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PharmGKB Summary: Very Important Pharmacogene information for *N*-acetyltransferase 2

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Background

Function and expression

Arylamine *N*-acetyltransferases (NATs) are xenobiotic metabolizing enzymes for which three distinct enzymatic activities have been described [1]. The first (EC 2.3.1.5) involves the acetyl coenzyme A (CoA) dependent *N*-acetylation of arylamines and arylhydrazines, a reaction usually associated with xenobiotic detoxification. The second (EC 2.3.1.118) is also acetyl-CoA dependent and involves *O*-acetylation of *N*-hydroxyarylamines [2], typically generated through *N*-oxidation of arylamines by cytochrome P450 enzymes. The third (EC 2.3.1.56) is an acetyl-CoA independent *N*,*O*-acetyltransfer performed on *N*-arylhydroxamic acids, generating highly reactive mutagenic compounds that bind to DNA. NATs have important roles in the metabolism and detoxification of xenobiotics and therapeutic drugs, and are implicated in cancer risk due to their role in the activation or detoxification of carcinogens and their interaction with environmental chemicals [3–5].

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Disclaimers

Two *NAT* genes (*NAT1* and *NAT2*) have been characterized in humans, which differ in gene structure, extent of genetic variation, pattern of developmental and tissue expression [6–8]. Their protein products have different physiological roles, and despite being structurally similar, differences in key residues result in different substrate profiles/ affinities [6, 7, 9]. NAT1 is ubiquitously expressed, and therefore may be involved in homeostasis and development, though levels of expression vary between cell types and tissues [3, 8, 10–12]. NAT2 expression is found predominantly in the liver, small intestine and colon tissues and thus is regarded as a typical xenobiotic metabolizing enzyme [3, 8, 10, 12, 13], though basal *NAT2* mRNA levels can be found in most tissues [2].

Genomic locus organization and protein structure

The genes *NAT1*, *NAT2* and the nonfunctional pseudogene *NATP* (*AACP*) are found on chromosome 8p22 [2, 3, 14, 15]. *NAT1* and *NAT2* share 87.5% coding sequence homology, and around 80% with the corresponding sequence in *NATP* [14]. The *NAT1* gene contains eight non-coding exons upstream of the intronless open reading frame (ORF), resulting in differentially spliced transcripts with the same coding region that can be found in different tissues [16–18]. The *NAT2* gene has one non-coding exon around 8.6kb upstream of the intronless ORF [13, 17, 19]. The two genes have ORFs of 870 nucleotides in length and they encode similar size proteins of 290 amino acids (~30 kDa) (Gene ID 9 and 10) [20, 21]. The crystal structure of human NAT1 and NAT2 proteins, 3-dimensional modeling and docking simulations have provided insight into the functional properties of the two different isoenzymes, revealing a larger substrate binding pocket with a lip in NAT2 compared to NAT1, likely contributing to different substrate specificities [9, 22].

Genetic polymorphisms and phenotype

Both *NAT1* and *NAT2* are polymorphic genes – to date 28 *NAT1* alleles and 88 *NAT2* alleles have been assigned official symbols by the Arylamine *N*-acetyltransferase Gene Nomenclature Committee, according to consensus guidelines [23–25]. *NAT1*4* and *NAT2*4* are the reference (or “wildtype”) alleles for the respective genes, and most variant alleles differ from these by one or more single nucleotide polymorphisms (SNPs).

Many *NAT1* alleles result in a phenotype equivalent to that of reference *NAT1*4* (**20, *21, *23, *24, *25, *27*), some confer a ‘slow’ acetylation phenotype (**14A, *14B, *17, *22*), or result in truncated proteins with no enzymatic activity (**15, *19A, *19B*), and others are undetermined [26]. Despite these polymorphisms, looking across global human populations the *NAT1* sequence seems to be highly conserved, though variation in the 3’-untranslated region (3’UTR) has been maintained [27–29].

In contrast, the *NAT2* gene has a high frequency of functional variation, differing amongst populations that are ethnically diverse, and has high levels of haplotype diversity [27, 28]. SNPs within the *NAT2* gene can affect NAT2 function by resulting in reduced enzyme stability, altered affinity for substrate, or a protein that is targeted for proteosome degradation [2, 30]. *NAT2* genotypes can be grouped into three different phenotypes; ‘slow acetylator’ (two slow alleles), ‘intermediate acetylator’ (1 slow and 1 rapid allele), and ‘rapid’ acetylator (2 rapid alleles, sometimes referred to as ‘fast’) [3]. Some papers simply

report rapid (any genotypes containing *NAT2*4*) and slow (any non-carriers of *NAT2*4*) acetylators, for example; [31]. However, rapid alleles additional to *NAT2*4* have been identified recently (e.g. **11A, *12A-C, *13A, *18*), and heterozygous (intermediate) genotypes seem to display differences in phenotype compared to homozygous rapid (for examples see *Section 4. Caffeine*). In addition, within the slow acetylator genotype group there is heterogeneity in phenotype due to variation in enzyme activity conferred by different alleles [2, 32–34], which may affect the ability to detect significant associations [35].

Early studies report a bimodal pattern of drug acetylation in a given population, and sulfamethazine (SMZ) was described as a suitable probe drug to divide individuals into a slow or rapid acetylator phenotype by plotting serum, urine or liver cytosol acetylation percentages [36–39]. Now, many studies genotype *NAT2* variants to define acetylator phenotype instead, and the SNPs investigated can vary between studies. An economic 4-SNP genotyping panel was reported to accurately predict NAT2 acetylator phenotype in different populations; rs1801280, rs1799930, rs1799931 and rs1801279 (Table 1) [40–42]. Early genotyping methods based on PCR-RFLP typically used *Kpn*I (cuts wildtype allele C at position 481 rs1799929), *Taq*1 (cuts wildtype allele G at position 590 rs1799930) and *BamH*I (cuts wildtype allele G at position 857 rs1799931) enzymes to distinguish *NAT2*4* from the slow alleles described as **5*, **6* and **7*, respectively (for example [43, 44]) or defined as **5B*, **6A*, and **7B*, respectively (for example [45, 46]). However, such approaches may lead to misclassification as the three SNPs they detect are present in numerous *NAT2** alleles (see Table 1). The methodology is also unable to detect other *NAT2* slow alleles, such as *NAT2*14A* and **14B*.

