HHS Public Access

Author manuscript

Pharmacogenet Genomics. Author manuscript; available in PMC 2019 October 01.

Published in final edited form as:

Pharmacogenet Genomics. 2018 October ; 28(10): 238–244. doi:10.1097/FPC.0000000000000350.

Functional expression of human arylamine N-acetyltransferase *NAT1*10* and *NAT1*11* alleles: a minireview

David W. Hein[a], **Giannoulis Fakis**[b], and **Sotiria Boukouvala**[b]

[a]Department of Pharmacology & Toxicology, University of Louisville Health Sciences Center, Louisville, Kentucky, USA.

[b]Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, Greece.

Abstract

The arylamine N-acetyltransferase (NAT) nomenclature committee assigns functional phenotypes for human *NAT1* alleles in those instances in which the committee determined a consensus has been achieved in the scientific literature. In the most recent nomenclature update, the committee announced that functional phenotypes for *NAT1*10* and *NAT1*11* alleles were not provided due to lack of consensus. Phenotypic inconsistencies observed among various studies for *NAT1*10* and *NAT1*11* may be due to variable allelic expression among different tissues, the limitations of the genotyping assays (which mostly relied on techniques not involving direct DNA sequencing), the differences in recombinant protein expression systems used (bacteria, yeast, mammalian cell lines) and/or the known inherent instability of human NAT1 protein which requires very careful handling of native and recombinant cell lysates. Three recent studies provide consistent evidence of the mechanistic basis underlying the functional phenotype of *NAT1*10* and *NAT1*11* as "increased-activity" alleles. Some *NAT1* variants (e.g. *NAT1*14*, *NAT1*17,* and *NAT1*22*) may be designated as "decreased-activity" alleles and other *NAT1* variants (e.g., *NAT1*15* and *NAT1*19*) may be designated as "no-activity" alleles compared to the *NAT1*4* reference allele. We propose that phenotypic designations as "rapid" and "slow" acetylator should be discontinued for *NAT1* alleles, although these designations remain very appropriate for N-acetyltransferase 2 (*NAT2*) alleles.

Genetic variants of the arylamine N-acetyltransferases are expressed in human populations and a consensus nomenclature for arylamine N-acetyltransferase 1 (*NAT1*) and 2 (*NAT2*) alleles or haplotypes was initially published in *Pharmacogenetics* over 20 years ago [1]. Subsequently, additional *NAT1* and *NAT2* alleles appeared in the scientific literature. In order to achieve consensus for identification and naming of new *NAT1* and *NAT2* alleles, an arylamine N-acetyltransferase nomenclature committee was initiated [2] to establish, publish and maintain consensus listings of *NAT1* and *NAT2* alleles on a website originally housed at the University of Louisville and presently housed at Democritus University of Thrace (http://

Correspondence to: David W. Hein, PhD, Kosair Charities Clinical and Translational Research Building, Room 303, 505 South Hancock Street, Louisville, KY USA 40202-1617, Tel: 001 502-852-6252, d.hein@louisville.edu.

Conflicts of interest

There are no conflicts of interest.

nat.mbg.duth.gr). As globally the most common functional allele, *NAT1*4* has been assigned reference allelic status [1]. Therefore, studies comparing the sequence and phenotypic impact of other *NAT1* alleles use *NAT1*4* as a reference for comparison.

Updates and discussions of N-acetyltransferase gene nomenclature have been conducted at each of the seven N-acetyltransferase workshops held at three year intervals. At the 4th *N*-acetyltransferase workshop held in Alexandroupolis, Greece [3], the N-acetyltransferase nomenclature committee was asked to post functional phenotypes for the human *NAT1* and *NAT2* alleles in those instances in which the committee determined a consensus has been achieved in the scientific literature. In the most recent N-acetyltransferase nomenclature update [4], the committee announced that functional phenotypes for *NAT1* and *NAT2* alleles were provided where consensus is evident in the scientific literature, but that functional phenotypes for some alleles such as *NAT1*10* and *NAT1*11* were not provided due to lack of consensus.

*NAT1*10* allele

The *NAT1*10* allele was first described by Vatsis and Weber [5] and is defined by two single nucleotide polymorphisms (SNPs) in the 3'-untranslated region (UTR) of the *NAT1* gene, namely 1088T>A (c.*215T>A, rs1057126) and 1095C>A (c.*222C>A, rs15561) (Table 1). It is the most common *NAT1* variant allele, with an average global population frequency of about 35–40%. Its allelic prevalence is highest (~53%) in East Asian populations and lowest (15–25%) in Europeans [6, 7] (also see the dbSNP database for rs1057126). The 3'-UTR polymorphisms cause no amino acid changes, but SNP 1088T>A (c.*215T>A) causes a change in polyA-1 (AATAAA→AAAAAA), one of multiple active consensus polyadenylation signals of human *NAT1* gene [8–10]. *NAT1*10* has been reported to be associated with slightly elevated NAT1 activity levels in human bladder [11, 12], colon [12], liver [9, 13], and white blood cells [9, 14]. Some studies also detected higher levels of carcinogen-DNA adducts in bladder and breast tissue of individuals carrying the *NAT1*10* allele [11, 15]. As described by Hein et al. [16], urinary metabolites were measured in 547 healthy individuals administered caffeine. Probit plots of the caffeine urinary metabolites 5-acetylamino-6-formylamino-3-methyluracil/1-methylxanthine (AFMU/1X) are normally used to separate rapid from slow acetylator phenotypes for NAT2 using a cut-point of 0.6, with the remaining activity being attributed to NAT1 [17]. This attribute was used to plot probits of AFMU/1X according to *NAT1*4* homozygous, *NAT1*10* homozygous and *NAT1*4/*10* heterozygous genotypes. Presence of the *NAT1*10* allele resulted in a gene-dose increase in acetylation *in vivo* with *NAT1*10/*10 > NAT1*4/*10 > NAT1*4/*4* [16]. This trend could, however, be at least partially due to the reported linkage disequilibrium between *NAT1*10* and *NAT2*4* alleles [18, 19], with the observed gene-dose increase in acetylation potentially attributed to the "rapid acetylator" *NAT2*4* allele frequently co-localizing with *NAT1*10* on the same haplotype. In contrast, in other studies, *NAT1*10* did not confer higher N-acetylation in blood cells [20–24] or healthy tissue of bladder [25] and breast [26]. Similar observations were made *in vivo* [23, 25, 27] and when measuring carcinogen-hemoglobin [28] or carcinogen-DNA [26, 29] adduct formation. Furthermore, transfection of *NAT1*10* did not increase acetylation activity in COS-1 cells [22, 30]. Consequently, numerous reviews [31–

