:559–65. [PubMed: 1356735]

Probst-Hensch NM, Bell DA, Watson MA, Skipper PL, Tannenbaum SR, Chan KK, Ross RK, Yu MC. Cancer Epidemiol Biomarkers Prev 2000;9:619–23. [PubMed: 10868698]

Reilly TP, Lash LH, Doll MA, Hein DW, Woster PM, Svensson CK. J Invest Dermatol 2000;114:1164–73. [PubMed: 10844561]

Risch A, Wallace DM, Bathers S, Sim E. Hum Mol Genet 1995;4:231–6. [PubMed: 7757072]

Rodrigues-Lima F, Delomenie C, Goodfellow GH, Grant DM, Dupret JM. Biochem J 2001;356:327–34. [PubMed: 11368758]

Rodrigues-Lima F, Dupret JM. Biochem Biophys Res Commun 2002;291:116–23. [PubMed: 11829470]

Rothman N, Bhatnagar VK, Hayes RB, Zenser TV, Kashyap SK, Butler MA, Bell DA, Lakshmi V, Jaeger M, Kashyap R, Hirvonen A, Schulte PA, Dosemeci M, Hsu F, Parikh DJ, Davis BB, Talaska G. Proc Natl Acad Sci U S A 1996;93:5084–9. [PubMed: 8643532]

Rothman N, Stewart WF, Caporaso NE, Hayes RB. Cancer Epidemiol Biomarkers Prev 1993;2:299–303. [PubMed: 8348052]

Sandy J, Mushtaq A, Kawamura A, Sinclair J, Sim E, Noble M. J Mol Biol 2002;318:1071–83. [PubMed: 12054803]

Sinclair JC, Sandy J, Delgoda R, Sim E, Noble ME. Nat Struct Biol 2000;7:560–4. [PubMed: 10876241]

Smith TJ, Hanna PE. Carcinogenesis 1986;7:697–702. [PubMed: 3486051]

Smith CA, Wadelius M, Gough AC, Harrison DJ, Wolf CR, Rane A. J Med Genet 1997;34:758–60. [PubMed: 9321764]

Stabbert R, Schafer KH, Biefel C, Rustemeier K. Rapid Commun Mass Spectrom 2003;17:2125–32. [PubMed: 12955743]

Stanley LA, Mills IG, Sim E. Pharmacogenetics 1997;7:121–30. [PubMed: 9170149]

Su HJ, Guo YL, Lai MD, Huang JD, Cheng Y, Christiani DC. Pharmacogenetics 1998;8:187–90. [PubMed: 10022756]

Sugamori KS, Wong S, Gaedigk A, Yu V, Abramovici H, Rozmahel R, Grant DM. Mol Pharmacol 2003;64:170–9. [PubMed: 12815173]

Svensson CK and Hein DW. (2004). Drug Metabolism and Transport: Molecular Methods and Mechanisms: Methods in Pharmacology and Toxicology. Lash LH (ed.). Humana Press: Totowa, NJ, pp 173–195.

Taylor JA, Umbach DM, Stephens E, Castranio T, Paulson D, Robertson C, Mohler JL, Bell DA. Cancer Res 1998;58:3603–10. [PubMed: 9721868]

Trinidad A, Kirlin WG, Ogolla F, Andrews AF, Yerokun T, Ferguson RJ, Brady PK, Hein DW. Drug Metab Dispos 1989;17:238–47. [PubMed: 2568903]

Tsukino H, Nakao H, Kuroda Y, Imai H, Inatomi H, Osada Y, Katoh T. Eur J Cancer Prev 2004;13:509–14. [PubMed: 15548945]

Turesky RJ, Lang NP, Butler MA, Teitel CH, Kadlubar FF. Carcinogenesis 1991;12:1839–45. [PubMed: 1934265]

van der Hel OL, Peeters PH, Hein DW, Doll MA, Grobbee DE, Kromhout D, Bueno de Mesquita HB. Pharmacogenetics 2003;13:399–407. [PubMed: 12835615]

Vatsis KP, Martell KJ, Weber WW. Proc Natl Acad Sci U S A 1991;88:6333–7. [PubMed: 2068113]

Vatsis KP, Weber WW, Bell DA, Dupret JM, Evans DA, Grant DM, Hein DW, Lin HJ, Meyer UA, Relling MV, et al. Pharmacogenetics 1995;5:1–17. [PubMed: 7773298]

