Arch Toxicol (2015) 89:2445–2447
DOI 10.1007/s00204-015-1650-2

GUEST EDITORIAL

N-Acetyltransferase 2: ultra-slow acetylators enter the stage

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Published online: 25 November 2015

The polymorphic *N*-Acetyltransferase 2 (NAT2) is a well-characterised key enzyme in the phase II metabolism of aromatic amines and heterocyclic aromatic amines present in a wide range of xenobiotics such as pharmaceutical drugs and carcinogens from tobacco smoke, workplace, environment and food (Selinski et al. 2014). Patterns of polymorphisms—so-called *haplotypes* or *NAT2 alleles*—result in a reduced *N*-, *O*- and *N,O*-acetylation of aromatic and heterocyclic amines—in general rather due to a reduction in protein as due to structural changes in the enzyme (McDonagh et al. 2014; Hein et al. 2008). Persons with an impaired NAT2 capacity—so-called *slow acetylators*—have two slow copies of the gene, whereas the presence of one or two *rapid* or *wild-type* copies of the gene leads to an *intermediate* or *rapid* genotype—so-called *rapid acetylators*. The relationship between genotype and NAT2 activity has been investigated extensively in vitro using bacterial, yeast and eukaryotic cell systems (Selinski et al. 2014; Walraven et al. 2008; García-Closas et al. 2011). Furthermore, the caffeine test (using a further NAT2 substrate) allows the determination of the metabolic capacity of NAT2 in humans by the ratio of two caffeine metabolites in urine (Selinski et al. 2011).

Recent in vivo studies indicate remarkable differences in the subgroup of slow acetylators leading to a differentiation between "ultra-slow" and "all other slow" acetylators (Selinski et al. 2013b; Ruiz et al. 2012). Ultra-slow acetylators are persons with two copies of the frequent NAT*6A haplotype and/or perhaps other infrequent haplotypes from the NAT2*6 (sharing rs1799930, G590A with *6A) cluster or NAT2*7B (sharing rs1041983, C282A with *6A). Two independent studies in Europeans found a 35 (Ruiz et al. 2012) and 46 % (Selinski et al. 2013b), respectively, decreased NAT2 capacity in NAT2*6/*6 genotypes compared to slow *5/*5. From the functional perspective, the missense SNP rs1799930 (G590A) is more likely than the synonymous rs1041983 (C282A) to result in an even more reduced metabolic capacity than the "all other slow" NAT2*5 and further less frequent slow haplotypes (*11 and *14). However, NAT2*7B shows in vivo also a reduced metabolic capacity though data are sparse (frequency: 2–3 % in Europeans). New studies and re-evaluation of older studies indicate that this further reduced metabolic capacity of the ultra-slow acetylators may have a particular impact on a number of biological outcomes ranging from drug efficacy and side effects, bladder cancer risk to age-related impairments. Interestingly, in vitro studies show contrary results suggesting the NAT2*5 haplotype cluster (rs1801280, T341C) to result in clearly lower acetylation capacity than NAT2*6 and *7 haplotypes (Hein 2002).

NAT2 shows a remarkable interethnic variability (Garcia-Martin 2008; Selinski et al. 2013a) regarding the haplotype distribution and the proportion of slow acetylators. So, interpretation of NAT2 effects should consider these differences, e.g. absence of particular haplotypes in certain populations. These interethnic differences give rise to the

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question of evolutionary processes that might have been involved in the development and distribution of slow haplotypes. Slow haplotypes have a high prevalence (>50 %) in populations of European descent and particular African and South Asian populations, but are rather infrequent in East Asians (<25 %, Sabbagh et al. 2011). Change of subsistence from hunting and gathering to pastoralism and agriculture and the resulting change of ingested xenobiotics are hypothesised as selection factor in favour of slow haplotypes (Sabbagh et al. 2011; Luca et al. 2008), especially the ultra-slow haplotype NAT2*6A (Patillon et al. 2014). Possibly relevant NAT2 substrates in food are, for instance, heterocyclic amines present in well-done meat (Turesky and Le Marchand 2011). Interestingly, European and Northeast Asian populations have a similar frequency of ultra-slow NAT2*6A haplotypes but differ in the slow NAT2*5B, virtually absent in Northeast Asians but frequent in Europeans, and NAT2*7B that is much more frequent in East Asians than in Europeans (Sabbagh et al. 2011). Reasons for these large interethnic differences are still unclear but might be due to genetic drift or local selective pressures (Sabbagh et al. 2011).