37] conclude that these inconsistent findings reflect lack of consensus regarding *NAT1*10* phenotype.

*NAT1*11* allele

A similar lack of consensus exists for the *NAT1*11* allelic group comprising haplotypes *NAT1*11A, *11B* and *11C* [5, 38, 39]. Those three related but distinct haplotypes bear combinations of the following variations: c.−344C>T (rs4986988), c.−40A>T (rs4986989), c.445G>A (p.Val149Ile, rs4987076; not present in *NAT1*11C*), c.459G>A (p.Thr153=, rs4986990), c.640T>G (p.Ser214Ala, rs4986783), 1095C>A (c.*222C>A, rs15561; not present in *NAT1*11B*), and a 9 bp deletion between nucleotide positions 1065–1090 (c.*192-c.*217, rs367921464) affecting a stretch of eight TAA repeats adjacent to polyadenylation signal polyA-1 [8] (Table 1). Those allelic variants are rare with an average global population frequency of about 1.8% and higher prevalence observed in Eurasian populations [6] (also see the dbSNP database for rs367921464). Previous studies with recombinant *NAT1* variants bearing only the coding SNPs of *NAT1*11* alleles did not show any substantial effects on NAT1 enzymatic function when expression took place in bacterial or yeast cells [23, 38, 40, 41]. However, the results were inconsistent between studies when the same variants were expressed in mammalian COS-1 cells [22, 42] or when genotype-phenotype correlation was undertaken for *NAT1*11* in blood cells [14, 21]. Inclusion of the 3'-UTR SNPs in the *NAT1*11* recombinant constructs expressed in yeast cells did not resolve those ambiguities [22, 30]. Consequently, the functional phenotype for *NAT1*11* alleles has remained elusive [35] and no designation is presently posted on the N-acetyltransferase nomenclature committee database (http://nat.mbg.duth.gr).

Insights into the mechanistic basis of *NAT1*10* and *NAT1*11* allelic function

The aforementioned studies have attributed the phenotypic inconsistencies observed for *NAT1*10* and *NAT1*11* to the possible variable allelic expression among different tissues, the limitations of the genotyping assays (which mostly relied on techniques not involving direct DNA sequencing), the differences in recombinant protein expression systems used (bacteria, yeast, mammalian cell lines) and/or the known inherent instability of human NAT1 protein which requires very careful handling of native and recombinant cell lysates.

More recent studies may provide a mechanistic basis to identify *NAT1*10* and *NAT1*11* allelic function. Although the open reading frame of human *NAT1* gene is contained in a single 873 bp exon, the gene is transcribed into mRNAs with variable 5'- and 3'-UTRs formed via alternative splicing of eight upstream non-coding exons and differential utilization of at least three downstream polyadenylation signals [8–10, 43, 44] (Figure 1). Using recombinant constructs expressing the sequence of the major transcript of human *NAT1* gene (comprising upstream non-coding exons 4 and 8, as well as the coding exon and an adjacent 888 bp portion encompassing the 3'-UTR), Millner and colleagues [10] studied the effects of *NAT1*10* polymorphisms relative to *NAT1*4* reference allele in mammalian CHO cells subjected to transient or stable transfection. Although no differences between *NAT1*4* and *NAT1*10* polyadenylation pattern and no differences in mRNA stability were observed, nevertheless cells transfected with *NAT1*10* haplotype expressed higher *N*- and

O- acetylation activity, *NAT1* mRNA, and immunoreactive protein compared to cells transfected with *NAT1*4*. Incubation of these cells with the arylamine carcinogen 4-aminobiphenyl showed higher DNA adducts and mutants in cells transfected with *NAT1*10* compared to *NAT1*4*. Those effects were more pronounced in cells transfected with a third variant (named *NAT1*10B*) combining *NAT1*10* SNPs at positions 1088 (c.*215) and 1095 (c.*222) with additional downstream SNPs linked together in high allelic frequencies according to current population data. Such polymorphisms have not been examined by previous genotyping studies and could explain the inconsistencies reported for *NAT1*10* phenotype in different studies [10].