Vineis P, Alavanja M, Garte S. Int J Cancer 2004;108:2–7. [PubMed: 14618608]

Vineis P, Bartsch H, Caporaso N, Harrington AM, Kadlubar FF, Landi MT, Malaveille C, Shields PG, Skipper P, Talaska G, et al. Nature 1994;369:154–6. [PubMed: 7909916]

Vineis P, Marinelli D, Autrup H, Brockmoller J, Cascorbi I, Daly AK, Golka K, Okkels H, Risch A, Rothman N, Sim E, Taioli E. Cancer Epidemiol Biomarkers Prev 2001;10:1249–52. [PubMed: 11751441]

Weber WW, Hein DW. Pharmacol Rev 1985;37:25–79. [PubMed: 2860675]

Windmill KF, Gaedigk A, Hall PM, Samaratunga H, Grant DM, McManus ME. Toxicol Sci 2000;54:19–29. [PubMed: 10746928]

Woodhouse KW, Adams PC, Clothier A, Mucklow JC, Rawlins MD. Hum Toxicol 1982;1:443–5. [PubMed: 7173929]

Yerokun T, Kirlin WG, Trinidad A, Ferguson RJ, Ogolla F, Andrews AF, Brady PK, Hein DW. Drug Metab Dispos 1989;17:231–7. [PubMed: 2568902]

Yu MC, Skipper PL, Taghizadeh K, Tannenbaum SR, Chan KK, Henderson BE, Ross RK. J Natl Cancer Inst 1994;86:712–6. [PubMed: 8158701]

Zang Y, Zhao S, Doll MA and Hein DW. (2005). Proc Amer Assoc Cancer Res 46, Abstract #4060.

Zang Y, Zhao S, Doll MA, States JC, Hein DW. Pharmacogenetics 2004;14:717–23. [PubMed: 15564878]

Zhang X, Lambert JC, Doll MA, Walraven JM, Arteel GE, Hein DW. J Pharmacol Exp Ther 2006;316:289–294. [PubMed: 16192314]