Several studies examining the diversity of *NAT2* haplotypes between different populations and ethnicities support the hypothesis suggesting the *NAT2* slow acetylator phenotype was positively selected for in the transition to an agricultural/ pastoral lifestyle from a hunter-gatherer/ nomadic lifestyle, resulting in changes in diet and thus exposure to different xenobiotics [27, 47–51]. For example, slow acetylator status is higher amongst Tajik populations (agriculturists) compared to Kirghiz populations (nomads) in Central Asia [48], and a high frequency of rapid or intermediate status is observed in hunter-gatherer populations in Western/ Southern Africa (Kung San, Bakola Pygmy, Biaka Pygmy populations) [28, 47]. In India, the frequency of slow acetylators (based on genotype) is higher than rapid acetylators in areas where a vegetarian diet dominates, and the converse is observed in areas where non-vegetarian diet is more frequent [52]. Worldwide *NAT2* allele frequencies are detailed in Table 1, and more detailed information regarding allele frequencies in different populations can be found at http://www.pharmgkb.org/vip/PA18.

It should be noted that the phenotype associated with a particular variant or allele may be specific to particular drugs, and that the designated phenotypes of *NAT1* and *NAT2* alleles are not always consistent in all studies (discussed in detail in [30]). For example, compared with the product of the *NAT1*4* reference allele, the enzyme conferred by *NAT1*11* (as determined by genotyping 445G>A, 459G>A, 640T>G) displays increased acetylation activity against *p*-aminobenzoic acid. However, this effect seems to be substrate specific, as the difference in activity is not statistically significant with the carcinogen *N*-hydroxy-2-

amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (N-OH-PhIP) [53]. Other studies report contradicting results (as discussed in [53]). Another example of inconsistent phenotype is seen with the *NAT2*7* signature variant rs1799931 857G>A, which displays decreased *N*-acetylation of sulfamethazine (SMZ) and decreased *O*-acetylation of the carcinogen N-OH-4-aminobiphenyl *in vitro*, indicating a slow acetylator phenotype. However *O*-acetylation activity against N-OH-PhIP does not differ from NAT2 4 [54]. These results are also reflected in cells which express the *NAT2*7B* allele (rs1799931 857G>A and rs1041983 282C>T) [54]. Regulatory mechanisms, substrate interaction, exposure to xenobiotics and other environmental factors may also influence *NAT1* and *NAT2* allele expression and activity [8, 55].

Another issue is determining phenotype from genotype. *NAT2* alleles are often reported by examining a single SNP, however genotyping for other positions is required to confirm that it is the only variant in order to rule out other positions, and the number of SNPs covered by studies differs (also discussed in [56]). This is particularly important for SNPs that are in *NAT2* alleles with different phenotypes, for example rs1799929 allele T (the signature SNP for *NAT2*11*), is present in several slow and rapid alleles, but alone does not seem to affect acetylation activity (see Table 1) [54].

Pharmacogenetics

Below we describe some of the important pharmacogenetic associations between *NAT1* and *NAT2* genetic variants and drug response, arranged by drug indication. Pharmacogenetic associations between *NAT* polymorphisms and drug responses are predominantly described for NAT2, because of its role in the metabolism of numerous pharmaceuticals, and in Table 1 we focus on important genetic variants of *NAT2*. Further details of individual studies are provided at http://www.pharmgkb.org/gene/PA18. Please note; some studies do not mention *NAT* genotyping or the specific NAT enzyme involved in metabolism, simply reporting acetylation phenotype. However, where possible, we provide specific details for studies that do describe the specific enzyme or genetic variant.

1. Anti-infective agents

1.1 Isoniazid (INH)—The vast majority of *NAT2* pharmacogenetic studies are those that report an association (or lack of) with anti-tuberculosis (anti-TB) drug-induced hepatotoxicity (ATDH), liver injury (DILI), or hepatitis. Standard therapy for TB infection involves a treatment regimen of INH, pyrazinamide, and rifampicin, sometimes with ethambutol or streptomycin, for 2 months, then INH and rifampicin for an additional 4 months [57, 58]. Latent infections can be treated with INH alone [57]. NAT2 has a major role in the metabolism of INH, mediating its biotransformation to the metabolite acetyl-INH, which is hydrolyzed to isonicotinic acid or acetyl-hydrazine [58–62]. Acetyl-hydrazine can be further acetylated to the non-toxic diacetylhydrazine, or hydrolyzed to hydrazine [58–62]. Liver toxicity of INH treatment derives from INH itself (a hydrazine derivative) and its metabolites, including acetyl-hydrazine, hydrazine and ammonia, and is thought to involve the formation of reactive oxygen species that can cause necrosis and autoimmunity [58–60, 63, 64] and may also involve epigenetic effects [65].

Due to reduced metabolism, *NAT2* slow acetylators have reduced clearance and increased exposure to INH and hydrazine compared to rapid acetylators [63, 66–70]. *NAT2* slow acetylator profile (or two slow *NAT2* alleles) has therefore been associated with an increased risk of hepatotoxicity/ liver injury/ hepatitis induced by anti-TB drug treatment as compared to rapid acetylators (and sometimes intermediates) in many studies [43–46, 71–87]. Individual *NAT2* SNPs have also been associated with ATDH (see Table 1).

However, there are numerous contradictory studies that do not find an association between increased risk of ATDH and slow *NAT2* acetylator genotype in TB patients [46, 88–92], or *NAT2* genotype with INH-induced adverse reactions in healthy individuals, despite an association seen between genotype and acetylator phenotype [93]. Meta-analyses suggest there is a significantly increased risk of anti-TB drug induced liver injury/ hepatotoxicity in NAT2 slow acetylators [94–97], but a publication bias for positive results in smaller studies is reported [94, 95]. This, along with allele frequency, definition of hepatotoxicity, study exclusion criteria, drug combination, other genetic variants, population ethnicity, genotyping method, haplotype reconstruction/ allele definition method, and grouping of genotypes into acetylator status, are all factors that may contribute to the differences seen in study outcome.

Despite these inconsistencies, a recent randomized control trial that compared standard INH dosing (n=52) with pharmacogenetic-based dosing (n=47) in Japanese patients supports an association between acetylator status (determined by *NAT2* genotype) and INH treatment outcome. A significant decrease in the incidence of DILI in slow-acetylators and a reduced incidence of persistent positive TB culture (indicating efficacy) in rapid acetylators was observed compared to the corresponding genotype groups on standard dose [98]. Combined, the relative risk of unfavorable events was significantly lower in the pharmacogenetic-based treatment group compared to the standard treatment group, suggesting that *NAT2*–based dosing may be of clinical relevance to enhance INH treatment efficacy and reduce toxicity, though further and more extensive studies in other populations are required [98].