In another study, Wang and colleagues [9] measured transcription and translation of *NAT1*10* and *NAT1*11* alleles, assessing the influence of various transcription start sites, alternative splicing of 5'-UTR exons and differential usage of polyadenylation sites, employing liver biopsies, B-lymphocyte samples and transfected mammalian cell lines (HepG2 and HEK293). The determined allele frequencies in clinical samples were 19% for *NAT1*10* and 2.4% for *NAT1*11*. These alleles did not significantly affect total levels of *NAT1* mRNA in either tissue compared to the *NAT1*4* allele. The relative abundance of alternative transcripts, i.e. *NAT1* transcripts bearing variable 5'-UTR sequences (Figure 1), was also similar for all three alleles. The two *NAT1*11* polymorphisms found upstream of the gene coding region (c.−344C>T and c.−40A>T) had no effect on the transcription initiation site or the splicing pattern of the 5'-UTR. Moreover, no effect was evident for *NAT1*11* coding SNPs c.445G>A (p.Val149Ile) and c.640T>G (p.Ser214Ala) on mRNA or enzymatic activity levels, consistent with earlier studies outlined above.

The investigators then turned their attention to the region downstream of *NAT1* coding exon, focusing on the effects of *NAT1*10* and *NAT1*11* SNPs located within the 3'-UTR of the gene [9]. First, they undertook quantification of *NAT1*4* transcripts terminated after three active polyadenylation signals, located at 213 (polyA-1), 331 (polyA-2) and 734 (polyA-3) nucleotides downstream of the coding exon (Figure 1), and determined their relative amount to be 30, 60 and 10%, respectively, in both livers and B-lymphocytes. However, using a computational algorithm, an effect was predicted on transcriptional strength of polyA-1 signal due to the adjacent 9 bp deletion of the *NAT1*11* allele, unlike *NAT1*10* which was predicted to have no such effect. Consistent with these predictions, in ten *NAT1*4/*11* heterozygous samples (6 liver and 4 B-lymphocytes), allelic mRNA analyses showed *NAT1*11* to increase the amount of transcript terminated after polyadenylation signals polyA-2 (major) and potentially polyA-3, at the expense of the shorter transcript terminated after polyA-1, but without apparent change in the total *NAT1* mRNA levels expressed. Further luciferase reporter gene assays demonstrated that *NAT1*11* enhances translation by favoring formation of transcripts with intermediate or long 3'-UTRs, additionally implicating three *NAT1*11*-linked SNPs downstream of polyA-2 signal.

Undertaking a similar investigation for the *NAT1*10* allele [9], no apparent differences were observed between *NAT1*10* and *NAT1*4* allelic transcripts expressed, in terms of both the total amount of mRNA measured and the relative abundance of generated transcripts with variable 3'-UTR lengths. However, compared with *NAT1*4*, reporter gene assays produced higher levels of luciferase activity with *NAT1*10* 3'-UTR constructs (irrespectively of their

length), suggesting some enhancing effect on protein translation efficiency. The above findings were further corroborated by measurement of NAT1 protein/enzymatic activity in liver and B-cell samples genotyped as *NAT1*4/*4, NAT1*4/*10, NAT1*10/*10* and *NAT1*4/*11*. An increase was evident for samples carrying the *NAT1*10* allele, and this increase was even higher for carriers of the *NAT1*11* allele.

In a more recent study, Mascarenhas and colleagues [45] undertook allele-selective whole-transcriptome analysis to assess which allelic variants of genes are likely to be recruited more efficiently by the polysomes. Levels of polysome-bound mRNA (translatome) are better correlated with levels of expressed proteins, allowing more comprehensive insight into the possible effects of SNPs located within the 5'- and 3'-UTR of transcriptionally active genes. In the course of validating their methodology, the investigators undertook allelic RNA ratio analysis to compare total cellular to polysomal RNA using a heterozygous *NAT1*4/*10* lymphoblast cell line as model. No apparent differences were observed when measuring the cytoplasmic mRNA ratio for the two alleles, suggesting that *NAT1*10* has no significant effect on expression and processing of *NAT1* transcripts. However, when the analysis was focused on polysomal mRNA, it became evident that *NAT1*10* increased protein translation by enhancement of mRNA loading to the translational apparatus of cells [45]. This is a very significant finding, as it provides a mechanism by which the *NAT1*10* allele may enhance protein expression without affecting transcription of the *NAT1* gene [9].

Concluding remarks

Despite some minor differences, the three studies above [9, 10, 45] succeed to reach a consensus about the mechanistic basis underlying the functional phenotype of *NAT1*10* and *NAT1*11* as "increased-activity" alleles compared to the *NAT1*4* reference function allele. Some *NAT1* variants (e.g. *NAT1*14*, *NAT1*17* and *NAT1*22*) may be designated as "decreased-activity" alleles and other *NAT1* variants (e.g. *NAT1*15* and *NAT1*19*) may be designated as "no-activity" alleles compared to *NAT1*4*.

Designation of variants as "increased-function", "decreased-function" and "no-function" alleles is well established for *CYP2D6* gene [46]. We consider phenotypic designations described as "increased-activity", "decreased-activity" and "no-activity" to be most suitable for *NAT1* alleles. We also propose that phenotypic designations as "rapid" and "slow" acetylator should be discontinued for *NAT1* alleles, as they have been used inconsistently and in different contexts in the literature, often causing confusion. For instance, some investigators have used the designation "rapid" allele to describe *NAT1*4*, while others have used the same designation to describe *NAT1*10*. Similarly, the designation "slow" allele has been used to describe low activity alleles (like *NAT1*14*, *NAT1*17* and *NAT1*22*), but also prematurely terminated "null" alleles (like *NAT1*15* and *NAT1*19*). Moreover, we consider the term "ultra-rapid" allele to be inappropriate for *NAT1*10* and *NAT1*11*, in view of their apparently modest increases in activity. We thus propose that *NAT1* variants be grouped as "increased-activity", "decreased-activity" or "no-activity" alleles, with *NAT1*4* as the "reference" allele. These designations would sufficiently incorporate the different mechanisms by which various *NAT1* alleles may exert their phenotypic effects (e.g. via changes in transcription or translation, protein integrity or turnover, enzymatic activity etc.).