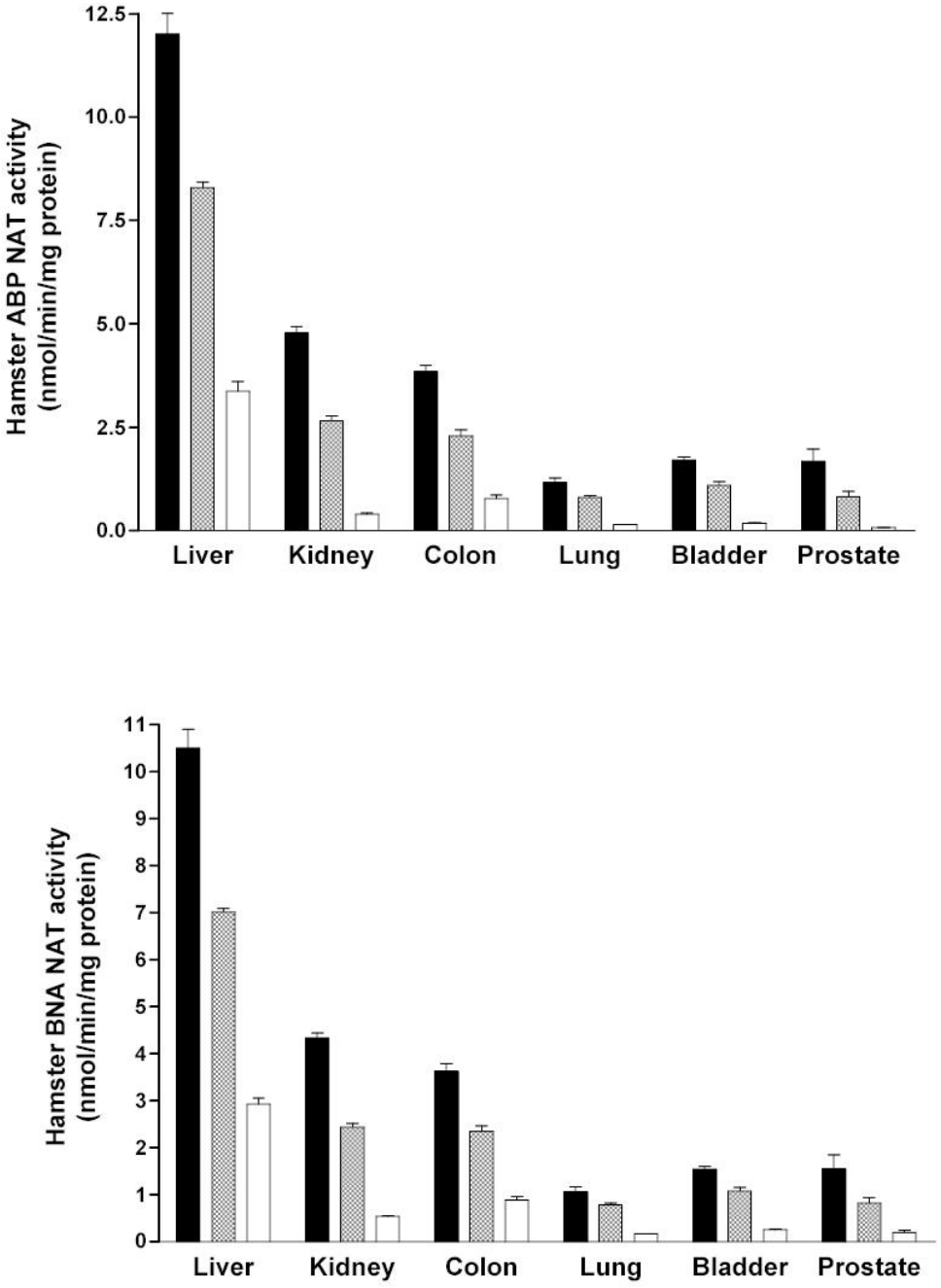


Figure 1.

Each bar represents Mean ± SE for cytosolic *N*-acetyltransferase activities towards the aromatic amine urinary bladder carcinogens 4-aminobiphenyl (ABP) and ß -naphthylamine (BNA) in congenic Syrian hamsters with homozygous rapid acetylator genotype (black), heterozygous acetylator genotype (gray) or homozygous slow acetylator genotype (white). Differences among the genotypes were highly significant ($p<0.0001$) following one way analysis of variance. Adapted from Hein *et al.*, 1994a; 2003.

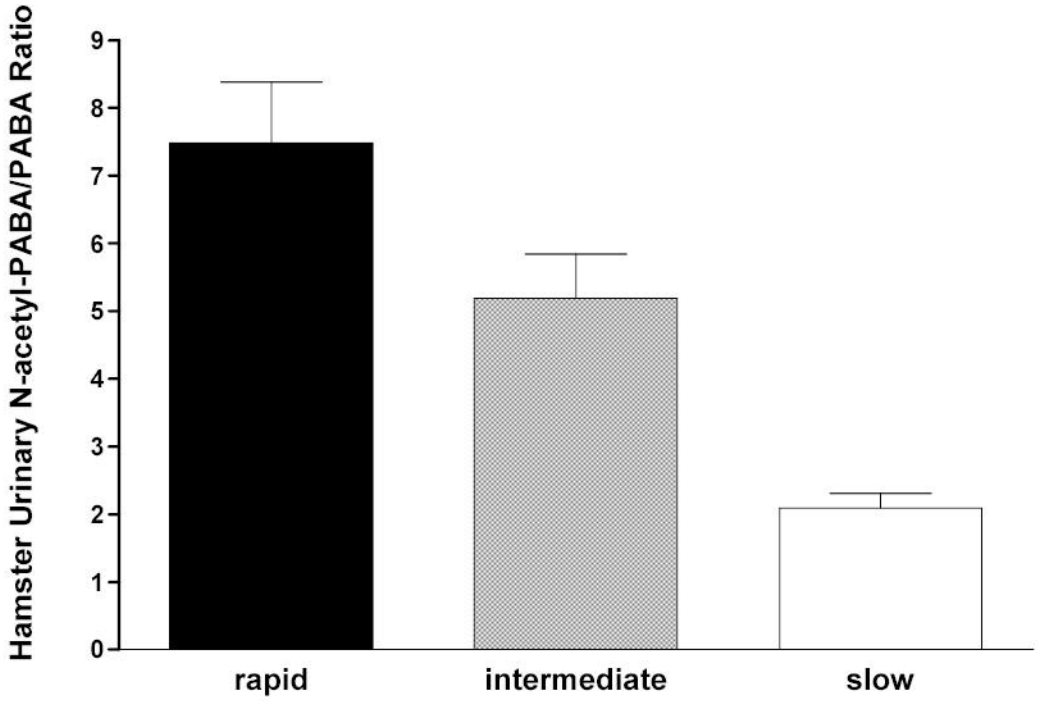


Figure 2.

