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Variability in the frequency of Single nucleotide polymorphisms of N-acetyl transferase (*NAT2*) gene in Vietnamese

Dinh Doan Long*, Pham Thi Hong Nhung, Tran Thi Thuy Anh, Hoang Hai Yen

Faculty of Biology, VNU University of Science, 334 Nguyen Trai, Hanoi, Vietnam

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Abstract. N-acetyltransferase 2 (NAT2) modifies drug toxicity and cancer risk due to its role in bioactivation and detoxification of arylamine and hydrazine drugs and carcinogens. Human *NAT2* alleles possess a combination of single nucleotide polymorphisms (SNPs) associated with slow acetylation phenotypes. To identify variations in genetic polymorphisms of drug-metabolizing enzyme in Vietnamese for the first time, three SNPs of NAT2, C341T (I114T), G590A (R197Q) and G857A (G286E) were determined by using PCR- RFLP technique. For *NAT2*, the 341T allele appear with a rather low frequency of 2% in our 100 subjects. The frequencies of alleles 590A and 857A were also examined in this study with 12.5% and 25%, respectively. The variation in the genetic polymorphisms of drug-metabolizing enzyme is worthy of futher study to understand different therapeutic and adverse drug responses in Vietnamese population.

Keywords: N-acetyltransferase 2 enzyme, single nucleotide polymorphism (SNP), PCR-RFLP.

1. Introduction

Variability in the human N-acetyltransferase 2 (NAT2; EC 2.3.1.5) phenotype was first identified as a modifier of toxic side-effects in patients prescribed the anti-tubercular drug isoniazid [1]. In addition to the metabolism of many aromatic amine and hydrazine drugs [2], NAT2 modifies cancer predisposition with roles in bioactivation and detoxification of aromatic and heterocyclic amine carcinogens [3]. In the metabolic scheme for these drugs and carcinogens, NAT2 catalyzes not only N-acetylation, but following N-hydroxylation also catalyzes subsequent O-acetylation and N, O-acetylation [4-6].

Human NAT2 alleles or haplotypes possess a combination of single nucleotide polymorphisms (SNPs), some of which are associated with slow acetylator phenotypes. NAT2 polymorphisms modify individual cancer risk and drug response, or susceptibility to adverse drug reactions [2–12]. Patients with low NAT2 activity have a higher risk of developing severe skin reactions and hepatitis when treated with sulphonamide and isoniazid, respectively. In addition, some evidence suggests that people with the slow acetylation genotype had the risk various cancers including urinary bladder, colorectal, breast, prostate, pancreas, lung, liver,

* Corresponding author. Tel.: 84-4-38584748.
E-mail: longdd_ksh@vnu.edu.vn

esophageal, and non-Hodgkin lymphoma. Therefore, it may be important to understand the functional NAT2 activity in each individual to avoid excessive exposure to certain drugs and environments. A number of single nucleotide polymorphisms (SNPs) of *NAT2* that influence NAT2 activity has been systematically classified and applied in the human clinical studies. Reduced enzyme activity is associated with some SNPs of *NAT2*, such as T341C, G590A, G857A. C481T is a silent mutation but it often linked to T341C, restriction enzyme *Kpn*I is applied to detect the existence of C481T as a tag for T341C.

The frequencies of the important allelic variants in the NAT2 genes have been extensively studied in many ethnic groups, and the accumulated data show the variation in the distribution of these variants. However, no information is available for the Vietnamese population. In the present study, we applied PCR-RFLP in investigating the frequency of such SNPs in Vietnamese subjects, providing a basis for future clinical studies concerning variability in the response and/or toxicity to drugs known to be substrates for NAT2.

2. Materials and methods

2.1. Materials

The Vietnamese population sample is composed of 100 Vietnamese individuals collected randomly from patients in the Hanoi Huu Nghi Hospital and National Transfusion, Vietnam. Venous bood samples were collected in vials containing EDTA and stored in -20^0C for a year to 2 years.

2.2. Methods

DNA extraction. Genomic DNA was extracted from blood samples by using standard precipitation described by Sambrook *et al.* (2001) with some minor modifications. The extracted DNA products were analyzed on a 1% agarose gel and measured at OD_{280} and OD_{260}. $OD_{260/280}$ was calculated to identify the extraction efficacy and intactness of the genomic DNA.