However, “rapid” and “slow” acetylator remain very appropriate phenotypic designations for N-acetyltransferase 2 (*NAT2*) alleles, where genotype-phenotype correlations are much more straightforward.

Whether or not the presence of *NAT1*10* and/or *NAT1*11* increased-activity alleles is sufficient to modify disease risk (particularly cancer) is subject to ongoing investigations discussed by several recent reviews and meta-analyses [37, 47–51]. The NAT1 isoenzyme is expressed in many tissues, where it is likely to compete with other xenobiotic metabolizing enzymes [52]. Therefore, it is difficult to predict how toxicity of xenobiotic compounds may be modulated by *NAT1*10* and *NAT1*11*, as the moderate phenotypic impact of those alleles is likely to be influenced by a range of other factors. Furthermore, current evidence implicates NAT1 in carcinogenesis via mechanisms not directly relevant to allelic variation [37, 53–61].

Acknowledgements

The authors gratefully acknowledge the contribution of the Erasmus+ International Credit Mobility programme (2016–2017 and 2017–2018) for faculty and student exchanges funded by the European Union. DWH is supported by United States Public Health Service grants R25-CA134283, P20-GM113226 and P42-ES023716.

References

[1]. Vatsis KP, Weber WW, Bell DA, Dupret JM, Evans DA, Grant DM, et al. Nomenclature for N-acetyltransferases. Pharmacogenetics 1995;5:1–17. [PubMed: 7773298]

[2]. Hein DW, Grant DM, Sim E. Update on consensus arylamine N-acetyltransferase gene nomenclature. Pharmacogenetics 2000;10:291–2. [PubMed: 10862519]

[3]. Boukouvala S, Westwood IM, Butcher NJ, Fakis G. Current trends in N-acetyltransferase research arising from the 2007 International NAT Workshop. Pharmacogenomics 2008;9:765–71. [PubMed: 18518853]

[4]. Hein DW, Boukouvala S, Grant DM, Minchin RF, Sim E. Changes in consensus arylamine N-acetyltransferase gene nomenclature. Pharmacogenet Genomics 2008;18:367–8. [PubMed: 18334921]

[5]. Vatsis KP, Weber WW. Structural heterogeneity of Caucasian N-acetyltransferase at the NAT1 gene locus. Arch Biochem Biophys 1993;301:71–6. [PubMed: 8442668]

[6]. Patin E, Barreiro LB, Sabeti PC, Austerlitz F, Luca F, Sajantila A, et al. Deciphering the ancient and complex evolutionary history of human arylamine N-acetyltransferase genes. Am J Hum Genet 2006;78:423–36. [PubMed: 16416399]

[7]. Mortensen HM, Froment A, Lema G, Bodo JM, Ibrahim M, Nyambo TB, et al. Characterization of genetic variation and natural selection at the arylamine N-acetyltransferase genes in global human populations. Pharmacogenomics 2011;12:1545–58. [PubMed: 21995608]

[8]. Boukouvala S, Sim E. Structural analysis of the genes for human arylamine N-acetyltransferases and characterisation of alternative transcripts. Basic Clin Pharmacol Toxicol 2005;96:343–51. [PubMed: 15853926]

[9]. Wang D, Para MF, Koletar SL, Sadee W. Human N-acetyltransferase 1 *10 and *11 alleles increase protein expression through distinct mechanisms and associate with sulfamethoxazole-induced hypersensitivity. Pharmacogenet Genomics 2011;21:652–64. [PubMed: 21878835]

[10]. Millner LM, Doll MA, Stepp MW, States JC, Hein DW. Functional analysis of arylamine N-acetyltransferase 1 (NAT1) NAT1*10 haplotypes in a complete NATb mRNA construct. Carcinogenesis 2012;33:348–55. [PubMed: 22114069]

[11]. Badawi AF, Hirvonen A, Bell DA, Lang NP, Kadlubar FF. Role of aromatic amine acetyltransferases, NAT1 and NAT2, in carcinogen-DNA adduct formation in the human urinary bladder. Cancer Res 1995;55:5230–7. [PubMed: 7585581]

[12]. Bell DA, Badawi AF, Lang NP, Ilett KF, Kadlubar FF, Hirvonen A. Polymorphism in the N-acetyltransferase 1 (NAT1) polyadenylation signal: association of NAT1*10 allele with higher N-acetylation activity in bladder and colon tissue. Cancer Res 1995;55:5226–9. [PubMed: 7585580]

[13]. Zenser TV, Lakshmi VM, Rustan TD, Doll MA, Deitz AC, Davis BB, et al. Human N-acetylation of benzidine: role of NAT1 and NAT2. Cancer Res 1996;56:3941–7. [PubMed: 8752161]

[14]. Zhangwei X, Jianming X, Qiao M, Xinhua X. N-Acetyltransferase-1 gene polymorphisms and correlation between genotype and its activity in a central Chinese Han population. Clin Chim Acta 2006;371:85–91. [PubMed: 16600204]

[15]. Ambrosone CB, Abrams SM, Gorlewska-Roberts K, Kadlubar FF. Hair dye use, meat intake, and tobacco exposure and presence of carcinogen-DNA adducts in exfoliated breast ductal epithelial cells. Arch Biochem Biophys 2007;464:169–75. [PubMed: 17601487]

[16]. Hein DW, McQueen CA, Grant DM, Goodfellow GH, Kadlubar FF, Weber WW. Pharmacogenetics of the arylamine N-acetyltransferases: a symposium in honor of Wendell W. Weber. Drug Metab Dispos 2000;28:1425–32. [PubMed: 11095579]