Each bar represents Mean ± SE for urinary excretion ratio of *N*-acetyl-p-aminobenzoic acid to p-aminobenzoic acid in Syrian hamsters with homozygous rapid acetylator genotype (black), heterozygous acetylator genotype (gray) or homozygous slow acetylator genotype (white). Differences among the genotypes were highly significant ($p<0.0001$) following one way analysis of variance. Adapted from Hein *et al.*, 1994a.

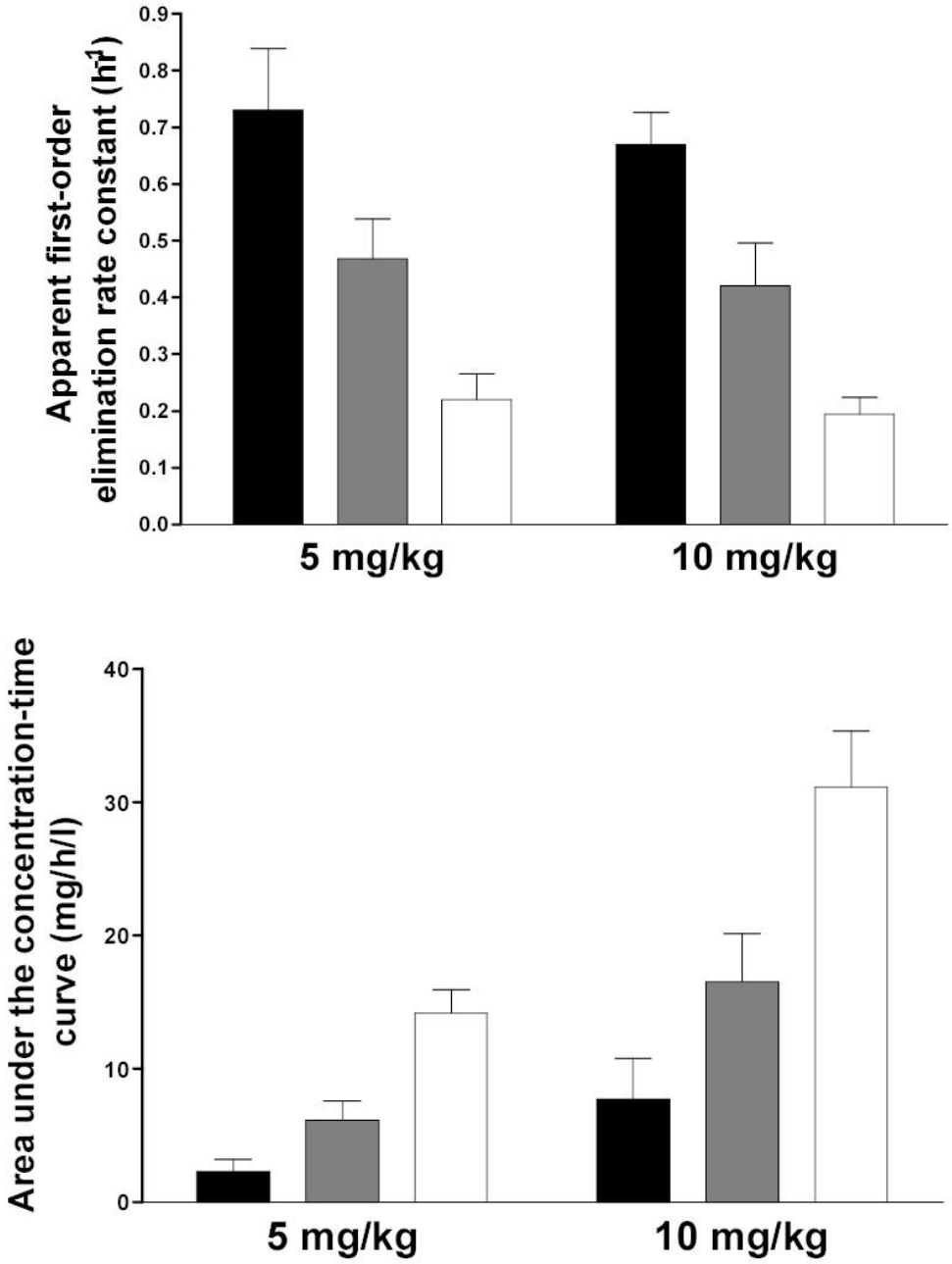


Figure 3.

