

Editorial:

IMPROVED GENOTYPING OF N-ACETYLTRANSFERASE 2: ROLE OF THE ULTRA-SLOW ACETYLATORS

Meinolf Blaszkewicz

Leibniz Research Centre for Working Environment and Human Factors, Dortmund/Germany
Blaszkewicz@ifado.de

N-acetyltransferase 2 polymorphisms are of high relevance in clinical toxicology (Lück et al., 2009; Bing et al., 2011; Costa et al., 2012). The slow acetylator genotype of NAT2 has been demonstrated to be associated with an increased risk of anti-tuberculosis drug-induced liver damage (Cai et al., 2012; Lv et al., 2012; An et al., 2012; Ben Mahmoud et al., 2012; Bose et al., 2011). Moreover, many urinary bladder carcinogens are substrates of NAT2 (Golka et al., 1996; 2002; Vineis et al., 2001; Hung et al., 2004; Moore et al., 2011). Large meta-analyses have clearly shown an association between slow acetylation genotypes and increased risk of bladder cancer (Garcia-Closas et al., 2005; 2011; Sanderson et al., 2007; Agúndez et al., 2008; Hein, 2002, 2006, 2009; Hein and Doll, 2012a, b). However, at the level of individual studies the results remain controversial. Of 46 studies included into one of the recent meta-analysis 35 did not reach statistical significance (Moore et al., 2011).

To clarify the situation a recent study has been performed to identify the role of 'extreme' genotypes (Selinski et al., 2013). This study is based on a population of 344 individuals that have been phenotyped by the caffeine test (Blaszkewicz, 2004; Hakooz, 2009; Jetter et al., 2009). This test quantitatively determines the activity of NAT2 *in vivo*. A subgroup with an 'ultra-slow' *in vivo* metabolism of caffeine was identified.

Interestingly, these individuals with the ultra-slow NAT2 phenotype carried several

slow acetylator alleles and could be identified as *6A/*6A, *6A/*7B and *7B/*7B genotypes. This combination of slow alleles, the 'ultra-slow genotype' was further tested in 1,712 bladder cancer cases and 2,020 controls. Remarkably, individuals with the 'ultra-slow' genotype showed an increased odds ratio for bladder cancer risk (OR=1.31, P=0.012) whereas the slow acetylators in general were not significantly associated with cancer risk.

Currently, a huge number of studies is performed to understand the association between genetic variations and phenotype (Daly, 2013; Stewart and Marchan, 2012; Partosch et al., 2013; Sobin et al., 2011; Tumer et al., 2012; Zeller et al., 2012; Escobar-García et al., 2012). A special focus are drug metabolizing enzymes and their role in carcinogenesis (Chen et al., 2012; Hanioka et al., 2011; Santovito et al., 2011; Fujihara et al., 2011; Lankisch et al., 2008; Ulusoy et al., 2007). Genome-wide association studies have identified to which degree genetic variants influence bladder cancer risk (Golka et al., 2011; Selinski et al., 2011, 2012a, b; Safarinejad et al., 2011; Lehmann et al., 2010). However, most of these approaches considered only the genotype in relation to disease. The present study (Selinski et al., 2013) demonstrates the importance of understanding the association of haplotypes with enzyme activity and the relevance of extreme phenotypes.