FDA-approved drug labels for INH differ slightly between manufacturers. One does not directly mention the *NAT2* gene, but does mention that slow acetylation may result in higher levels of the drug and therefore an increase in toxic reactions (Remedyrepack Inc.) [99]. Another mentions that rate of acetylation is genetically determined, different ethnicities display differences in rate of inactivation, and that slow acetylation may result in higher blood levels of the drug and therefore an increase in toxic reactions (Mikart Inc.) [100]. Rifater drug labels (a combination of rifampin, INH, pyrazinamide) contain similar information [101]. All labels contain a boxed warning regarding hepatitis associated with INH treatment, but none mention this with regard to *NAT2* or genetic testing.

1.2 Sulfamethoxazole—Sulfamethoxazole is acetylated to *N*-acetylsulfamethoxazole, or oxidized to sulfamethoxazole hydroxylamine by CYP450 enzymes (a reactive metabolite which may result in toxicity) [102]. Recent studies have shown an association between *NAT2* genotypes and sulfamethoxazole pharmacokinetics (PK). In renal transplant patients treated with an immunosuppressive regimen, significantly higher sulfamethoxazole concentrations in slow acetylators (defined as homozygotes or compound heterozygotes for *NAT2*5*, **6*, or **7* variants) are seen compared to rapid acetylators (homozygous *NAT2*4/*

**4*), though the clinical relevance of this is not clear as toxic side effects in this study were not observed [103].

Pneumocystis fungi is commonly found in the respiratory tract of most healthy individuals, however it can cause pneumonia in those who are immune-compromised or receiving immunosuppressive drugs, and is one of the most common infections associated with acquired immunodeficiency syndrome (AIDS) in HIV-infected patients [104]. Co-trimoxazole (sulfamethoxazole combined with trimethoprim) is the choice medication for prophylaxis and treatment of *Pneumocystis* pneumonia, however it is associated with several significant side effects including skin rash, Stevens-Johnson syndrome and hepatic impairment [104]. Different rates of co-trimoxazole induced adverse reactions are reported between ethnicities/ races (higher in Caucasians/ White patients), indicating a possible underlying pharmacogenetic association [105, 106]. Susceptibility to toxicity has been investigated in relation to *NAT2* genotype due to the role of NAT2 in sulfamethoxazole PK. In a study of 48 Caucasian children under 3 years of age, 60% developed adverse reactions when treated with co-trimoxazole for pneumonia infection [107]. *NAT2* variants rs1799930 allele A and rs1799931 allele A were independently found at a significantly higher frequency in children with co-trimoxazole-induced adverse drug reactions (ADRs) compared to those without. Conversely, a significantly higher number of children with no variant alleles were found in the group without ADRs (absence of variant alleles rs1799929 481T, rs1208 803G, rs1799930 590A, rs1799931 857A) [107]. In systemic lupus erythematosus (SLE) patients in Japan who were treated with co-trimoxazole, slow acetylator status (determined in this study by *NAT2* genotypes **6A/*6A*, **6A/*7B*, **7B/*7B*) was associated with an increased risk of adverse events, compared to rapid acetylators (genotypes *NAT2*4/*4*, **4/*5B*, **4/*5E*, **4/*6A*, **4/*7B*) [31]. However, when sequencing the *NAT2* gene, a matched case-control study excluding immuno-compromised patients found no association with individual *NAT2* variants or slow acetylator genotype and risk of hypersensitivity to co-trimoxazole [108]. Some adverse reactions with underlying auto-immune responses are not concentration-dependent, for example carbamazepine-induced Stevens Johnson Syndrome for which individuals with the *HLA-B*5201* allele are at high risk [109]. This may therefore be a factor underlying the lack of association between *NAT2* genotype and hypersensitivity to co-trimoxazole.

Side effects of co-trimoxazole are higher in those with HIV infection compared to those without (Septra drug label) [108, 110], though association with *NAT2* acetylator status and toxicity in HIV patients has been inconsistent. In the majority of studies, no association with co-trimoxazole hypersensitivity (fever and/ or rash, including Stevens-Johnson syndrome) and *NAT2* slow acetylator genotype or individual *NAT2* slow allele frequencies in HIV patients is reported [111–114]. A significant association with risk of co-trimoxazole–induced cutaneous reactions was however seen in AIDS patients with a combined *NAT2* slow acetylator and *GSTM1* null/null genotype [114]. Using dapsone or caffeine as a probe drug, no association with slow acetylator phenotype and co-trimoxazole hypersensitivity is observed in HIV patients [112–115] though one study reports HIV patients who experienced co-trimoxazole hypersensitivity were significantly more likely to have a slow acetylator phenotype than patients who did not experience toxicity [116]. Meta-analyses show no

significant difference in the frequency of slow acetylator phenotype (combining 4 studies) or genotype (combining 3 studies) in HIV patients with or without hypersensitivity to co-trimoxazole [111, 112].

It should be noted that discordance between *NAT2* acetylator genotype and acetylator phenotype has been reported in HIV patients [112–114]. Lower NAT2 activity has been observed in HIV-infected subjects compared to uninfected subjects [117, 118]. Genotyping may also be a factor influencing this discrepancy. In one study, discrepancy between genotype and phenotype (as measured by dapsone as a probe drug) in 8 patients could be resolved in half of the cases by sequencing for other variants, the others were slow genotypes with a borderline rapid phenotype – highlighting the importance of looking at variation across the *NAT2* gene rather than a handful of variants [112].

2. Cardiovascular and hematology agents

Hydralazine—Hydralazine is a vasodilator used to treat hypertension [119, 120]. More recently, due to its epigenetic effects, one group has investigated its use in combination with valproic acid in clinical trials with the hypothesis of reducing tumor resistance and increasing anti-cancer chemotherapy efficacy [121–123]. Its beneficial epigenetic effects in cancer cells are thought to be as an inhibitor of DNA methyltransferase (DNMT) enzymes in order to reactivate tumor suppressor genes silenced by DNA methylation [124], and may also inhibit histone methyltransferase activity [123] and histone acetyltransferases [65]. Hydralazine is thought to be metabolized by two pathways, both of which involve acetylation [125]. One is via direct acetylation, forming the metabolite 3-methyl-s-triazolo [3,4-a]-phthalazine (MTP), and 3-OH-MTP [125, 126]. Another is via oxidation to form an unstable intermediate compound that is acetylated to form *N*-acetylhydrazinophthalazine (NAcHPZ) [125].