Each bar represents Mean ± SD for the isoniazid elimination rate constant (top) or the area under the concentration-time curve (bottom) following a single oral dose of 5 or 10 mg/kg isoniazid in individuals with homozygous rapid acetylator genotype (black), heterozygous acetylator genotype (gray) or homozygous slow acetylator genotype (white). NAT2 genotypes and phenotypes were 100% concordant and differences among the genotypes were highly significant ($p<0.0001$) following one way analysis of variance. Adapted from Parkin *et al.*, 1997.

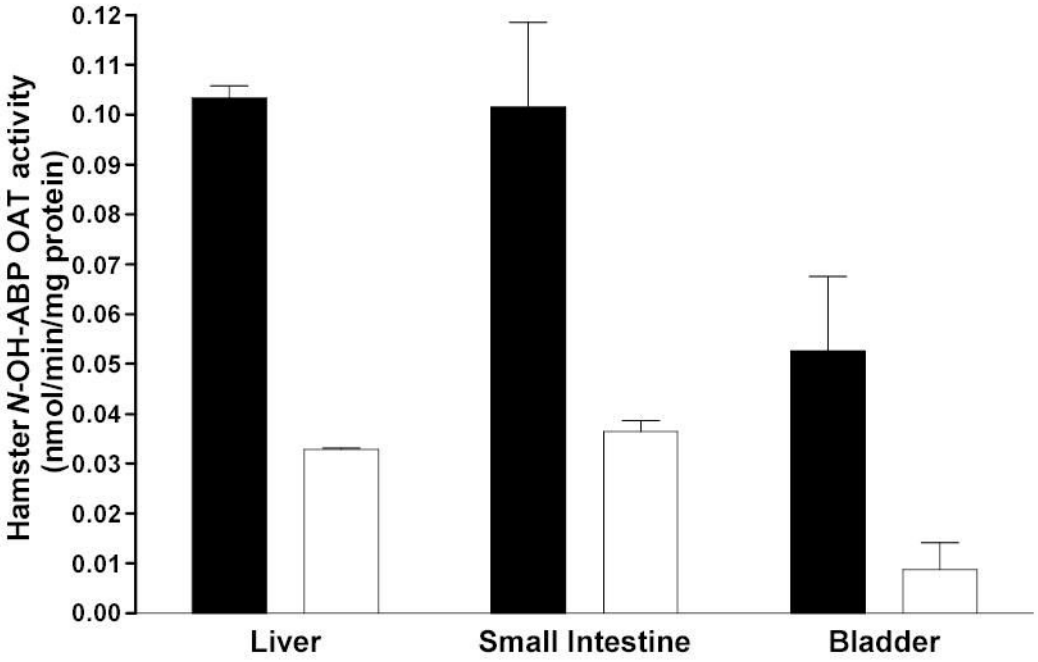


Figure 4.

Each bar represents Mean ± SE for cytosolic *O*-acetyltransferase activities towards *N*-hydroxy-4-aminobiphenyl (*N*-OH-ABP) in congenic Syrian hamsters with homozygous rapid acetylator genotype (black) or homozygous slow acetylator genotype (white). Differences between rapid and slow acetylators were significant in each tissue. Modified from Hein et al., 2006.