REFERENCES

- Agúndez JA, Golka K, Martínez C, Selinski S, Blaszkewicz M, García-Martín E. Unraveling ambiguous NAT2 genotyping data. *Clin Chem* 2008;54:1390-4.
- An HR, Wu XQ, Wang ZY, Zhang JX, Liang Y. NAT2 and CYP2E1 polymorphisms associated with antituberculosis drug-induced hepatotoxicity in Chinese patients. *Clin Exp Pharmacol Physiol* 2012;39:535-43.
- Ben Mahmoud L, Ghozzi H, Kamoun A, Hakim A, Hachicha H, Hammami S et al. Polymorphism of the N-acetyltransferase 2 gene as a susceptibility risk factor for antituberculosis drug-induced hepatotoxicity in Tunisian patients with tuberculosis. *Pathol Biol (Paris)* 2012;60:324-30.
- Bing C, Xiaomeia C, Jinhenga L. Gene dose effect of NAT2 variants on the pharmacokinetics of isoniazid and acetylisoniazid in healthy Chinese subjects. *Drug Metabol Drug Interact* 2011;26:113-8.
- Blaszkewicz M. N-Acetyltransferase 2 (phenotyping: caffeine test) In: Angerer J, Müller M, Weiss T et al. (eds): *Analyses of hazardous substances in biological materials*, Vol 9. Special issue: Markers of susceptibility (pp 165-82). Weinheim: Wiley-VCH, 2004.
- Bose PD, Sarma MP, Medhi S, Das BC, Husain SA, Kar P. Role of polymorphic N-acetyl transferase 2 and cytochrome P4502E1 gene in antituberculosis treatment-induced hepatitis. *J Gastroenterol Hepatol* 2011;26:312-8.
- Cai Y, Yi J, Zhou C, Shen X. Pharmacogenetic study of drug-metabolising enzyme polymorphisms on the risk of anti-tuberculosis drug-induced liver injury: a meta-analysis. *PLoS One* 2012;7(10):e47769.
- Chen SC, Chen CC, Kuo CY, Huang CH, Lin CH, Lu ZY et al. Elevated risk of hypertension induced by arsenic exposure in Taiwanese rural residents: possible effects of manganese superoxide dismutase (MnSOD) and 8-oxoguanine DNA glycosylase (OGG1) genes. *Arch Toxicol* 2012;86:869-78.
- Costa GN, Magno LA, Santana CV, Konstantinovas C, Saito ST, Machado M et al. Genetic interaction between NAT2, GSTM1, GSTT1, CYP2E1, and environmental factors is associated with adverse reactions to anti-tuberculosis drugs. *Mol Diagn Ther* 2012;16:241-50.
- Daly AK. Optimal dosing of warfarin and other coumarin anticoagulants: the role of genetic polymorphisms. *Arch Toxicol* 2013;87:407-20.
- Escobar-García DM, Del Razo LM, Sanchez-Peña LC, Mandeville PB, Lopez-Campos C, Escudero-Lourdes C. Association of glutathione S-transferase Ω 1-1 polymorphisms (A140D and E208K) with the expression of interleukin-8 (IL-8), transforming growth factor beta (TGF- β), and apoptotic protease-activating factor 1 (Apaf-1) in humans chronically exposed to arsenic in drinking water. *Arch Toxicol* 2012;86:857-68.
- Fujihara J, Yasuda T, Kato H, Yuasa I, Panduro A, Kunito T et al. Genetic variants associated with arsenic metabolism within human arsenic (+3 oxidation state) methyltransferase show wide variation across multiple populations. *Arch Toxicol* 2011;85:119-25.
- García-Closas M, Malats N, Silverman D, Dosemeci M, Kogevinas M, Hein DW et al. NAT2 slow acetylation, GSTM1 null genotype, and risk of bladder cancer: results from the Spanish Bladder Cancer Study and meta-analyses. *Lancet* 2005;366(9486):649-59.
- García-Closas M, Hein DW, Silverman D, Malats N, Yeager M, Jacobs K et al. A single nucleotide polymorphism tags variation in the arylamine N-acetyltransferase 2 phenotype in populations of European background. *Pharmacogenet Genomics* 2011;21:231-6.
- Golka K, Prior V, Blaszkewicz M, Cascorbi I, Schöps W, Kierfeld G et al. Occupational history and genetic N-acetyltransferase polymorphism in urothelial cancer patients of Leverkusen, Germany. *Scand J Work Environ Health* 1996;22:332-8.
- Golka K, Prior V, Blaszkewicz M, Bolt HM: The enhanced bladder cancer susceptibility of NAT2 slow acetylators towards aromatic amines: a review considering ethnic differences. *Toxicol Lett* 2002;128:229-41.
- Golka K, Selinski S, Lehmann ML, Blaszkewicz M, Marchan R, Ickstadt K et al. Genetic variants in urinary bladder cancer: collective power of the "wimp SNPs". *Arch Toxicol* 2011;85:539-54.
- Hakooz NM. Caffeine metabolic ratios for the in vivo evaluation of CYP1A2, N-acetyltransferase 2, xanthine oxidase and CYP2A6 enzymatic activities. *Curr Drug Metab* 2009;10:329-38.