PCR-RFLP genotyping. The fragment 1093 bp of *NAT2* gene was amplified by PCR with primers of sequences, 5'-GGA ACA AAT TGG ACT TGG-3' and 5'-TCT AGC ATG AAT CAC TCT GC-3' . PCR mixture was composed of 50 ng/ml DNA template, 0.3 μM of each primer (Bioneer), 0.3 mM dNTPs, 2 mM $MgCl_2$, 1u Taq polymerase and deionized water in a final volume of 35 μl. PCR program settings were preheating at 94°C for 4 min, 35 cycle of 94°C for 30s, 57°C for 45s, 72°C for 90s, and then extension at 72°C for 10 min. Following amplification, the reaction mixture was digested with restriction enzymes, which bought from Fermentas, *Kpn*I (C341T) and *Bam*HI (G857A) for 14-16h. The digested products were resolved by electrophoresis in 2% agarose gel at 80 V. For G590A, we use Fast Digest *Taq*I to cut PCR products for 5min and resolved its by electrophoresis in 10% acrylamide gel at 80 V.

Verifying the allele frequency distribution of the SNPs was compared with that as expected from Hardy-Weinberg equilibrium by $\chi 2$ tests.

3. Results and discussion

DNA extraction. Genomic DNA was extraction from blood samples anticoagulated with either EDTA by using the methods described by Sambrook *et al* (2001). In our study, the results showed that genomic DNA was extracted successfully with this method. Whole genomic DNA appears as a sharp, bring

band in agarose gel of electrophoresis. Optical density assay showed relatively purified products of OD $_{260/280}$ values ranging from 1.6 to 2 and the concentration of DNA were 30-600 µg/ml. The DNA samples subsequently were diluted to concentration of 50 µg/ml for further PCR experiments.

Polymerase chain reactions. The optimization of primer annealing was performed on purified DNA samples. The annealing temperature was identified as 57°C for the best result. For amplification of 1093bp fragment to analyze the SNPs in *NAT2* gene, PCRs were operated at the size of 1093bp as expected according to its theoretical calculation. The results were illustrated in Fig. 1.

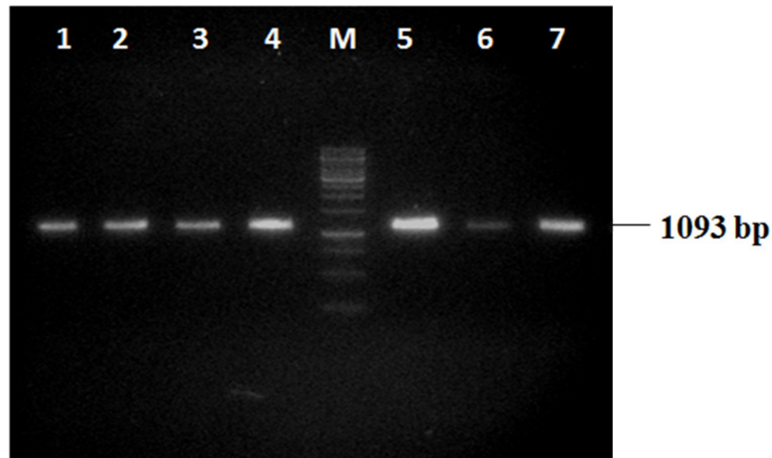

Fig 1. Electrophoresis of PCR products NAT2 gene. Lane 1 – 7: PCR products. M: DNA marker 100bp.

Genotyping and data analysis. Genomic DNA was amplified by PCR and digested by restriction enzymes. Slow acetyltor mutation were examined: C481T, by use of *Kpn*I, yields 659 and 434bp bands for wild-type alleles and a single 1093bp band for the mutant alleles; G590A, by use of a *Taq*I, yields 381, 316, 226, and 170 bp bands for wild-type alleles and 396, 381, and 316bp bands for the mutant alleles; and G857A, by use of *Bam*Hl, yields 810 and 283bp bands for the wild-type alleles and a single 1093 bp band for the mutant alleles.

For C481T, in 100 samples in this study, frequency of individuals with homozygotic genotype 481C/C was 0.96, heterozygotic genotype 481C/T was 0.04 and there was no 481T/T. From that, we calculated allele frequencies of 481C and 481T as 0.98 and 0.02, respectively. These results confirmed by χ^2 test (χ^2 = 3.96 lower than the χ^2 value of statistical significant at p= 0.05 which is 5.99), showing that the frequencies of these alleles reached to balanced state and there were no deviation from Hardy-Weinberg expectations in the population. Because SNP C481T always linked to T341C, using frequent data of C481T we can identify the allele frequency of SNP T341C. So allele frequencies of 481C and 481T are 0.98 and 0.02. The comparisons of 341C frequencies between Vietnamese and other populations indicated that, the frequency of this rare allele Vietnamese is of average to other Asian populations and relatively low compared to other populations world-wide[14] (Fig.2).