[17]. Cribb AE, Isbrucker R, Levatte T, Tsui B, Gillespie CT, Renton KW. Acetylator phenotyping: the urinary caffeine metabolite ratio in slow acetylators correlates with a marker of systemic NAT1 activity. Pharmacogenetics 1994;4:166–70. [PubMed: 7920698]

[18]. Smelt VA, Mardon HJ, Sim E. Placental expression of arylamine N-acetyltransferases: Evidence for linkage disequilibrium between *NAT1*10* and *NAT2*4* alleles of the two human arylamine N-acetyltransferase loci *NAT1* and *NAT2*. Pharmacol Toxicol 1998;83:149–57. [PubMed: 9820875]

[19]. Cascorbi I, Brockmöller J, Mrozikiewicz PM, Müller A, Roots I. Arylamine N-acetyltransferase activity in man. Drug Metab Rev 1999;31:489–502. [PubMed: 10335449]

[20]. Payton MA, Sim E. Genotyping human arylamine N-acetyltransferase type 1 (NAT1): the identification of two novel allelic variants. Biochem Pharmacol 1998;55:361–6. [PubMed: 9484803]

[21]. Bruhn C, Brockmoller J, Cascorbi I, Roots I, Borchert HH. Correlation between genotype and phenotype of the human arylamine N-acetyltransferase type 1 (NAT1). Biochem Pharmacol 1999;58:1759–64. [PubMed: 10571250]

[22]. de Leon JH, Vatsis KP, Weber WW. Characterization of naturally occurring and recombinant human N-acetyltransferase variants encoded by NAT1. Mol Pharmacol 2000;58:288–99. [PubMed: 10908296]

[23]. Hughes NC, Janezic SA, McQueen KL, Jewett MA, Castranio T, Bell DA, et al. Identification and characterization of variant alleles of human acetyltransferase NAT1 with defective function using p-aminosalicylate as an in-vivo and in-vitro probe. Pharmacogenetics 1998;8:55–66. [PubMed: 9511182]

[24]. Kukongviriyapan V, Prawan A, Warasiha B, Tassaneyakul W, Aiemsa-ard J. Polymorphism of N-acetyltransferase 1 and correlation between genotype and phenotype in a Thai population. Eur J Clin Pharmacol 2003;59:277–81. [PubMed: 12879167]

[25]. Vaziri SA, Hughes NC, Sampson H, Darlington G, Jewett MA, Grant DM. Variation in enzymes of arylamine procarcinogen biotransformation among bladder cancer patients and control subjects. Pharmacogenetics 2001;11:7–20. [PubMed: 11207033]

[26]. Williams JA, Stone EM, Fakis G, Johnson N, Cordell JA, Meinl W, et al. N-Acetyltransferases, sulfotransferases and heterocyclic amine activation in the breast. Pharmacogenetics 2001;11:373–88. [PubMed: 11470991]

[27]. Sy SK, de Kock L, Diacon AH, Werely CJ, Xia H, Rosenkranz B, et al. N-acetyltransferase genotypes and the pharmacokinetics and tolerability of para-aminosalicylic acid in patients with drug-resistant pulmonary tuberculosis. Antimicrob Agents Chemother 2015;59:4129–38. [PubMed: 25963985]

[28]. Probst-Hensch NM, Bell DA, Watson MA, Skipper PL, Tannenbaum SR, Chan KK, et al. N-acetyltransferase 2 phenotype but not NAT1*10 genotype affects aminobiphenyl-hemoglobin adduct levels. Cancer Epidemiol Biomarkers Prev 2000;9:619–23. [PubMed: 10868698]

[29]. Pfau W, Stone EM, Brockstedt U, Carmichael PL, Marquardt H, Phillips DH. DNA adducts in human breast tissue: association with N-acetyltransferase-2 (NAT2) and NAT1 genotypes. Cancer Epidemiol Biomarkers Prev 1998;7:1019–25. [PubMed: 9829711]

[30]. Zhu Y, States JC, Wang Y, Hein DW. Functional effects of genetic polymorphisms in the N-acetyltransferase 1 coding and 3' untranslated regions. Birth Defects Res A Clin Mol Teratol 2011;91:77–84. [PubMed: 21290563]

[31]. Butcher NJ, Boukouvala S, Sim E, Minchin RF. Pharmacogenetics of the arylamine N-acetyltransferases. Pharmacogenomics J 2002;2:30–42. [PubMed: 11990379]

[32]. Boukouvala S, Fakis G. Arylamine N-acetyltransferases: what we learn from genes and genomes. Drug Metab Rev 2005;37:511–64. [PubMed: 16257833]

[33]. Sim E, Westwood I, Fullam E. Arylamine N-acetyltransferases. Expert Opin Drug Metab Toxicol 2007;3:169–84. [PubMed: 17428149]

[34]. Sim E, Fakis G, Laurieri N, Boukouvala S. Arylamine N-acetyltransferases--from drug metabolism and pharmacogenetics to identification of novel targets for pharmacological intervention. Adv Pharmacol 2012;63:169–205. [PubMed: 22776642]

[35]. Hein DW. N-acetyltransferase SNPs: emerging concepts serve as a paradigm for understanding complexities of personalized medicine. Expert Opin Drug Metab Toxicol 2009;5:353–66. [PubMed: 19379125]

[36]. Walker K, Ginsberg G, Hattis D, Johns DO, Guyton KZ, Sonawane B. Genetic polymorphism in N-Acetyltransferase (NAT): Population distribution of NAT1 and NAT2 activity. J Toxicol Environ Health B Crit Rev 2009;12:440–72. [PubMed: 20183529]