NAT2 polymorphisms were initially discovered as risk factor for drug-induced liver injury (DILI) in tuberculosis patients receiving isoniazid therapy (Hughes et al. 1954). Studies on anti-tuberculosis drug-induced hepatotoxicity (ATDH) are mostly conducted in East Asian and South American populations where tuberculosis is a major health concern (Daly 2013; Du et al. 2013). A recent meta-analysis indicated that ultra-slow acetylators are at highest risk of ATDH (Selinski et al. 2014) though results have to be interpreted with care due to the absence of NAT2*5B in East Asians.

The impact of slow NAT2 status on bladder cancer risk in persons exposed to carcinogenic NAT2 substrates, e.g. aromatic amines from tobacco smoke, is well investigated since decades (Figueroa et al. 2014; Moore et al. 2011; Risch et al. 1995). In recent studies, the effect of slow NAT2 status is diminishing even in smokers, possibly due to reductions in the general environmental and occupational exposure (Ovsiannikov et al. 2012). However, ultra-slow acetylators seem to be still susceptible to bladder cancer when exposed (Selinski et al. 2013b). This can be seen, for instance, in a large case–control study (1692 cases, 1995 controls, Selinski et al. 2013b) where slow acetylators had no particular bladder cancer risk in general (OR 1.02, 95 % CI 0.89–1.17) or when smoking (current smokers OR 1.07, 95 % CI 0.81–1.41), whereas ultra-slow acetylators had still higher bladder cancer risks (OR 1.29, 95 % CI 1.03–1.62), especially when smoking (current smokers OR 1.51, 95 % CI 0.99–2.31).

Though an effect of NAT2 on ageing itself can be excluded (Agundez et al. 1997; Korrapati et al. 1997; Muiras et al. 1998), age-related impairments have been subject of a number of studies. Age-related cataract was reported to be more frequent in slow acetylators (Tamer et al. 2005; Meyer et al. 2003) though the sample size in both studies was quite small. Slow, especially ultra-slow, acetylators seem to be more susceptible to age-related hearing impairment than rapid acetylators (for review, see Uchida et al. 2014), although this effect could not be confirmed in all study groups with sufficient sample size (Dawes et al. 2015). To date, the mechanism how NAT2 is involved in age-related impairments is unclear. An effect of the decreased antioxidant capacity of the slow NAT2 genotype is hypothesised (Uchida et al. 2014).

In addition to age-related declines in basic sensory hearing functions, a recent study also suggests an association of ultra-slow NAT2 with higher cognitive auditory functions in elderly persons (Selinski et al. 2015). Using an auditory distraction paradigm and electrophysiological measures, it could be demonstrated that ultra-slow acetylators are more prone to distracting sounds than rapid and slow acetylators. Moreover, the analysis of event-related potentials indicated that ultra-slow acetylators show deficits specifically in the automatic detection of changes in the acoustic environment and in the re-focusing of attention after a distracting event. Future research will show whether NAT2 effects are confined to auditory functions, or can also be found for further cognitive functions, like visual perception and attention.

In essence, recent studies show that in parallel genotyping and phenotyping still lead to new insights in NAT2-mediated metabolism. Furthermore, it is of high importance to differentiate between the “all other slow” and “ultra-slow” acetylators as both groups may still differ in the strength of the induced biological effects. Finally, it has to be considered that acetylation—similar to methylation—is a common metabolic process that has been investigated only in a few fields such as adverse drug reactions and urinary bladder cancer risk. Recent studies indicate the impact of NAT2-mediated acetylation is broader.

References

Agundez JA, Rodriguez I, Olivera M, Ladero JM, Garcia MA, Ribera JM, Benitez J (1997) CYP2D6, NAT2 and CYP2E1 genetic polymorphisms in nonagenarians. Age Ageing 26(2):147–151

Daly AK (2013) Pharmacogenomics of adverse drug reactions. Genome Med 5(1):5. doi:10.1186/gm409

Dawes P, Platt H, Horan M, Ollier W, Munro K, Pendleton N, Payton A (2015) No association between apolipoprotein E or *N*-Acetyltransferase 2 gene polymorphisms and age-related hearing loss. Laryngoscope 125(1):E33–E38. doi:10.1002/lary.24898

Du H, Chen X, Fang Y, Yan O, Xu H, Li L, Li W, Huang W (2013) Slow *N*-Acetyltransferase 2 genotype contributes to anti-tuberculosis drug-induced hepatotoxicity: a meta-analysis. Mol Biol Rep 40(5):3591–3596. doi:10.1007/s11033-012-2433-y