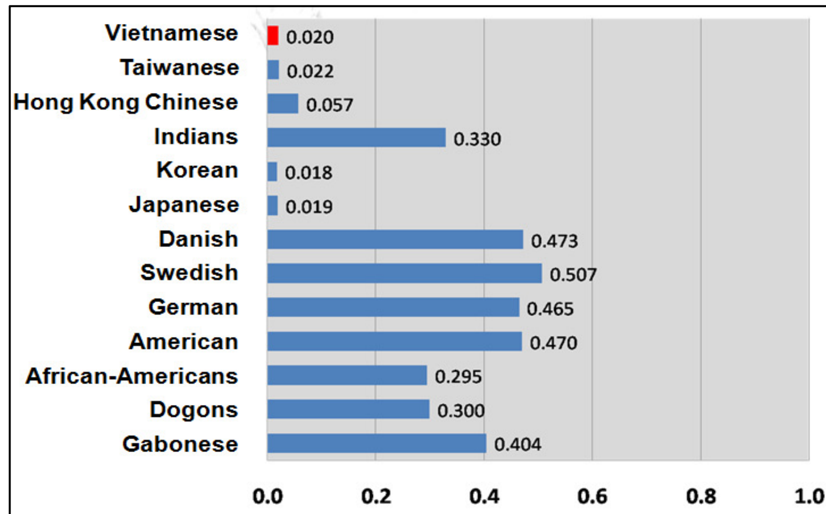

Fig. 2. Frequencies of 341C in various population groups in the world: Asians (Vietnamese, Taiwanese, Hong Kong Chinese, Indians, Korean, Japanese), Caucasians (Danish, Swedish, German, American), African-Americans, Native Africans (Gogons, Gabonese).

Genotyping of *NAT2* gene for analysis of allele frequencies of G590A was performed by digestion reaction of PCR *Taq*I. Among of 100 samples, 79% are GG homozygous, 17% are GA heterozygous and 4% are AA homozygous. The frequencies of these alleles are 0.87(G) and 0.13 (A). These results were tested by using χ^2 test (χ^2 = 1.6 lower than the χ^2 value of statistical significant at p= 0.05 which is 5.99). This result also indicated that there was no deviation from Hardy-Weinberg expectations for distribution of these alleles in this Vietnamese population. The frequency of this allele in Vietnam and other populations is compared in Fig. 3.

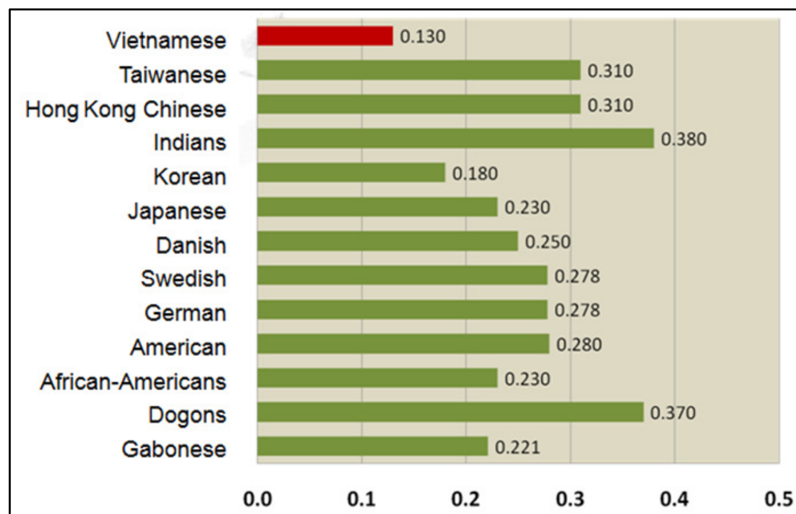

Fig. 3. Frequencies of 590A in various population groups in the world: Asians (Vietnamese, Taiwanese, Hong Kong Chinese, Indians, Korean, Japanese), Caucasians (Danish, Swedish, German, American), African-Americans, Native Africans (Gogons, Gabonese).

For the G857A polymorphism, which is best common in these polymorphisms, frequency of individuals with homozygotic genotype GG is 54%, heterozygotic genotype GA is 41% and homozygotic genotype AA is 5%. So, allele frequencies of 857G and 857A are 0.75 and 0.25, respectively. These results were tested by using χ^2 test (χ^2 = 0.67 lower than the χ^2 value of statistical significant at p= 0.05). The distribution of the combined NAT2 genotypes in this population did not differ significantly, as derived from Hardy-Weinberg equilibrium. When comparing with other populations in the world, the frequency of this allele Vietnamese is the highest [14] (Fig.4).