[37]. Butcher NJ, Minchin RF. Arylamine N-acetyltransferase 1: a novel drug target in cancer development. Pharmacol Rev 2012;64:147–65. [PubMed: 22090474]

[38]. Doll MA, Jiang W, Deitz AC, Rustan TD, Hein DW. Identification of a novel allele at the human NAT1 acetyltransferase locus. Biochem Biophys Res Commun 1997;233:584–91. [PubMed: 9168895]

[39]. Johnson N, Bell P, Jonovska V, Budge M, Sim E. NAT gene polymorphisms and susceptibility to Alzheimer's disease: identification of a novel NAT1 allelic variant. BMC Med Genet 2004;5:6. [PubMed: 15142281]

[40]. Fretland AJ, Doll MA, Leff MA, Hein DW. Functional characterization of nucleotide polymorphisms in the coding region of N-acetyltransferase 1. Pharmacogenetics 2001;11:511–20. [PubMed: 11505221]

[41]. Fretland AJ, Doll MA, Zhu Y, Smith L, Leff MA, Hein DW. Effect of nucleotide substitutions in N-acetyltransferase-1 on N-acetylation (deactivation) and O-acetylation (activation) of arylamine carcinogens: implications for cancer predisposition. Cancer Detect Prev 2002;26:10–4. [PubMed: 12088197]

[42]. Zhu Y, Hein DW. Functional effects of single nucleotide polymorphisms in the coding region of human N-acetyltransferase 1. Pharmacogenomics J 2008;8:339–48. [PubMed: 17909564]

[43]. Husain A, Barker DF, States JC, Doll MA, Hein DW. Identification of the major promoter and non-coding exons of the human arylamine N-acetyltransferase 1 gene (NAT1). Pharmacogenetics 2004;14:397–406. [PubMed: 15226672]

[44]. Butcher NJ, Arulpragasam A, Goh HL, Davey T, Minchin RF. Genomic organization of human arylamine N-acetyltransferase Type I reveals alternative promoters that generate different 5'-UTR splice variants with altered translational activities. Biochem J 2005;387:119–27. [PubMed: 15487985]

[45]. Mascarenhas R, Pietrzak M, Smith RM, Webb A, Wang D, Papp AC, et al. Allele-Selective Transcriptome Recruitment to Polysomes Primed for Translation: Protein-Coding and Noncoding RNAs, and RNA Isoforms. PLoS One 2015;10:e0136798. [PubMed: 26331722]

[46]. Gaedigk A, Sangkuhl K, Whirl-Carrillo M, Klein T, Leeder JS. Prediction of CYP2D6 phenotype from genotype across world populations. Genet Med 2017;19:69–76. [PubMed: 27388693]

[47]. Dhaini HR, El Hafi B, Khamis AM. NAT1 genotypic and phenotypic contribution to urinary bladder cancer risk: a systematic review and meta-analysis. Drug Metab Rev 2018;50:208–19. [PubMed: 29258340]

[48]. Xu Z, Li X, Qin Z, Xue J, Wang J, Liu Z, et al. Association of N-acetyltransferase 1 polymorphism and bladder cancer risk: an updated meta-analysis and trial sequential analysis. Int J Biol Markers 2017;32:e297–e304. [PubMed: 28604993]

[49]. Zhang K, Gao L, Wu Y, Chen J, Lin C, Liang S, et al. NAT1 polymorphisms and cancer risk: a systematic review and meta-analysis. Int J Clin Exp Med 2015;8:9177–91. [PubMed: 26309576]

[50]. Wu K, Wang X, Xie Z, Liu Z, Lu Y. N-acetyltransferase 1 polymorphism and bladder cancer susceptibility: a meta-analysis of epidemiological studies. J Int Med Res 2013;41:31–7. [PubMed: 23569127]

[51]. Cai J, Zhao Y, Zhu CL, Li J, Huang ZH. The association of NAT1 polymorphisms and colorectal carcinoma risk: evidence from 20,000 subjects. Mol Biol Rep 2012;39:7497–503. [PubMed: 22327651]

[52]. Grant DM, Hughes NC, Janezic SA, Goodfellow GH, Chen HJ, Gaedigk A, et al. Human acetyltransferase polymorphisms. Mutat Res 1997;376:61–70. [PubMed: 9202739]

[53]. Tiang JM, Butcher NJ, Minchin RF. Small molecule inhibition of arylamine N-acetyltransferase Type I inhibits proliferation and invasiveness of MDA-MB-231 breast cancer cells. Biochem Biophys Res Commun 2010;393:95–100. [PubMed: 20100460]

[54]. Tiang JM, Butcher NJ, Cullinane C, Humbert PO, Minchin RF. RNAi-mediated knock-down of arylamine N-acetyltransferase-1 expression induces E-cadherin up-regulation and cell-cell contact growth inhibition. PLoS One 2011;6:e17031. [PubMed: 21347396]

[55]. Tiang JM, Butcher NJ, Minchin RF. Effects of human arylamine N-acetyltransferase I knockdown in triple-negative breast cancer cell lines. Cancer Med 2015;4:565–74. [PubMed: 25627111]

[56]. Stepp MW, Doll MA, Samuelson DJ, Sanders MA, States JC, Hein DW. Congenic rats with higher arylamine N-acetyltransferase 2 activity exhibit greater carcinogen-induced mammary tumor susceptibility independent of carcinogen metabolism. BMC Cancer 2017;17:233. [PubMed: 28359264]

