

Allele Genotyping of Arylamine N-acetyltransferase 2 Gene in Latvian Population: Comparison of Two Methods

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Introduction. Arylamine N-acetyltransferase 2 (NAT2) gene has a key role in metabolism of hydrazine and arylamine drugs and carcinogens. NAT2 is highly polymorphic, and polymorphisms in the NAT2 gene are related to the rapid, intermediate, and slow acetylator phenotypes. These polymorphisms have been studied as modifiers of drug toxicity and/or efficacy, and in relation to the incidences of cancer. Acetylator phenotypic categories in humans are often inferred from NAT2 haplotypes based on seven single nucleotide polymorphisms (SNPs). Recently, a single common tag SNP (rs1495741) located in the 3' end of NAT2 has been shown to accurately predict the NAT2 acetylator phenotype of an individual; however, it seems to be population-specific.

Aim, Materials and Methods. The aim of the study was to compare NAT2 genotype identification in Latvians based on the rs1495741 SNP genotyping and conventional 7-SNP (G191A, C282T, T341C, C481T, G590A, A803G and G857A) genotyping, and to assess the degree of concordance between these methods.

85 DNA samples were used in this study. All individuals were Caucasians. The samples and information were obtained from the Genome Database of the Latvian Population (VIGDB). The study protocol was approved by the Central Medical Committee of Ethics in Latvia. NAT2 acetylator status was inferred using two methods: (i) SNP rs1495741 was analysed using TaqMan Assay and Quant Studio™ Real Time PCR System Software v1.3; (ii) a 1093-bp DNA fragment which contains the entire coding region of the NAT2 gene was amplified by PCR and subsequently sequenced on both strands by Sanger method. The sequence analysis and 7-SNP panel identification was performed using CodonCode Aligner software with the sequence of human gene (EC 2.3.1.5) (GenBank: X14672.1) as the reference. The degree of agreement is quantified by kappa.

Results. Based on the results of both methods used, all individuals were classified as rapid (carrying two rapid alleles), intermediate (one rapid and one slow allele) or slow (two slow alleles) acetylators. The NAT2 haplotypes were assigned according to the database (http://nat.mbg.duth.gr/Human%20NAT2%20alleles_2013.htm). The rs1495741 (AA), (AG) and (GG) genotypes predicted slow, intermediate, and rapid NAT2 acetylation phenotypes, respectively.

In total, 35.3% of individuals were defined as intermediate acetylators, 44.7% as slow acetylators, and 3.5% – as rapid acetylators. Our results on distribution of NAT2 slow acetylation phenotype in Latvia fall in the range of NAT2 acetylation phenotype frequency in Caucasians (MAF 23.9%).

71 of 85 (83.53%) genotyped DNA samples coincided in two methods (Kappa = 0.692; 95% CI = 0.544–0.841). Discrepancy between the two methods was observed in 14 DNA samples; however, there was no misclassification for the extreme categories, i. e. misclassified individuals were always heterozygous for rs1495741 (AG genotype) or showed NAT2 7-SNP-intermediate genotype (one rapid and one slow allele).

Conclusions. A novel NAT2 tag SNP (rs1495741) was correlated with NAT2 haplotypes derived from the seven SNPs, and the strength of agreement is considered to be “good”. While the use of this SNP as a sole marker can be applied to predict the NAT2 acetylation genotype in Latvian population, 7-SNP genotyping remains the method of choice in this setting. Large-scale studies are needed with respect to both target and population diversity.

The research was conducted as part of the Latvian National Research Programme VPP “BIOMEDICINE”.