Study of the role of single nucleotide polymorphisms 152G>T, 203G>A and 458C>T in the phenotype of human N-acetyltransferase 2 by site-directed mutagenesis

Autores: Silva, E. A. S.[1], Lopes, M. Q. P.[1], Berredo-Pinho, M.M[2], Gomes, L. H. F. [3], Mendonça-Lima, L.[4], Suffys, P. N.[1], Santos, A. R.[1], Teixeira, R. L. F.[1]

Instituição: [1]Laboratório de Biologia Molecular Aplicada a Micobactérias; [2]Laboratório de Microbiologia Celular; [4]Laboratório de Genômica Funcional e Bioinformática – FIOCRUZ (Avenida Brasil, 4365. Manguinhos. 21.040-360. Rio de janeiro – RJ); [3]Instituto Fernandes Figueira – FIOCRUZ (Avenida Rui Barbosa, 716. Flamengo. 22.250-020. Rio de Janeiro – RJ).

Human N-acetyltransferase 2 (NAT2) is encoded by *NAT2* gene and plays a significant role in the clearance and biotransformation of many drugs and carcinogens. Point mutations, known as single nucleotide polymorphisms (SNPs), within coding region of the *NAT2* can alter its enzymatic activity resulting in the individual phenotypes of slow, intermediate and fast acetylation. Genetic variability in *NAT2* gene can contribute to individual differences in drug response or toxicity and have been attributed to therapeutic failure and adverse drug reactions (ADRs) in the treatment of different disease models such as leprosy and tuberculosis. In Brazil, recent studies have shown a high allelic diversity of *NAT2*, with the description of new alleles and SNPs with unknown functional effects. Molecular modeling and *in silico* structural analysis suggested that Gly51Val (152G>T) variant may have an important effect on substrate recognition by NAT2, amino acid change Cys68Tyr (203G>A) would affect acetylating activity since Cys68 is part of catalytic site and other variants, such like Thr153Ile (458C>T) and Thr193Met (578C>T) may lead to the presence of hydrophobic residues on NAT2 surface involved in protein aggregation and/or targeted degradation. The objective of this study was to characterize the functional role of these new SNPs, through heterologous expression system in *E. coli*, and investigate the mechanism by which these SNPs may result in a slow acetylation phenotype. Human *NAT2* wild type gene (*NAT2*4*) was amplified by PCR and cloned into pBAD/TOPO expression vector. Site directed mutagenesis assays were used to introduce individual SNPs into *NAT2*4* containing plasmid and the effects of these SNPs on NAT2 expression were evaluated through the comparison of recombinant human NAT2 enzymes with the active enzyme in terms of immunoreactive protein levels and mRNA levels. Preliminary results show that 152G>T, 203G>A and 458C>T reduced the amount of NAT2 seen in Western blot with specific antibody. These findings suggested that these polymorphisms could reduce the catalytic activity of NAT2 contributing to slow acetylation phenotype. Knowledge of the functional role of variations in the *NAT2* coding sequence may contribute to a better understanding of the relationship between genotype and phenotype of human N-acetyltransferase 2 and contribute for prediction of unfavorable therapeutic outcomes for diseases treated with drugs metabolized by this enzyme.

Palavras-chaves: N-acetyltransferase 2, SNPs, heterologous expression system

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