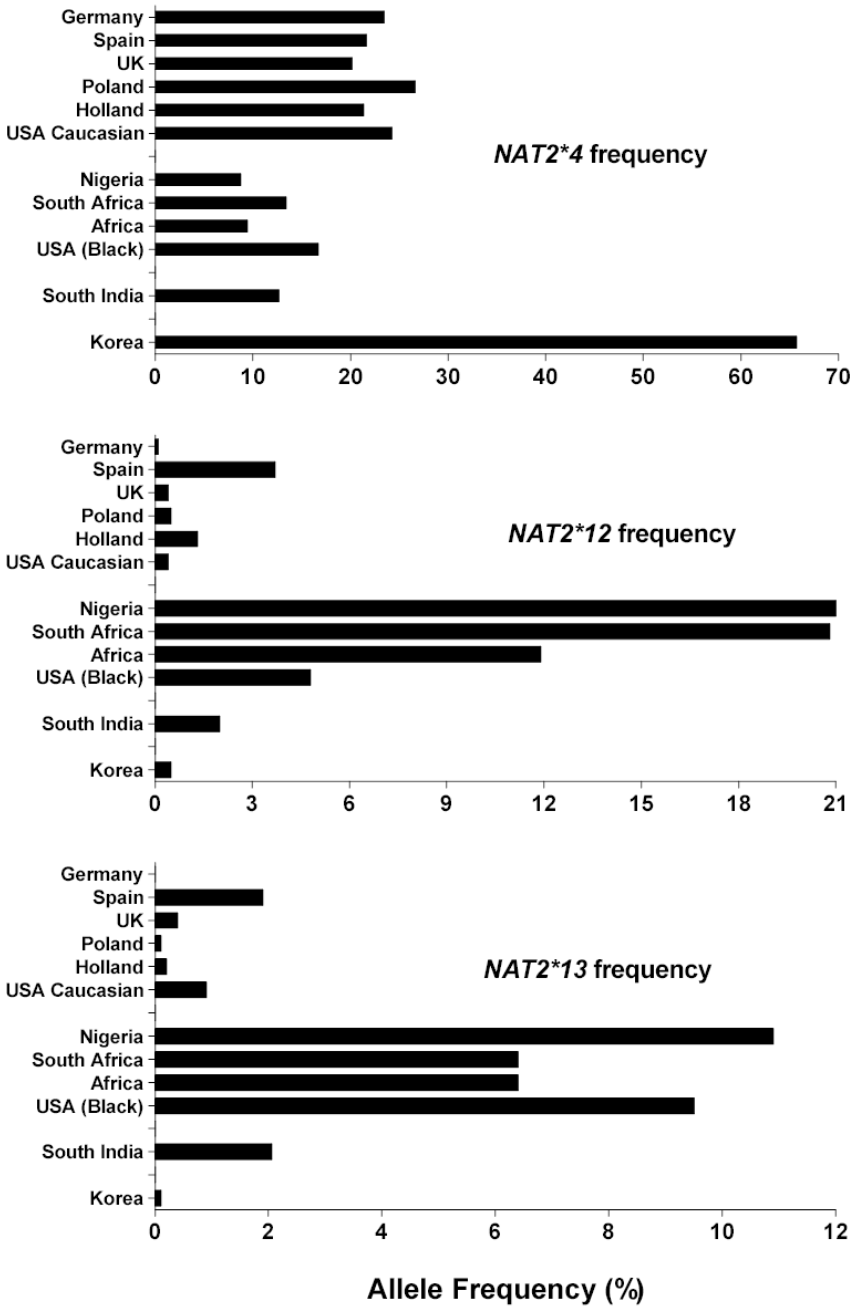
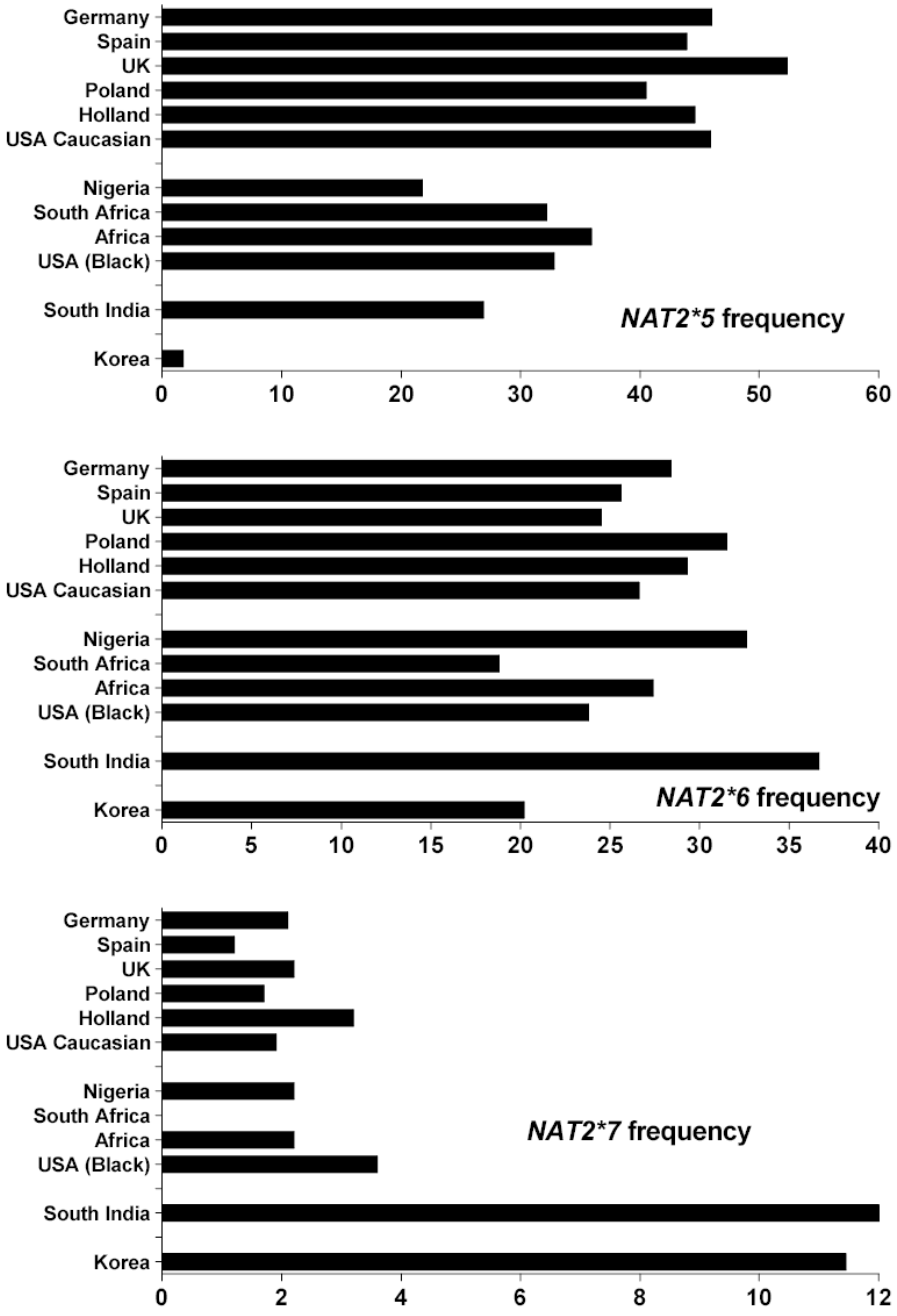