[57]. Stepp MW, Doll MA, Carlisle SM, States JC, Hein DW. Genetic and small molecule inhibition of arylamine N-acetyltransferase 1 reduces anchorage-independent growth in human breast cancer cell line MDA-MB-231. Mol Carcinog 2018;57:549–558. [PubMed: 29315819]

[58]. Johansson I, Nilsson C, Berglund P, Lauss M, Ringnér M, Olsson H, et al. Gene expression profiling of primary male breast cancers reveals two unique subgroups and identifies N-acetyltransferase-1 (NAT1) as a novel prognostic biomarker. Breast Cancer Res 2012;14:R31. [PubMed: 22333393]

[59]. Endo Y, Yamashita H, Takahashi S, Sato S, Yoshimoto N, Asano T, et al. Immunohistochemical determination of the miR-1290 target arylamine N-acetyltransferase 1 (NAT1) as a prognostic biomarker in breast cancer. BMC Cancer 2014;14:990. [PubMed: 25528056]

[60]. Minchin RF, Butcher NJ. Trimodal distribution of arylamine N-acetyltransferase 1 mRNA in breast cancer tumors: association with overall survival and drug resistance. BMC Genomics 2018;19(1):513. [PubMed: 29969986]

[61]. Carlisle SM, Hein DW. Retrospective analysis of estrogen receptor 1 and N-acetyltransferase gene expression in normal breast tissue, primary breast tumors, and established breast cancer cell lines. Int J Oncol 2018;53:694–702. [PubMed: 29901116]

[62]. Kalman LV, Agúndez J, Appell ML, Black JL, Bell GC, Boukouvala S, et al. Pharmacogenetic allele nomenclature: International workgroup recommendations for test result reporting. Clin Pharmacol Ther 2016;99:172–85. [PubMed: 26479518]

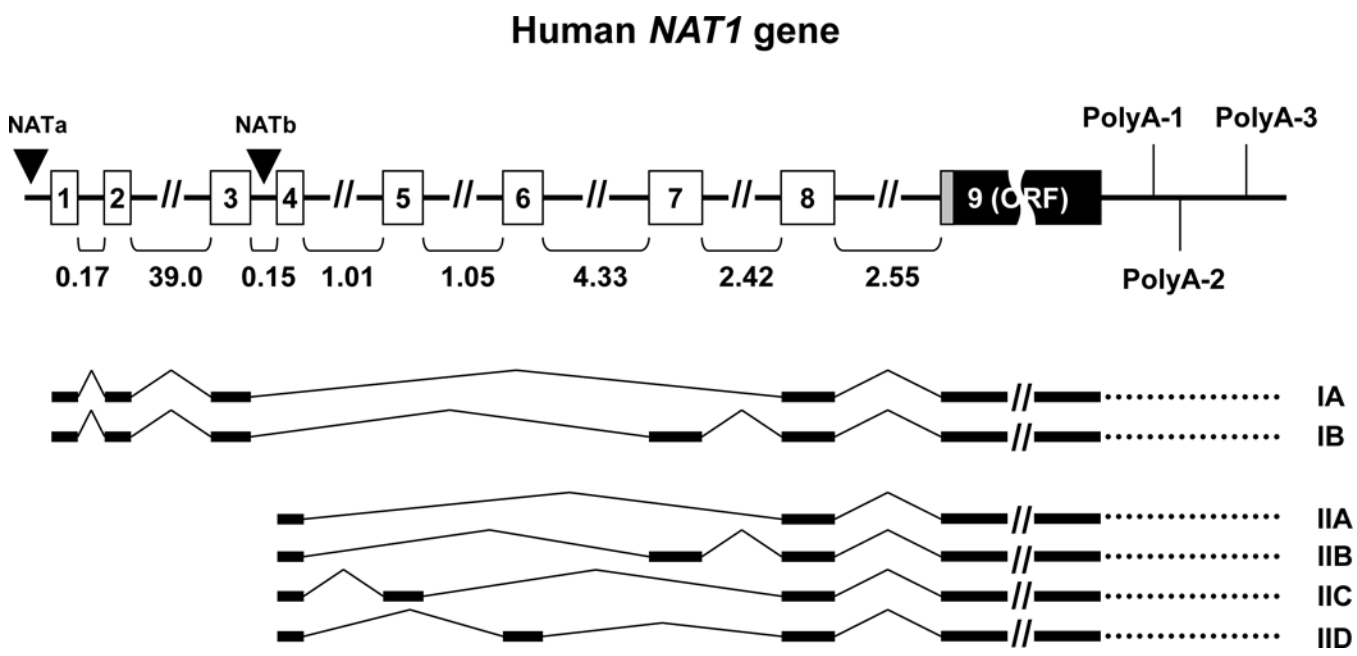

Figure 1:

Overview of *NAT1* gene structure and main alternative transcripts. The gene comprises 8 non-coding exons differentially spliced to generate alternative transcripts with variable 5'-UTRs. Exon 9 contains the entire open reading frame (ORF) of the gene, as well as the adjacent 3'-UTR terminated after three differentially utilized polyadenylation signals located at 213 (polyA-1), 331 (polyA-2; major) and 734 (polyA-3) nucleotides downstream of the coding exon. Type I transcripts (A,B) are initiated by promoter NATa located upstream of non-coding exon 1. Type II transcripts (A-D) are initiated by promoter NATb (major) located upstream of non-coding exon 4. The size of introns (in kilobases) is indicated below the gene. The figure was compiled from information previously published [7–9, 12] (figure not drawn to scale).

Table 1:

Description of *NAT1* haplotypes **4, *10* and **11A-C*. The current NAT nomenclature is aligned to the consensus Human Genome Variation Society (HGVS) nomenclature used by public electronic databases to localize SNPs in the human genome[1–3].