Acetylation status has been associated with PK parameters of hydralazine. After oral dose, rapid acetylators display lower hydralazine plasma concentrations and area under the concentration-time curve (but no real difference in drug half life) compared to slow acetylators [119, 125, 127]. MTP/ hydralazine ratio can be used to divide a population into slow and rapid acetylators, with a lower and higher ratio, respectively [128]. In one study, patients with a slow acetylator genotype displayed significant reductions in blood pressure measurements at 24 hours before and after hydralazine, whereas significant effects were not observed in rapid or intermediate acetylators [129]. Three out of a total of four patients who presented hydralazine-induced adverse reactions had a slow acetylator genotype [129]. However, evidence for hydralazine dose adjustment based on acetylator status is not clear. In recent clinical trials in cancer patients, rapid acetylators (according to SMZ-acetylator phenotype) are given more than double the dose of hydralazine than that of slow acetylators. This resulted in similar plasma levels between the two acetylator groups in two studies [122, 127], but significantly higher plasma levels in rapid acetylators in a third study by the same group [121]. In a separate study examining blood pressure and cardiac output, using half doses of hydralazine in SMZ-slow acetylators was ineffective at changing peripheral resistance [130]. A model incorporating multiple clinical factors including acetylator status may better predict dose required for better response to hydralazine [131]. The FDA-

approved BiDil® (contains isosorbide dinitrate and hydralazine hydrochloride) is indicated for the treatment of heart failure in self-identified Black patients (though the genetics behind the mode of efficacy is to our knowledge currently unknown), and the drug label contains information regarding acetylation status explaining that rapid acetylators have lower exposure to the drug, however changes to dosing according to this are not mentioned [132].

Hydralazine treatment is associated with an increased risk of systemic lupus erythematosus (SLE) [133, 134], and this has been associated with acetylator status, though again lacks clear evidence (discussed in [38]). Acetylator status may be related to disease severity, with an increased number of lesions seen in slow SMZ acetylators with discoid LE and SLE [38]. Studies using bacterial strains suggest that hydralazine is detoxified by acetylation to MTP [126]. Other studies also suggest that drug-induced toxic side effects are likely due to hydralazine itself rather than its metabolites – hydralazine and INH both inhibit complement component C4, whereas MTP and *N*-acetyl INH have little inhibitory effect - inhibitory effects on the complement system may contribute to impaired clearance of immune complexes and thus to SLE [7, 135, 136]. Development of anti-nuclear antibody positivity in patients treated with hydralazine has been reported to be more likely and more rapid in slow acetylators compared to rapid acetylators, with occurrence of lupus more likely in slow acetylators [119, 125]. However, further evidence and studies determining the genetic variants behind this association are required. Another potential mechanism behind hydralazine-induced lupus is the reduction of B cell receptor gene rearrangements required for self-tolerance shown in mice models, and transfer of hydralazine treated bone marrow B cells to naïve mice caused autoantibody production compared to vehicle control transferred cells [137]. Slow acetylators may have reduced clearance of hydralazine and thus higher repression of this mechanism compared to rapid acetylators, but again this requires investigation. Another theory suggests hydralazine-derivative (including todralazine and INH) -induced liver injury is due to inhibition of histone acetylation (carried out by histone acetyltransferase (HAT) enzymes), affecting transcription and inhibiting proliferation and thus impairing liver regeneration after hepatotoxicity has occurred [65]. This is supported by slow acetylator mouse models in which todralazine treatment did not induce liver failure on its own, however in mice with anti-CD95 induced liver injury, resulted in mortality, smaller livers and impaired histone acetylation compared to controls despite similar alanine transaminase (ALT) levels [65]. The role of HATs, their cofactors, and histone acetylation in liver regeneration after toxic injury has been shown in other studies [138, 139]. This may be another contributing factor to drug-induced liver injury that affects association with *NAT2* genotype. Toxicity of hydralazine and related compounds is likely a combination of formation of reactive species, triggering of immune responses/ autoimmunity, and epigenetic effects.

3. Pain, anti-inflammatory and immunomodulating agents

Sulfasalazine—Sulfasalazine is indicated for the treatment of ulcerative colitis, Crohn's disease and as a second-line treatment for arthritis (DrugBank [140–142]), [143]. It is a combination of 5-aminosalicyclic acid and sulfapyridine linked together by an azo bond [125, 143]. Gut bacteria split the bond, a mechanism thought to deliver the two compounds at higher concentrations to the colon than if administered alone [143, 144]. The effective

derivative of sulfasalazine is considered to be 5-aminosalicylic acid, the majority of which remains in the colon where it is subject to *N*-acetylation by NAT1 [125, 145]. The second derivative, sulfapyridine, is readily absorbed and converted to *N*-acetyl-sulfapyridine, a process influenced by NAT2 acetylator status [125, 143].

Sulfasalazine PK is not influenced by *NAT2* polymorphisms, however, metabolism of sulfapyridine to *N*-acetyl-sulfapyridine is significantly reduced in slow acetylators (carriers of two variant alleles *NAT2*5B*, *6A*, *7B* or *5*, *6* and *7*) compared to both intermediate (one variant and one *NAT2*4* allele) and rapid acetylators (*NAT2*4/*4*) [146, 147]. Slow acetylators have higher concentrations and elimination half-life of sulfapyridine (based on genotyping *NAT2* SNPs rs1041983, rs1801280, rs1799929, rs1799930, rs1799931) [148]. Plotting of the metabolic ratio *N*-acetyl-sulfapyridine/ sulfapyridine against *NAT2* genotype gives two distinct groups – rapid and slow acetylators [148]. There may therefore be an association between increased risk of sulfasalazine-induced toxicity and higher concentrations of sulfapyridine observed in slow acetylators [125, 143]. A prospective study in Japan of female rheumatoid arthritis (RA) patients treated with sulfasalazine identified 4 patients who had adverse events in a one year period - none had the *NAT2*4* allele, each carrying two variant alleles [149].

4. Caffeine

Paraxanthine, a metabolite of caffeine, can undergo acetylation by NAT2 to form 5-acetylamino-6-formylamino-3-methyluracil (AFMU) (see PharmGKB Caffeine Pathway, Pharmacokinetics http://www.pharmgkb.org/pathway/PA165884757) [150]. Caffeine can be used as a non-toxic probe drug *in vivo* for predicting acetylator phenotype; by measuring metabolite ratio AFMU/1-methyl xanthine (1X) in urine after caffeine consumption, a bi- or tri-modal pattern in a given population is observed [39, 115, 151]. AFMU/AFMU+1X+1-methyluric acid (1U), AFMU+5-acetylamino-6-amino-3-methyluracil (AAMU)/AFMU +AAMU+1X+1U or AAMU/ AAMU+1X+1U metabolite ratios can also be used to determine acetylator phenotype [152–156]. Variability in NAT2 activity (as determined by caffeine AFMU/AFMU+1X+1U ratio) between different populations exists - significantly higher NAT2 activity is observed in Koreans compared to Swedes, and this may be due to a higher proportion of the *NAT2*4* rapid allele in Koreans and the higher frequency of slow acetylator genotype in Swedes [153]. Some studies report good concordance between acetylator phenotype determined by caffeine metabolite ratio and *NAT2* genotype [155, 157], however others show discordance [114, 154, 158–161]. These discrepancies may be due to differences in sample collection and handling, laboratory techniques and conditions, genotyping method, differences in assignment of slow/intermediate/rapid to genotypes based on *NAT2* allele combinations, whether heterozygotes are analyzed independently, as well as other genetic, disease state, environmental factors or use of drugs that could affect the caffeine metabolism pathway (as discussed in [30, 158, 160, 162–164]). In one study, up to 54% of the variation in acetylation activity determined by caffeine test could be explained by *NAT2* genotype (homozygous wildtype, homozygous variant or heterozygous determined by PCR-RFLP), though phenotype variation was seen with homozygous wildtype [162].