Figure 5.

Rapid acetylator *NAT2* allelic (haplotype) frequencies reported in various populations. Data for each population was derived from the following sources: Germany (Cascorbi *et al.*, 1999); Spain (Agundez *et al.*, 1996); United Kingdom (UK); (Loktionov *et al.*, 2002); Poland (Lan *et al.*, 2003); Holland (van der Hel *et al.*, 2003); USA Caucasian (Deitz *et al.*, 2000); Nigeria (unpublished data from author's laboratory); South Africa (Loktionov *et al.*, 2002); Africa (Delomenie *et al.*, 1996); USA Black (O'Neill *et al.*, 2000); South India (Anitha and Banerjee, 2003) and Korea (Lee *et al.*, 2002).



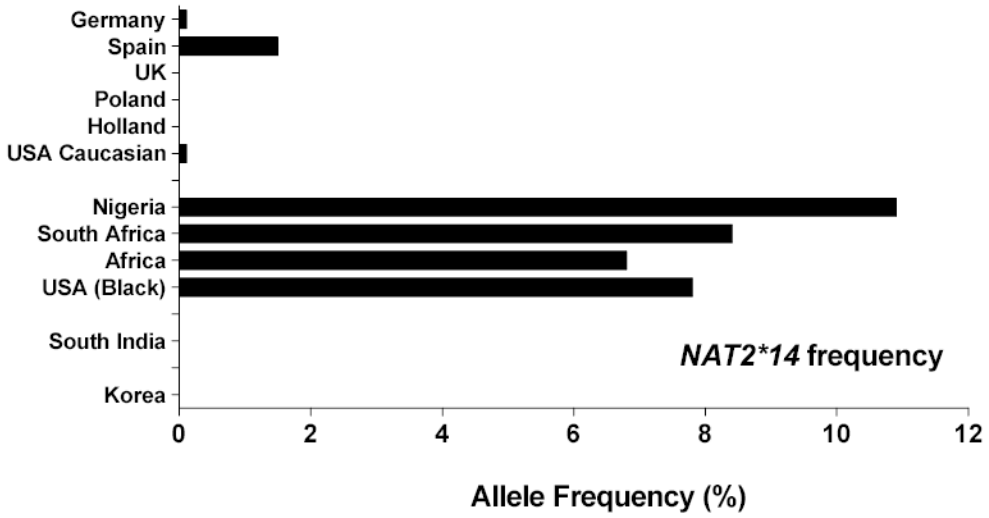


Figure 6.
Slow acetylator *NAT2* allelic (haplotype) frequencies reported in various populations. Data for each population was derived from the same sources listed in Figure 5.