***Homo sapiens* chromosome 8 GRCh38.p12 primary assembly[4], GeneID: 9[5], HGNC:7645[6], official gene symbol: *NAT1* (arylamine N-acetyltransferase 1)[6]**									
Description of nucleotide change (Current NAT nomenclature)[7]		−344C>T	−40A>T	445G>A	459G>A	640T>G	Δ9 between 1065–1090	1088T>A	1095C>A
Description of nucleotide change (HGVS nomenclature)[8]		c.−344C>T	c.−40A>T	c.445G>A	c.459G>A	c.640T>G	c.*192_c.*217del AATAATAAT	c.*215T>A	c.*222C>A
Reference (Allele)[3]	*NAT1*4* (AJ307007.1)	C	A	G	G	T	No deletion	T	C
Variant 1	*NAT1*10*	C	A	G	G	T	No deletion	A	A
Variant 2	*NAT1*11A*	T	T	A	A	G	Deletion	T	A
Variant 3	*NAT1*11B*	T	T	A	A	G	Deletion	T	C
Variant 4	*NAT1*11C*	T	T	G	A	G	Deletion	T	A
Location in Gene		Intron 8, upstream of exon 9 (coding)	Intron 8, upstream of exon 9 (coding)	Exon 9 (coding)	Exon 9 (coding)	Exon 9 (coding)	3'-untranslated region (polyA-1)	3'-untranslated region (polyA-1)	3'-untranslated region
Description of amino acid change (Current NAT nomenclature)[7]		N/A	N/A	V149I	T153T	S214A	N/A	N/A	N/A
Description of amino acid change (HGVS nomenclature)[8]		N/A	N/A	p.Val149Ile	p.Thr153=	p.Ser214Ala	N/A	N/A	N/A
Reference (SNP)[9]		rs4986988	rs4986989	rs4987076	rs4986990	rs4986783	rs367921464	rs1057126	rs15561
Reference (Genomic)[10]	NC_000008.11	g.18221704C>T	g.18222008A>T	g.18222492G>A	g.18222506G>A	g.18222687T>G	g.18223127_18223135delAATAATAAA	g.18223135A>T	g.18223142A>C
Reference (Gene)[11]	NG_012245.2	g.56243C>T	g.56547A>T	g.57031G>A	g.57045G>A	g.57226T>G	g.57666_57674delAATAATAAA	g.57674A>T	g.57681A>C
Reference (Transcript)[12]	NM_000662.7 (Exons 4,8,9)	c.−6−338C>T	c.−6−34A>T	c.445G>A	c.459G>A	c.640T>G	c.*207_*215delAATAATAAA	c.*215A>T	c.*222A>C
Reference (Protein)[13]	NP_000653.3	N/A	N/A	p.Val149Ile	p.Thr153=	p.Ser214Ala	N/A	N/A	N/A
Minor Allele Frequency/Minor Allele Count (1000 Genomes)[14]		T=0.0168/84	T=0.0168/84	A=0.0170/85	A=0.0170/85	G=0.0170/85	−=0.0170/85	A=0.4006/2006	A=0.4373/2190

[1]Harmonization of allele nomenclature was recently proposed for all human genes relevant to pharmacogenomics research and clinical practice, and designations should follow the consensus HGVS nomenclature [62].

[2]All websites/databases were accessed on May 14, 2018.

[3]Note that all sequences derived from the Human Genome Reference Consortium (see footnotes 9–12 below) correspond to *NAT1* gene variant *NAT1*10*, instead of the most common functional *NAT1* allele found in global populations, designated as *NAT1*4* (Nucleotide ID: AJ307007.1) and considered as the reference allele for human *NAT1* (see NAT website, http://nat.mbg.duth.gr/background_2013.html).

[4]Genome Reference Consortium Human Build 38 patch release 12 (https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.38/#/st).

[5]NCBI *Gene* Database page for human *NAT1* gene (https://www.ncbi.nlm.nih.gov/gene?cmd=Retrieve&dopt=Graphics&list_uids=9).

[6] HUGO Gene Nomenclature Committee (HGNC) page for human *NAT1* gene (https://www.genenames.org/cgi-bin/gene_symbol_report?hgnc_id=HGNC:7645).

[7] NAT database page for human *NAT1* alleles (http://nat.mbg.duth.gr/).

[8] Sequence variant nomenclature according to current recommendations of the Human Genome Variation Society (HGVS) (http://varnomen.hgvs.org/).

[9] Reference SNP (rs) numbers available from the NCBI *dbSNP* Database (https://www.ncbi.nlm.nih.gov/snp/?term=).

[10] *Homo sapiens* chromosome 8 NCBI reference sequence NC_000008.11 (GRCh38.p12) (https://www.ncbi.nlm.nih.gov/nuccore/NC_000008.11).

[11] *Homo sapiens NAT1* NCBI RefSeqGene NG_012245.2 (https://www.ncbi.nlm.nih.gov/nuccore/NG_012245.2).

[12] *Homo sapiens NAT1* major transcript comprising non-coding exons 4 and 8, together with coding exon 9 [8, 43, 44] (NCBI RefSeq NM_000662.7, https://www.ncbi.nlm.nih.gov/nuccore/NM_000662.7).

[13] *Homo sapiens* NAT1 protein, allozyme NAT1_4 expressed from reference allele *NAT1*4* (NCBI RefSeq NP_000653.3 (https://www.ncbi.nlm.nih.gov/protein/NP_000653.3).

[14] Reported by the NCBI *dbSNP* Database in the context of allele frequencies determined by the 1000 Genomes project.