Cancer: NAT1 and NAT2 association with risk, treatment responses, treatment resistance and as drug targets

Due to their role in the activation or deactivation of xenobiotics, the NAT1 and NAT2 enzymes have been implicated in chemical carcinogenesis pathways. Polymorphisms in the *NAT1* and *NAT2* genes have therefore been investigated for an association with cancer risk, though findings are inconsistent likely due to the complex nature of cancer etiology and the multiple factors that contribute to susceptibility.

For studies examining the risk of bladder cancer, some report a significant association with NAT2 slow acetylator genotype/ phenotype (e.g. [165]), others do not after adjusting for multiple factors [35, 166]. Recent GWAS meta-analyses reveal multiple risk loci, including *NAT2* [167]. A meta-analysis of cases in the general population (n=5594) showed a significant association between NAT2 slow acetylation with risk of bladder cancer (OR=1.37, C.I.=1.22–1.54, $p=2\times10^{-7}$) [168]. The rs1495741 AA genotype (located downstream of the *NAT2* gene and associated with the slow acetylator status) was significantly associated with increased risk of bladder cancer in Europeans compared to those with AG or GG genotype [169], and a GWAS meta-analysis consisting of 12,270 cases and 55,059 controls confirmed the association with the A risk allele, along with numerous other SNPs at other loci that contribute to risk [167]. Furthermore, both an additive and multiplicative association was shown between smoking and rs1495741 allele A with risk of bladder cancer [167]. This GWAS meta-analysis study did not identify risk alleles associated with *NAT1*, and a meta-analysis of 11 studies (n=3311 cases, n=3906 controls) found no association between bladder cancer and the *NAT1*10* allele [170]. However, *NAT1*14A* has been associated with increased risk of bladder cancer in Lebanese men [171–173].

Associations between *NAT1/2* variants and susceptibly to other cancers also lack clarity or require further study [5, 12, 174, 175]. For example, NAT2 slow acetylator genotype may be a small, low penetrance risk factor for head and neck cancer [176]. Mixed results are reported for *NAT2* genotype and risk of breast cancer [177, 178] and esophageal cancer [179–181]. The InterLymph Consortium found no association between NAT2 phenotype (based on genotype, 4421 cases, 4095 controls) or *NAT1*10* (1528 cases, 1586 controls) and risk of non-hodgkin lymphoma [182].

Gene-environment interactions for cancer risk have been reported in an attempt to identify risk factors [175]. For example, individuals with a *NAT1* rapid acetylator genotype (defined as homozygous for alleles *NAT1*10, *11*, or these alleles in combination with *NAT1*3, *4*), and *AHR* rs2066853 genotype GA or AA, and high meat consumption were found to have an increased risk of concurrent adenomatous and hyperplastic colorectal polyps [183]. Conversely, meta-analyses show no statistically significant interaction between NAT1 acetylator phenotype and meat intake (2 studies), or NAT2 acetylator phenotype and meat intake (3 studies), with relation to risk of colorectal cancer [184], though this may be due to low penetrance and the need to include multiple genetic risk factors.

As well as combinatorial environmental/ genetic factors, reaction context is also an important consideration - examining the site of action and specific reaction by NAT1/ NAT2

may make these associations clearer and more consistent. For example, *O*-acetylation by NAT1 can result in the formation of nitrenium ions from the unstable *N*-acetoxyarylamine which can react with DNA to cause mutations, whereas *N*-acetylation by NAT1 detoxifies aromatic amines [2, 185]. Similarly, *O*-acetylation of *N*-hydroxy-heterocyclic amine carcinogens by NAT2 in the colon can explain the association between rapid acetylator phenotype and colorectal cancer risk in those who consume well-done meat, whereas association with slow acetylator phenotype and bladder cancer in smokers or those exposed to chemical dyes can be explained by *N*-acetylation competing with *N*-hydroxylation by cytochrome P450 enzymes that produce aromatic amine carcinogens in the liver [2]. *N*-acetylation of an aryldiamine (for example benzidine) could increase risk of bladder cancer due to enhancement of *N*-hydroxylation, whereas *N*-acetylation of an arylmonoamine may have the opposite effect [2, 168]. It should also always be kept in mind that ‘slow’ and ‘rapid’ acetylator phenotype is not homogenous, and that if the underlying genetic polymorphisms affect enzyme-substrate affinity, then the resulting association may only be seen with some drugs/ chemicals and exposure levels [2]. NAT1 activity is influenced by substrate-dependent down-regulation, the redox state of cells, and epigenetic regulation [12], thus these may contribute to the lack of consistency seen between a direct association between *NAT1* genotype and cancer risk, along with interacting environmental factors, other genetic polymorphisms, inconsistencies in allele-phenotype definitions or genotyping methods. For instance, attributing the rapid acetylation phenotype to the *NAT1*10* and **11* alleles remains an issue of controversy among investigators and the phenotypic effects of many *NAT1* polymorphisms (especially those in the 3' untranslated region of the gene) are still not well understood [2]. Cell-specific expression of alleles, alternative *NAT1* transcripts driven by different promoters or alternative polyadenylation site use may also be a factor, or if SNPs are missed in genotyping, for example misclassification of *NAT1*10B* for *NAT1*10* [2, 185].

Overexpression of *NAT1* is a common finding in estrogen receptor positive breast tumors [7, 186]. Cells over-expressing NAT1 display resistance to etoposide *in vitro* [187], and thus NAT1 activity may have implications in response to anti-cancer therapy - polymorphisms in the *NAT1* gene that result in changes in enzyme activity could affect drug response, though this needs to be investigated. These, and studies that show an association between increased NAT1 expression/ activity and cancer cell proliferation, support the use of specific NAT1 probes as potential diagnostic tools and the development of direct NAT1 inhibitors as potential leads for cancer therapeutics [4, 7, 12, 187–191]. Though not their primary target, several current chemotherapeutics have been shown to inhibit NAT1 or *N*-acetyltransferase activity *in vitro* in human cancer cells; cisplatin [192], tamoxifen [193, 194].