Figueroa JD, Han SS, Garcia-Closas M, Baris D, Jacobs EJ, Kogevinas M, Schwenn M, Malats N, Johnson A, Purdue MP, Caporaso N, Landi MT, Prokunina-Olsson L, Wang Z, Hutchinson A, Burdette L, Wheeler W, Vineis P, Siddiq A, Cortessis VK, Kooperberg C, Cussenot O, Benhamou S, Prescott J, Porru S, Bueno-de-Mesquita HB, Trichopoulos D, Ljungberg B, Clavel-Chapelon F, Weiderpass E, Krogh V, Dorronsoro M, Travis R, Tjønneland A, Brenan P, Chang-Claude J, Riboli E, Conti D, Gago-Dominguez M, Stern MC, Pike MC, Den Berg Van, David Yuan J, Hohensee C, Rodabough R, Cancel-Tassin G, Roupret M, Comperat E, Chen C, de Vivo I, Giovannucci E, Hunter DJ, Kraft P, Lindstrom S, Carta A, Pavanello S, Arici C, Mastrangelo G, Karagas MR, Schned A, Armenti KR, Hosain GM, Monawar Haiman CA, Fraumeni JF, Chanock SJ, Chatterjee N, Rothman N, Silverman DT (2014) Genome-wide interaction study of smoking and bladder cancer risk. Carcinogenesis 35(8):1737–1744. doi:10.1093/carcin/bgu064

García-Closas M, Hein DW, Silverman D, Malats N, Yeager M, Jacobs K, Doll MA, Figueroa JD, Baris D, Schwenn M, Kogevinas M, Johnson A, Chatterjee N, Moore LE, Moeller T, Real FX, Chanock S, Rothman N (2011) A single nucleotide polymorphism tags variation in the arylamine *N*-Acetyltransferase 2 phenotype in populations of European background. Pharmacogenet Genomics 21(4):231–236. doi:10.1097/FPC.0b013e32833e1b54

Garcia-Martin E (2008) Interethnic and intraethnic variability of NAT2 single nucleotide polymorphisms. Curr Drug Metab 9(6):487–497

Hein DW (2002) Molecular genetics and function of NAT1 and NAT2: role in aromatic amine metabolism and carcinogenesis. Mutat Res 506–507:65–77

Hein DW, Boukouvala S, Grant DM, Minchin RF, Sim E (2008) Changes in consensus arylamine *N*-Acetyltransferase gene nomenclature. Pharmacogenet Genomics 18(4):367–368. doi:10.1097/FPC.0b013e3282f60db0

Hughes HB, Biehl JP, Jones AP, Schmidt LH (1954) Metabolism of isoniazid in man as related to the occurrence of peripheral neuritis. Am Rev Tuberc 70(2):266–273

Korrapati MR, Sorkin JD, Andres R, Muller DC, Loi CM, Vesell ES, Vestal RE (1997) Acetylator phenotype in relation to age and gender in the Baltimore Longitudinal Study of Aging. J Clin Pharmacol 37(2):83–91

Luca F, Bubba G, Basile M, Brdicka R, Michalodimitrakis E, Rickards O, Vershubsky G, Quintana-Murci L, Kozlov AI, Novelletto A (2008) Multiple advantageous amino acid variants in the NAT2 gene in human populations. PLoS ONE 3(9):e3136. doi:10.1371/journal.pone.0003136

McDonagh EM, Boukouvala S, Aklillu E, Hein DW, Altman RB, Klein TE (2014) PharmGKB summary: very important pharmacogene information for *N*-Acetyltransferase 2. Pharmacogenet Genomics 24(8):409–425. doi:10.1097/FPC.0000000000000062

Meyer D, Parkin DP, Seifart HI, Maritz JS, Engelbrecht AH, Werely CJ, van Helden Paul D (2003) NAT2 slow acetylator function as a risk indicator for age-related cataract formation. Pharmacogenetics 13(5):285–289. doi:10.1097/01.fpc.0000054077.64000.3a

Moore LE, Baris DR, Figueroa JD, Garcia-Closas M, Karagas MR, Schwenn MR, Johnson AT, Lubin JH, Hein DW, Dagnall CL, Colt JS, Kida M, Jones MA, Schned AR, Cherala SS, Chanock SJ, Cantor KP, Silverman DT, Rothman N (2011) GSTM1 null and NAT2 slow acetylation genotypes, smoking intensity and bladder cancer risk: results from the New England bladder cancer study and NAT2 meta-analysis. Carcinogenesis 32(2):182–189. doi:10.1093/carcin/bgq223

Muiras ML, Verasdonck P, Cottet F, Schachter F (1998) Lack of association between human longevity and genetic polymorphisms in drug-metabolizing enzymes at the NAT2, GSTM1 and CYP2D6 loci. Hum Genet 102(5):526–532