- Hanioka N, Oka H, Nagaoka K, Ikushiro S, Narimatsu S. Effect of UDP-glucuronosyltransferase 2B15 polymorphism on bisphenol A glucuronidation. *Arch Toxicol* 2011;85:1373-81.
- Hein DW. Molecular genetics and function of NAT1 and NAT2: role in aromatic amine metabolism and carcinogenesis. *Mutat Res.* 2002;506-507:65-77.
- Hein DW. N-acetyltransferase 2 genetic polymorphism: effects of carcinogen and haplotype on urinary bladder cancer risk. *Oncogene* 2006;25:1649-58.
- 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.
- Hein DW, Doll MA. A four-SNP NAT2 genotyping panel recommended to infer human acetylator phenotype. *Pharmacogenomics* 2012a;13:855.
- Hein DW, Doll MA. Accuracy of various human NAT2 SNP genotyping panels to infer rapid, intermediate and slow acetylator phenotypes. *Pharmacogenomics* 2012b;13:31-41.
- Hung RJ, Boffetta P, Brennan P, Malaveille C, Hautefeuille A, Donato F et al. GST, NAT, SULT1A1, CYP1B1 genetic polymorphisms, interactions with environmental exposures and bladder cancer risk in a high-risk population. *Int J Cancer* 2004;110:598-604.
- Jetter A, Kinzig M, Rodamer M, Tomalik-Scharte D, Sörgel F, Fuhr U. Phenotyping of N-acetyltransferase type 2 and xanthine oxidase with caffeine: when should urine samples be collected? *Eur J Clin Pharmacol* 2009;65:411-7.
- Lankisch TO, Gillman TC, Erichsen TJ, Ehmer U, Kalthoff S, Freiberg N et al. Aryl hydrocarbon receptor-mediated regulation of the human estrogen and bile acid UDP-glucuronosyltransferase 1A3 gene. *Arch Toxicol* 2008;82:573-82.
- Lehmann ML, Selinski S, Blaszkewicz M, Orlich M, Ovsianikov D, Moormann O et al. Rs710521[A] on chromosome 3q28 close to TP63 is associated with increased urinary bladder cancer risk. *Arch Toxicol* 2010;84:967-78.
- Lück H, Kinzig M, Jetter A, Fuhr U, Sörgel F. Mesalazine pharmacokinetics and NAT2 phenotype. *Eur J Clin Pharmacol* 2009;65:47-54.
- Lv X, Tang S, Xia Y, Zhang Y, Wu S, Yang Z et al. NAT2 genetic polymorphisms and anti-tuberculosis drug-induced hepatotoxicity in Chinese community population. *Ann Hepatol* 2012;11:700-7.
- Moore LE, Baris DR, Figueroa JD, Garcia-Closas M, Karagas MR, Schwenn MR et al. 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* 2011;32:182-9.
- Partosch F, Mielke H, Gundert-Remy U. Functional UDP-glucuronosyltransferase 2B15 polymorphism and bisphenol A concentrations in blood: results from physiologically based kinetic modelling. *Arch Toxicol* 2013;87:1257-64.
- Safarinejad MR, Shafiei N, Safarinejad SH. The association between bladder cancer and a single nucleotide polymorphism (rs2854744) in the insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) gene. *Arch Toxicol* 2011;85:1209-18.
- Sanderson S, Salanti G, Higgins J. Joint effects of the N-acetyltransferase 1 and 2 (NAT1 and NAT2) genes and smoking on bladder carcinogenesis: a literature-based systematic HuGE review and evidence synthesis. *Am J Epidemiol* 2007;166:741-51.
- Santovito A, Schilirò T, Castellano S, Cervella P, Bigatti MP, Gilli G et al. Combined analysis of chromosomal aberrations and glutathione S-transferase M1 and T1 polymorphisms in pathologists occupationally exposed to formaldehyde. *Arch Toxicol* 2011;85:1295-302.
- Selinski S, Blaszkewicz M, Lehmann ML, Ovsianikov D, Moormann O, Guballa C et al. 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* 2011;21:673-8.
- Selinski S, Lehmann ML, Blaszkewicz M, Ovsianikov D, Moormann O, Guballa C et al. Rs11892031[A] on chromosome 2q37 in an intronic region of the UGT1A locus is associated with urinary bladder cancer risk. *Arch Toxicol* 2012a;86:1369-78.
- Selinski S, Lehmann ML, Blaszkewicz M, Ovsianikov D, Moormann O, Guballa C et al. Urinary bladder cancer risk in relation to a single nucleotide polymorphism (rs2854744) in the insulin-like growth factor-binding protein-3 (IGFBP3) gene. *Arch Toxicol* 2012b;86:195-203.

Selinski S, Blaszkewicz M, Ickstadt K, Hengstler JG, Golka K. Refinement of the prediction of N-acetyltransferase 2 (NAT2) phenotypes with respect to enzyme activity and urinary bladder cancer risk. *Arch Toxicol* 2013;87:2129-39.

Sobin C, Parisi N, Schaub T, Gutierrez M, Ortega AX. δ -Aminolevulinic acid dehydratase single nucleotide polymorphism 2 and peptide transporter 2*2 haplotype may differentially mediate lead exposure in male children. *Arch Environ Contam Toxicol* 2011;61:521-9.

Stewart JD, Marchan R. Polymorphisms hit the headlines. *Arch Toxicol* 2012;86:1799-801.

Tumer TB, Sahin G, Arinç E. Association between polymorphisms of EPHX1 and XRCC1 genes and the risk of childhood acute lymphoblastic leukemia. *Arch Toxicol* 2012;86:431-9.

Ulusoy G, Arinç E, Adali O. Genotype and allele frequencies of polymorphic CYP2E1 in the Turkish population. *Arch Toxicol* 2007;81:711-8.

Vineis P, Marinelli D, Autrup H, Brockmoller J, Cascorbi I, Daly AK et al. Current smoking, occupation, N-acetyltransferase-2 and bladder cancer: a pooled analysis of genotype-based studies. *Cancer Epidemiol Biomarkers Prev* 2001;10:1249-52.

Zeller J, Högel J, Linsenmeyer R, Teller C, Speit G. Investigations of potential susceptibility toward formaldehyde-induced genotoxicity. *Arch Toxicol* 2012;86:1465-73.