Amonafide has anti-cancer properties but is no longer in clinical development due to failing to reach phase III clinical trial primary end points [195]. The drug displayed variable and unpredictable toxic effects [196]. *NAT2* phenotype was one of the underlying genetic factors contributing to variation in myelosuppression severity; rapid acetylators (determined by caffeine test) were susceptible to greater toxicity and counterintuitively displayed higher plasma concentrations of amonafide. This was thought to be due to production of the metabolite *N*-acetyl amonafide which inhibits the oxidation of amonafide by CYP1A2 [196–

198]. Thus, higher and lower doses from the standard dosage were recommended in slow and rapid acetylators, respectively, and a pharmacodynamic model incorporating acetylator phenotype, gender and pre-treatment white blood cell count was developed [199, 200]. The story from this drug highlighted the importance of genetic influence on both drug pharmacokinetics and pharmacodynamics [196, 200].

NAT2 polymorphisms/ acetylator phenotype has been associated with risk of other complex multifactorial diseases (including asthma, Parkinson's Disease and diabetes), however the associations are inconclusive and further discussion of these is beyond the scope of this review [201, 202].

Summary

NAT1 and NAT2 are polymorphic enzymes with important roles in the deactivation or activation of numerous xenobiotics in humans. Due to expression of the isoenzyme in the liver, the genetic variants of *NAT2* have primarily been associated with drug metabolism, response and toxicity. *NAT2* genotype confers a slow, intermediate or rapid acetylation phenotype, resulting in differences in drug metabolic rates and susceptibility to drug toxicity. However, studies show inconsistencies for which *NAT2* and *NAT1* variants are genotyped and in the pooling of variants into phenotype groups, thus these factors along with how a patient's disease phenotype is defined, environmental factors, drug-drug interactions, and acetylation reaction context may contribute to the contradictory evidence for some pharmacogenetic and disease associations. Further studies are required to help determine whether genotyping of *NAT2* is clinically useful for determining a patient's dosage for efficacy of treatment and to avoid drug toxicity.

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Table 1

Important *NAT2* variants with pharmacogenetic associations.

***NAT2* Variant**[a,b,c]	**Signature allelic group**[d]	***NAT2* alleles**[a]	**NAT2 acetylator phenotype**[a]	**Global Allele Frequency**[e]	**Pharmacogenetic Associations and other information**
NA	Reference allele	**4*	Rapid	0.28	Often referred to as "wild type". Studies often genotype for several variants, and if these are not seen an individual is said to have the **4* allele, for example in [31, 103]. Therefore the *NAT2*4* group may include more rare variants that have not been sequenced/ covered. *NAT2*4* is found at a lower frequency in patients with anti-TB drug-induced hepatotoxicity compared to patients without hepatotoxicity [86].
rs1801280 341T>C Ile114Thr	**5*	**5A*-**5V*, **14C*, and **14F*.	Slow acetylator; **5A*-**5J*, **14C*, **14F*. Not known; **5K*-**5V*. This variant is often grouped for analysis with other *NAT2* variants that also confer a slow acetylation phenotype.	**5A* and **5C*; 0.022 and 0.028, respectively. **5B;* 0.32. **5D, *5E, *5M* are very rare alleles, with global frequencies of 0.00025 (**5D*), and 0.00013 (**5E* and **5M*).	This SNP is part of a genotyping panel of 4 SNPs for predicting acetylator phenotype [40–42]. This variant either on its own or when combined with other variants in the *NAT2*5B* haplotype (variants rs1799929 481T and rs1208 803G that alone do not confer reduced activity) results in a slow acetylator phenotype when assayed with SMZ, and carcinogens N-OH-4-aminobiphenyl and N-OH-PhIP in COS-1 cells [54]. Molecular dynamic simulation suggests that the conformation of the enzyme's catalytic residues and cofactor binding site are not dramatically different from wild type protein [203], and mRNA expression or thermostability of the protein is not decreased [2, 54]. Reduction in enzyme activity may therefore be due to increased degradation of the protein [2, 54]. In a cohort of Brazilian TB patients, rs1801280 allele C (**5* group) and rs1799930 allele A (**6* group), as well as the non-functional rs1041983 allele T and rs1799929 allele T (in linkage disequilibrium with the functional alleles), were all found at a significantly higher frequency in patients with anti-TB drug-induced hepatotoxicity (ATDH) compared to those without [71]. Clonazepam metabolism involves acetylation of the metabolite 7-amino clonazepam (7-AM), and slow acetylators (as determined by SMZ probe drug) excrete more 7-amino clonazepam and less 7-acetamidoclonazepam compared to rapid acetylators [37]. *In vitro* studies have confirmed the role of the NAT2 enzyme in 7-AM acetylation, and that *NAT2*5B* and

NAT2 Variant[a,b,c]	Signature allelic group[d]	*NAT2* alleles[a]	NAT2 acetylator phenotype[a]	Global Allele Frequency[e]	Pharmacogenetic Associations and other information
					*NAT2*6A* have significantly impaired metabolism have significantly impaired metabolism [204].
rs1799930, 590G>A, Arg197Gln	**6*	**6A-*6T, *5E, *5J, *5P-*5R, *5U* and **14D.*	Slow acetylator; **5E, *5J, *6A-*6E, *14D*. Unknown; all other alleles. This variant is often grouped with other variants that confer a slow acetylator phenotype.	**6A;* 0.26. **6B* and **6C;* 0.0020 and 0.00025, respectively.	This SNP is part of a genotyping panel of 4 SNPs for predicting acetylator phenotype [40–42]. This variant either on its own or when in the *NAT2*6A* haplotype (with variant rs1041983 282T that alone does not confer reduced activity) results in a slow acetylator phenotype when assayed with SMZ, and carcinogens N-OH-4-aminobiphenyl and N-OH-PhIP in COS-1 cells [54]. This variant results in a distorted enzyme cofactor binding site and reduced thermostability [30, 54, 203]. This variant has been associated with risk of ATDH in several studies, due to the role of NAT2 in INH metabolism. Korean patients with TB and genotype AA or GA displayed significantly reduced acetylation and clearance of INH compared to those with genotype GG, and allele A was associated with increased risk of ATDH [205]. Genotypes AA and GA are associated with increased risk of ATDH in Turkish and Tunisian TB patients [45, 78]. This association was also observed in Chinese patients, but was not statistically significant after controlling for gender [84]. In a cohort of Brazilian TB patients, rs1801280 allele C (*5 group) and rs1799930 allele A (*6 group), as well as the non-functional rs1041983 allele T and rs1799929 allele T (in linkage disequilibrium with the functional SNPs), were all found at a significantly higher frequency in patients with anti-TB drug-induced hepatotoxicity (ATDH) compared to those without [71]. A *NAT2* haplotype made up of two promoter SNPs rs4646244 allele A and rs4646267 allele A, and one coding region SNP rs1799930 allele A (conferring a slow acetylator phenotype), is significantly associated with anti-TB drug-induced hepatitis, and correlates with decreased acetylation and clearance of INH [205]. Clonazepam metabolism involves acetylation of the metabolite 7-amino clonazepam (7-AM), and slow acetylators (as determined by SMZ probe drug) excrete more 7-amino clonazepam and less 7-acetamidoclonazepam compared to rapid acetylators [37]. *In vitro* studies have