Ovsiannikov D, Selinski S, Lehmann M, Blaszkewicz M, Moormann O, Haenel MW, Hengstler JG, Golka K (2012) Polymorphic enzymes, urinary bladder cancer risk, and structural change in the local industry. J Toxicol Environ Health A 75(8–10):557–565. doi:10.1080/15287394.2012.675308

Patillon B, Luisi P, Poloni ES, Boukouvala S, Darlu P, Genin E, Sabbagh A (2014) A homogenizing process of selection has maintained an "ultra-slow" acetylation NAT2 variant in humans. Hum Biol 86(3):185–214. doi:10.13110/humanbiology.86.3.0185

Risch A, Wallace DM, Bathers S, Sim E (1995) Slow N-acetylation genotype is a susceptibility factor in occupational and smoking related bladder cancer. Hum Mol Genet 4(2):231–236

Ruiz JD, Martínez C, Anderson K, Gross M, Lang NP, García-Martín E, Agúndez José A G (2012) The differential effect of NAT2 variant alleles permits refinement in phenotype inference and identifies a very slow acetylation genotype. PLoS ONE 7(9):e44629. doi:10.1371/journal.pone.0044629

Sabbagh A, Darlu P, Crouau-Roy B, Poloni ES (2011) Arylamine *N*-Acetyltransferase 2 (NAT2) genetic diversity and traditional subsistence: a worldwide population survey. PLoS ONE 6(4):e18507. doi:10.1371/journal.pone.0018507

Selinski S, Blaszkewicz M, Lehmann M, Ovsiannikov D, Moormann O, Guballa C, Kress A, Truss MC, Gerullis H, Otto T, Barski D, Niegisch G, Albers P, Frees S, Brenner W, Thüroff JW, Angeli-Greaves M, Seidel T, Roth G, Dietrich H, Ebbinghaus R, Prager HM, Bolt HM, Falkenstein M, Zimmermann A, Klein T, Reckwitz T, Roemer HC, Löhlein D, Weistenhöfer W, Schöps W, Rizvi Hassan, Adibul Syed, Aslam M, Bánfi G, Romics I, Steffens M, Ekici AB, Winterpacht A, Ickstadt K, Schwender H, Hengstler JG, Golka K (2011) Genotyping NAT2 with only two SNPs (rs1041983 and rs1801280) outperforms the tagging SNP rs1495741 and is equivalent to the conventional 7-SNP NAT2 genotype. Pharmacogenet Genomics 21(10):673–678. doi:10.1097/FPC.0b013e3283493a23

Selinski S, Blaszkewicz M, Agundez Jose A G, Martinez C, Garcia-Martin E, Hengstler JG, Golka K (2013a) Clarifying haplotype ambiguity of NAT2 in multi-national cohorts. Front Biosci (Schol Ed) 5:672–684

Selinski S, Blaszkewicz M, Ickstadt K, Hengstler JG, Golka K (2013b) Refinement of the prediction of *N*-Acetyltransferase 2 (NAT2) phenotypes with respect to enzyme activity and urinary bladder cancer risk. Arch Toxicol 87(12):2129–2139. doi:10.1007/s00204-013-1157-7

Selinski S, Blaszkewicz M, Ickstadt K, Hengstler JG, Golka K (2014) Improvements in algorithms for phenotype inference: the NAT2 example. Curr Drug Metab 15(2):233–249

Selinski S, Getzmann S, Gajewski PD, Blaszkewicz M, Hengstler JG, Falkenstein M, Golka K (2015) The ultra-slow NAT2*6A haplotype is associated with reduced higher cognitive functions in an elderly study group. Arch Toxicol. doi:10.1007/s00204-015-1635-1

Tamer L, Yilmaz A, Yildirim H, Ayaz L, Ates NA, Karakas S, Oz O, Yildirim O, Atik U (2005) *N*-Acetyltransferase 2 phenotype may be associated with susceptibility to age-related cataract. Curr Eye Res 30(10):835–839. doi:10.1080/02713680591003799

Turesky RJ, Le Marchand L (2011) Metabolism and biomarkers of heterocyclic aromatic amines in molecular epidemiology studies: lessons learned from aromatic amines. Chem Res Toxicol 24(8):1169–1214. doi:10.1021/tx200135s

Uchida Y, Sugiura S, Sone M, Ueda H, Nakashima T (2014) Progress and prospects in human genetic research into age-related hearing impairment. Biomed Res Int 2014:390601. doi:10.1155/2014/390601

Walraven JM, Zang Y, Trent JO, Hein DW (2008) Structure/function evaluations of single nucleotide polymorphisms in human *N*-Acetyltransferase 2. Curr Drug Metab 9(6):471–486