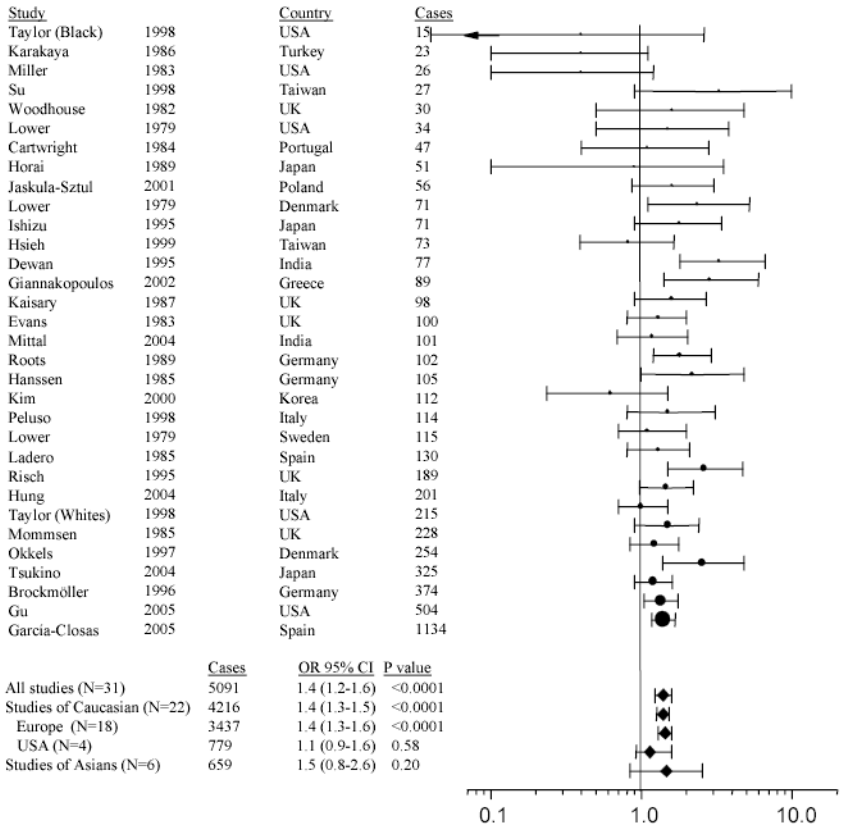


Figure 7.

Meta-analysis of *NAT2* slow acetylator genotype and bladder cancer risk. Odds ratios (circles) with 95% confidence limits (bars) represent the association of slow NAT2 acetylator phenotype/genotype with urinary bladder cancer reported in various studies throughout the world. Studies carried out in various countries are listed in ascending order of case size which is represented visually by circle size. Group analyses of the world total, and of European, American, and Asian subgroups are shown. Modified with permission from Garcia-Closas *et al.*, 2005.

Table 1

Common human *NAT2* alleles (haplotypes)

Allele (haplotype)[a]	Nucleotide Change(s)[b]	Amino Acid Change(s)[c]	Catalytic activity[d]
*NAT2*4*	None	None	High
NAT2*5A	**341T>C**; 481C>T	I114T	Low
NAT2*5B	**341T>C**; 481C>T; 803A>G	I114T; K268R	Low
NAT2*5C	**341T>C**; 803A>G	I114T; K268R	Low
NAT2*6A	282C>T ; **590G>A**	R197Q	Low
NAT2*6B	**590G>A**	R197Q	Low
NAT2*7A	**857G>A**	G286E	Low
NAT2*7B	282C>T; **857G>A**	G286E	Low
*NAT2*12A*	**803A>G**	K268R	High
*NAT2*12B*	282C>T; **803A>G**	K268R	High
*NAT2*12C*	481C>T; **803A>G**	K268R	High
*NAT2*13*	**282C>T**	None	High
NAT2*14A	**191G>A**	R64Q	Low
NAT2*14B	**191G>A**; 282C>T	R64Q	Low

[a] Common *NAT2* alleles (haplotypes) associated with low catalytic activity[d] and slow acetylator phenotype are bolded. Individuals homozygous for these alleles are slow acetylators.

[b] Signature SNP for each allele cluster is bolded.

[c] Amino acid substitutions that confer reduced NAT2 activities are underlined.