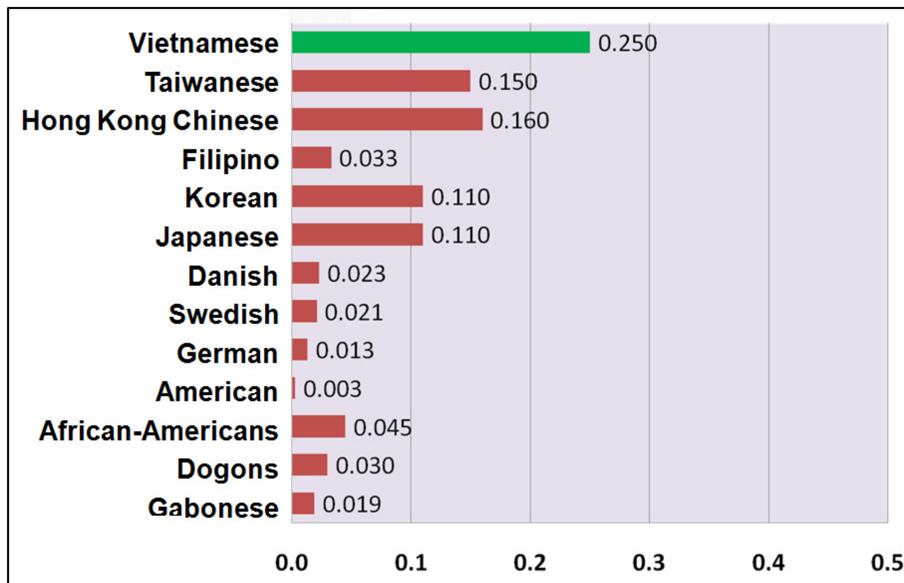

Fig.4. Frequencies of 857A in various population groups in the world: Asians (Vietnamese, Taiwanese, Hong Kong Chinese, Filipino, Korean, Japanese), Caucasians (Danish, Swedish, German, American), African-Americans, Native Africans (Gogons, Gabones).

4. Conclusion

Our experiments on assessing the frequencies of three single nucleotide polyrmorphisms of *NAT2* gene in 100 Vietnamese subjects showed that, both of these SNPs were found with identified frequencies. The 481T variant was found with very low frequency of 2% while the frequencies of 590A and 857A were 0.13 and 0.25, respectively. In the analyzed loci, the frequencies of genotypes are followed Hardy-Weinberg expectations. This means that, the genetic compositions of these alleles are quite balanced, at least in our 100 individuals of this study.

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Nghiên cứu tần số của đa hình đơn nucleotit gen N-acetyltransferase 2 (NAT2) ở người Việt Nam

Đinh Đoàn Long, Phạm Thị Hồng Nhung, Trần Thị Thùy Anh, Hoàng Hải Yến

Khoa Sinh học, Trường Đại học Khoa học Tự nhiên, ĐHQGHN, 334 Nguyễn Trãi, Hà Nội, Việt Nam

N-acetyltransferase 2 (NAT2) là enzym có vai trò quan trọng giúp cơ thể tránh khỏi các phản ứng quá khích với thuốc và môi trường. NAT2 có khả năng hoạt hoá sinh học, giải độc nhiều thuốc và các hợp chất độc hại, bao gồm cả các hợp chất gây ung thư. Nhằm xác định tần số các đa hình đơn nucleotit (SNP) có vai trò dược lý quan trọng, chúng tôi đã sử dụng phương pháp PCR-RFLP tiến hành phân tích 3 SNP có liên quan đến kiểu hình NAT2 acetyl hóa chậm là C341T (I114T), G590A (R197Q) và G857A (G286E). Nghiên cứu được thực hiện trên một nhóm mẫu gồm 100 người Việt Nam. Kết quả phân tích cho thấy alen 341T có tần số khá thấp là 2%. Tần số của các alen 590A và 857A lần lượt là 12.5% và 25%. Các kết quả của nghiên cứu này sẽ cung cấp những thông tin cơ bản định hướng cho các nghiên cứu sắp tới về sử dụng thuốc trong điều trị và ngăn ngừa nguy cơ mắc các bệnh liên quan trực tiếp đến ô nhiễm môi trường, trong đó đáng chú ý là các bệnh ung thư ở quần thể người Việt Nam.