NAT2 Variant[a,b,c]	Signature allelic group[d]	*NAT2* alleles[a]	NAT2 acetylator phenotype[a]	Global Allele Frequency[e]	Pharmacogenetic Associations and other information
					confirmed the role of the NAT2 enzyme in 7-AM acetylation, and confirmed the role of the *NAT2**5 and *NAT2**6A have significantly impaired metabolism [204]. A study investigating environmental and genetic effects on fecundability in women at risk of pregnancy, found alcohol consumption and smoking were shown to significantly reduce fecundability only in slow acetylators (those without *NAT2**4* as determined by genotyping rs1799929 allele T, rs1799930 allele A and rs1208 allele G), and this interaction with *NAT2* acetylator status was not seen for caffeine [206].
rs1799931 857G>A, Gly286Glu	**7*	**7A*-**7G*, **5S*, **6I*, **6J*, **6S* and **6T*.	**7A* and **7B*; slow acetylator phenotype but this may be dependent on substrate. Unknown phenotype; all other alleles. This variant is often grouped for analysis with variants that confer a slow acetylation phenotype.	**7A*; 0.0010, **7B;* 0.040.	This SNP is part of a genotyping panel of 4 SNPs for predicting acetylator phenotype [40–42]. This variant confers an enzyme with a slightly distorted substrate binding pocket and reduced activity for some substrates, decreased thermostability and protein levels [2, 30, 54, 203]. The phenotype conferred by this variant seems to be dependent on substrate – NAT2 857A is a slow acetylator of SMZ and N-OH-4-aminobiphenyl, but no difference in O-acetylation activity against N-OH-PhIP is seen compared to NAT2 4 in transfected COS-1 cells [54]. The frequency of allele A is significantly higher in Taiwanese patients with ATDH compared to those without [73]. A TB patient who developed severe ATDH requiring a liver transplant was found to have *NAT2* rs1799929 genotype CT and rs1799931 genotype AG along with *ABCB1* rs1045642 genotype AG [207]. However, other studies have seen no statistically significant association with the SNP and ATDH [84, 93, 205, 208]. This SNP is associated with toxicity of docetaxel and thalidomide treatment, or docetaxel treatment alone, in an investigative genotyping screen in patients with castration-resistant prostate cancer (though the risk allele was not described) [209].
rs1799929 481C>T Leu161Leu	**11*	**11A*-**11B*, also found in **5A*, **5B*, **5F*-**5I*, **5L*-**5P*, **5U*, **5V*, **6E*, **6N*, **6R*, **6T*, **7E*,	This variant is in alleles with different phenotypes. Slow acetylator; **5A*, **5B*, **5F*, **5G*, **5H*, **5I*, **6E*, **14C*. Rapid acetylator; **11A*, **12C*. Not known; all other alleles.		In transfected COS-1 cells, NAT2 protein with this SNP displays similar protein levels and activity against SMZ, and carcinogens N-OH-4-aminobiphenyl and N-OH-PhIP [54] as NAT2 4 [2, 54], however may be associated with slow acetylator

NAT2 Variant[a,b,c]	Signature allelic group[d]	*NAT2* alleles[a]	NAT2 acetylator phenotype[a]	Global Allele Frequency[e]	Pharmacogenetic Associations and other information
		**7F, *12C, *12M, *14C* **7F, *12C, *12M, *14C* **14I.*			alleles due to the presence of a functional variant, for example combined with the rs1801280 341C variant in the *NAT2*5B* haplotype [54]. In a cohort of Brazilian TB patients, rs1801280 allele C (**5* group) and rs1799930 allele A (**6* group), as well as the non-functional rs1041983 allele T and rs1799929 allele T (in linkage disequilibrium with the functional SNPs), were all found at a significantly higher frequency in patients with anti-TB drug-induced hepatotoxicity (ATDH) compared to those without [71]. Genotype TT is reported to be associated with an increased risk of ATDH [45, 86], but this association is likely due to linkage with functional SNPs conferring a slow acetylator phenotype. A TB patient who developed severe ATDH requiring a liver transplant was found to have *NAT2* rs1799929 genotype CT and rs1799931 genotype AG along with *ABCB1* rs1045642 genotype AG [207]. Other studies find no association with INH-induced adverse reaction or ATDH and this SNP [93, 208]. A study investigating environmental and genetic effects on fecundability in women at risk of pregnancy, found alcohol consumption and smoking were shown to significantly reduce fecundability only in slow acetylators (those without *NAT2*4* as determined by genotyping rs1799929 allele T, rs1799930 allele A and rs1208 allele G), and this interaction with *NAT2* acetylator status was not seen for caffeine [206].
rs1208 803A>G Lys268Arg Please note: on dbSNP this is 803G>A Arg268Lys , however the *NAT2*4* reference allele has allele A at this position.	**12*	**12A-*12M, *5B, *5C, *5F-*5I, *5L-*5R, *5T, *5U, *6C, *6F, *6R, *7C, *7F, *14C, *14E-*14G* and **14I.*	This variant is in alleles with different phenotypes. Slow acetylator; **12D, *5B, *5C, *5F-*5I, *6C, *14C, *14E, *14F, *14G.* Rapid acetylator; **12A-*12C.* Not known; all other alleles.		The *NAT2*12* allele is associated with a rapid acetylator status, though there are some controversial reports, as discussed in [30, 158]. The rs1208 allele G is present in multiple *NAT2** alleles that confer different phenotypes, thus coverage of the other SNPs in these alleles is required. In transfected COS-1 cells, NAT2 protein with this SNP displays similar protein levels and activity against SMZ, and carcinogens N-OH-4-aminobiphenyl and N-OH-PhIP as NAT2 4 [2, 54], however may be associated with slow acetylator alleles due to the presence of a functional variant, for example combined with the rs1801280 341C variant in the *NAT2*5B* haplotype [54].

***NAT2* Variant**[a,b,c]	**Signature allelic group**[d]	***NAT2* alleles**[a]	**NAT2 acetylator phenotype**[a]	**Global Allele Frequency**[e]	**Pharmacogenetic Associations and other information**
					This SNP was not associated with increased risk of INH-induced a This SNP was not associated with increased risk of INH-induced a 93] A study investigating environmental and genetic effects on fecundability in women at risk of pregnancy, found alcohol consumption and smoking were shown to significantly reduce fecundability only in slow acetylators (those without *NAT2*4* as determined by genotyping rs1799929 allele T, rs1799930 allele A and rs1208 allele G), and this interaction with *NAT2* acetylator status was not seen for caffeine [206].
rs1041983 282C>T Tyr94Tyr	**13*	**13A-*13C, *5G, *5J, *5K, *5P, *5R, *5T-*5V, *6A, *6C, *6D, *6G-*6O, *6Q, *6R, *7B-*7G, *12B, *12E, *12M, *14B, *14D, *14G, *14H* and **14J*.	This variant is in alleles with different phenotypes. Slow acetylator; **5G, *5J, *6A, *6C, *6D, *7B* (substrate specific), **14B, *14D, *14G*. Rapid acetylator; **12B, *13A*. Not known; all other alleles.		Assignment of **13* as a rapid allele has been controversial, as discussed in [158]. In transfected COS-1 cells, NAT2 protein with this SNP displays similar protein levels and activity against SMZ, N-OH-4-aminobiphenyl, and N-OH-PhIP as NAT2 4 [2, 54]. In a cohort of Brazilian TB patients, rs1801280 allele C (**5* group) and rs1799930 allele A (**6* group), as well as the non-functional rs1041983 allele T and rs1799929 allele T (in linkage disequilibrium with the functional SNPs), were all found at a significantly higher frequency in patients with anti-TB drug-induced hepatotoxicity (ATDH) compared to those without [71]. Genotype TT has been associated with increased risk of ATDH in TB patients compared to the patients with the CC genotype [84], which is likely due to linkage with functional SNPs conferring a slow acetylator phenotype.
rs1801279 191G>A Arg64Gln	**14*	**7D, *14A-*14J*	Slow acetylator; **14A-*G*. Not known; **14H-J, *7D*.	**14A* and **14B* alleles; 0.0040 and 0.0045, respectively.	The rs1801279 SNP is part of a genotyping panel of 4 SNPs for predicting acetylator phenotype [40–42]. In transfected COS-1 cells with this SNP, cells have significantly reduced NAT2 protein (minimal levels) and thermostability compared to NAT2 4 cells, and the SNP confers a slow acetylator phenotype when NAT2 is assayed with SMZ, and carcinogens N-OH-4-aminobiphenyl and N-OH-PhIP [2, 54]. This SNP was not associated with increased risk of ATDH [208].
rs4646244 -1144T>A described as -9796T>A in [205]					This SNP is upstream of the *NAT2* gene and the A allele may result in decreased transcription, as shown in *in vitro* promoter assays [205]. Allele A is associated with an increased risk of drug-induced hepatitis in

NAT2 Variant[a,b,c]	Signature allelic group[d]	*NAT2* alleles[a]	NAT2 acetylator phenotype[a]	Global Allele Frequency[e]	Pharmacogenetic Associations and other information
					TB patients, correlating with decreased acetylation and clearance o TB patients, correlating with decreased acetylation and clearance o TB patients, correlating with decreased acetylation and clearance o TB patients, correlating with decreased acetylation and clearance o *NAT2* genotype) [205]. This effect may be influenced by linkage with the A allele of the rs1799930 SNP that confers a slow acetylator phenotype, which also showed a significant association with risk of drug-induced hepatitis in TB patients and decreased clearance of INH in the same study [205]. A haplotype containing allele A of both SNPs was significantly associated with increased risk of anti-TB drug-induced hepatitis [205].
rs1495741 G>A (NC_00000 8.10:g.182 72881)					This SNP is downstream of the *NAT1* and *NAT2* genes. This variant was identified as a tag SNP for predicting *NAT2* acetylator phenotype in individuals with European background [169], and may also apply to other populations as demonstrated by a study in Taiwan [79]. Genotype AA demonstrates linkage with the slow acetylator phenotype and GG with the rapid acetylator phenotype, when assayed with SMZ *in vitro* or INH *in vivo* [40, 79, 169]. However, compared to using combinations of *NAT2* alleles, this SNP is only around 78% accurate as a predictive marker for acetylation status [40]. Genotype AA was associated with an increased risk of ATDH in a group of 348 TB patients [79]. Genotype AA was associated with increased risk of bladder cancer compared to the GG or AG genotype in a European cohort of 1097 cases and 1077 controls [169]. These associations are likely indirect, tagging a NAT2 slow acetylator phenotype or linked to another functional region.
rs4271002 -594G>C (described as - 9246G>C in [210].					This SNP is upstream of the *NAT2* gene. An association between *NAT2* genotype and aspirin intolerance was shown in a study of Korean asthmatics that genotyped 14 *NAT2* SNPs – allele C of rs4271002 and a haplotype containing this allele as the only variant allele was associated with an increased risk of aspirin-intolerant asthma [210]. The functional consequence of this SNP is currently unknown, but it may affect transcription [210].

More information regarding *NAT1* and *NAT2* pharmacogenetic associations can be found at http://www.pharmgkb.org/gene/PA17 and http://www.pharmgkb.org/gene/PA18, respectively. Please note; associations in this table are those reported for the individual SNPs rather than studies that grouped SNPs and compared slow and rapid acetylators.

[a] Information regarding variant positions, rsIDs, alleles and phenotypes are from the Consensus Human Arylamine *N*-Acetyltransferase Gene Nomenclature website http://nat.mbg.duth.gr/ (accessed May 2013).

[b] All positions given use *NAT2* reference sequences: NM_000015.2:c, NP_000006.2:p, and NC_000008.10, unless otherwise stated.

[c] Some SNP position information was also added from dbSNP: http://www.ncbi.nlm.nih.gov/projects/SNP/

[d] Studies often genotype for several variants, and if these are not seen an individual is said to have the **4* allele, for example in [31, 103]. Therefore the *NAT2*4* group may include more rare variants that have not been sequenced/ covered. Many studies use the signature allelic group term for carriers of the particular SNP variant allele, though under the *NAT2* nomenclature each allele is denoted with a letter subcategory. Alleles consisting of one SNP variant are often reported, however genotyping for other positions is required to confirm that it is the only variant in order to rule out other alleles that have this variant. This is particularly important for SNPs that are in *NAT2* alleles with different phenotypes, for example rs1799929 allele T (the signature SNP for *NAT2*11*), is present in several slow and rapid alleles.

[e] All global allele frequencies were calculated from data given in [49], in which *NAT2*4* was defined as positions 191G, 282C, 341T, 481C, 590G, 803A, 857G.