NO ASSOCIATION BETWEEN *NAT2* GENOTYPE AND GENDER AMONG CAUCASIONS

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Summary

N-acetyltransferase-2 (NAT2) is a phase II drug metabolizing enzyme with a highly polymorphic gene. Genomic sample from 150 Caucasian volunteers were analyzed using polymerase chain reaction- restriction fragment length polymorphism assays (PCR-RFLP) to determine the association between major *NAT2* alleles and genotypes with gender. The results showed that proportions of *NAT2* allele and genotype were almost similar among both genders. The present study conclude that there is no association between *NAT2* genotype and gender

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Running title: NAT2 genotypes and gender

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Introduction

Acetylation constitutes an important metabolic route for drugs-containing primary hydrazides, and primary aliphatic amines [1]. The amide derivatives formed from acetylation of these amino are generally inactive and non-toxic. Since water solubility is not greatly enhanced by N-acetylation, it appears that the primary function of acetylation is one for termination of pharmacological activity and detoxication. However, some reports have indicated that acetylated metabolites may be as active (Nacetylprocainamide) or more toxic (N-acetylisoniazid) than their parent compounds [2, 3].

Acetylation exhibits a trimodal distribution within any given population. Individuals can be slow, intermediate or fast acetylators depending on their acetylation capacity for drugs [4]. There is a considerable interethnic difference in the proportion of acetylator phenotype [5]. A high proportion of Eskimos and Oriental-Asians are fast acetylators, whereas Caucasians and Africans are mainly slow acetylators.

The human N-acetyltransferase (NAT) isozymes are encoded at 3 separate loci, one of them contains multiple premature termination codons and most likely represents a non-expressed pseudogene [6]. The two expressed genes, *NAT1* and *NAT2,* are both located on chromosome 8 sharing 87% and 81% nucleotide and amino acid sequence identity, respectively [7].

The intronless coding region of N-acetyltransferase-2 gene (*NAT2*) maps on chromosome 8, at 8p22, covering 9.97 kilo base (kp) on the direct strand. Its protein has 290 amino acids (33.5 kilo Dalton), containing one N-acetyltransferase domain, and has a cytoplasmic subcellular location [8]. At least 15 and perhaps as many as 26 different haplotype variants of *NAT2* have been identified to date, and their frequency in the population provides a molecular explanation for the polymorphic metabolism of model substrates such as sulfamethazine and procainamide [9].

There are many studies about NAT2 genotype. However, there are limited studies regarding the association of NAT2 genotype and the gender of the Caucasian volunteers. Accordingly, the aim of the current study is to investigate the association between NAT2 genotype and the gender.

Methods

150 volunteers (78 males and 72 females) agreed to donate 3 ml venous blood and signed an informed consent. DNA was extracted and three most common *NAT2* single nucleotide polymorphisms (C481T, G590A, and G857A) were genotyped using PCR-RFLP by using the following primers:

Forward primer: 5' GCT GGG TCT GGA AGC TCC TC 3'

Reverse primer 5' TTG GGT GAT ACA TAC ACA AGG G 3'

After an initial denaturation at 94°C for 10 min, 35 cycles were performed consisting of denaturation step at 94°C for 1 minute, annealing step at 58°C for 1 minute and elongation step at 72°C for 1 minute, completed with a final cycle of elongation at 72°C for 10 min.

***NAT2*5* allele detection**

Homozygote *NAT2*5* genotype was represented by variant allele (547 bp length band)while heterozygote *NAT2*5* genotype was represented by variant allele (547 bp) and digested invariant allele (437 and 110 bp length-bands) on 2% agarose gel electrophoresis (Figure 1).

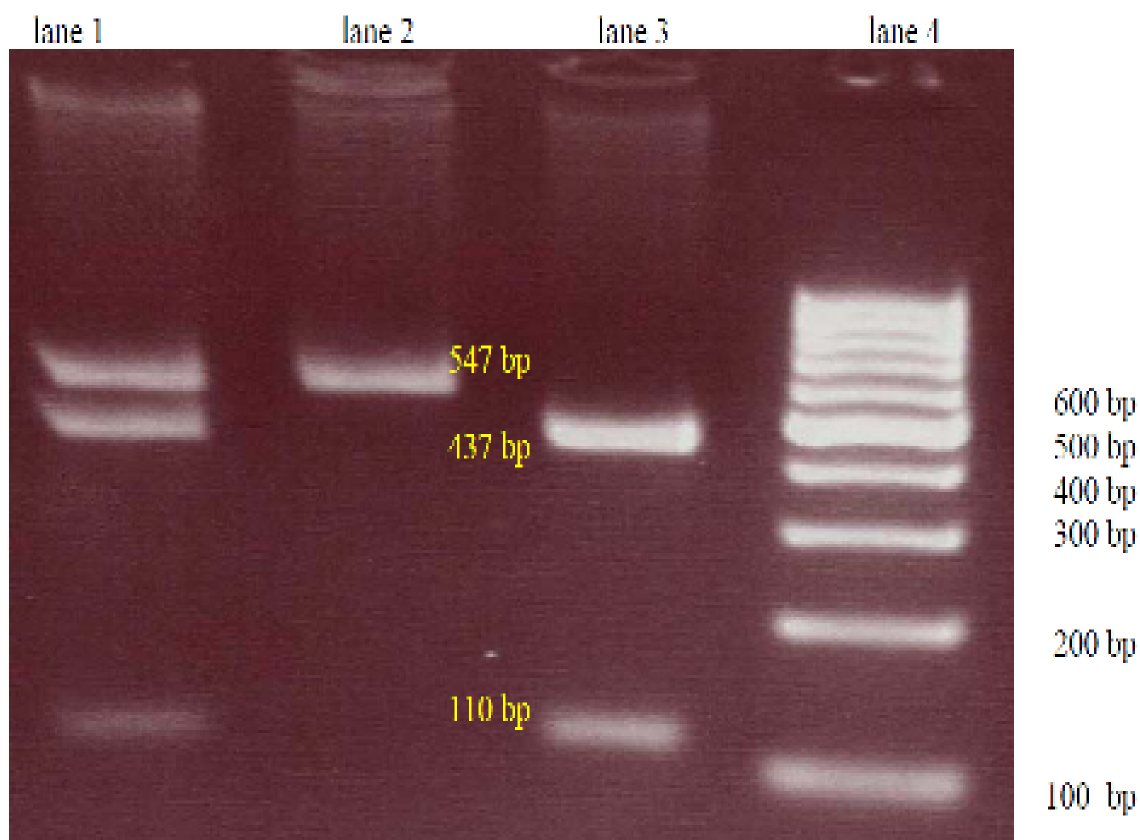

Figure 1. Detection of *NAT2*5* allele.
The analysis of three genomic DNA is shown: heterozygote *NAT2*5* genotype (lane 1), homozygote *NAT2*5* genotype (lane 2), *NAT2* genotype with no *NAT2*5* allele (lane 3) and DNA size marker (lane 4).

Detection of *NAT2*6* allele

Homozygote *NAT2*6* genotype was represented by variant allele (394 bp length-band), while heterozygote *NAT2*6* genotype was represented by four bands: variant allele (394 bp length band), and invariant allele (224, 170 and 153 bp length bands) on 3% agarose gel electrophoresis (Figure 2).

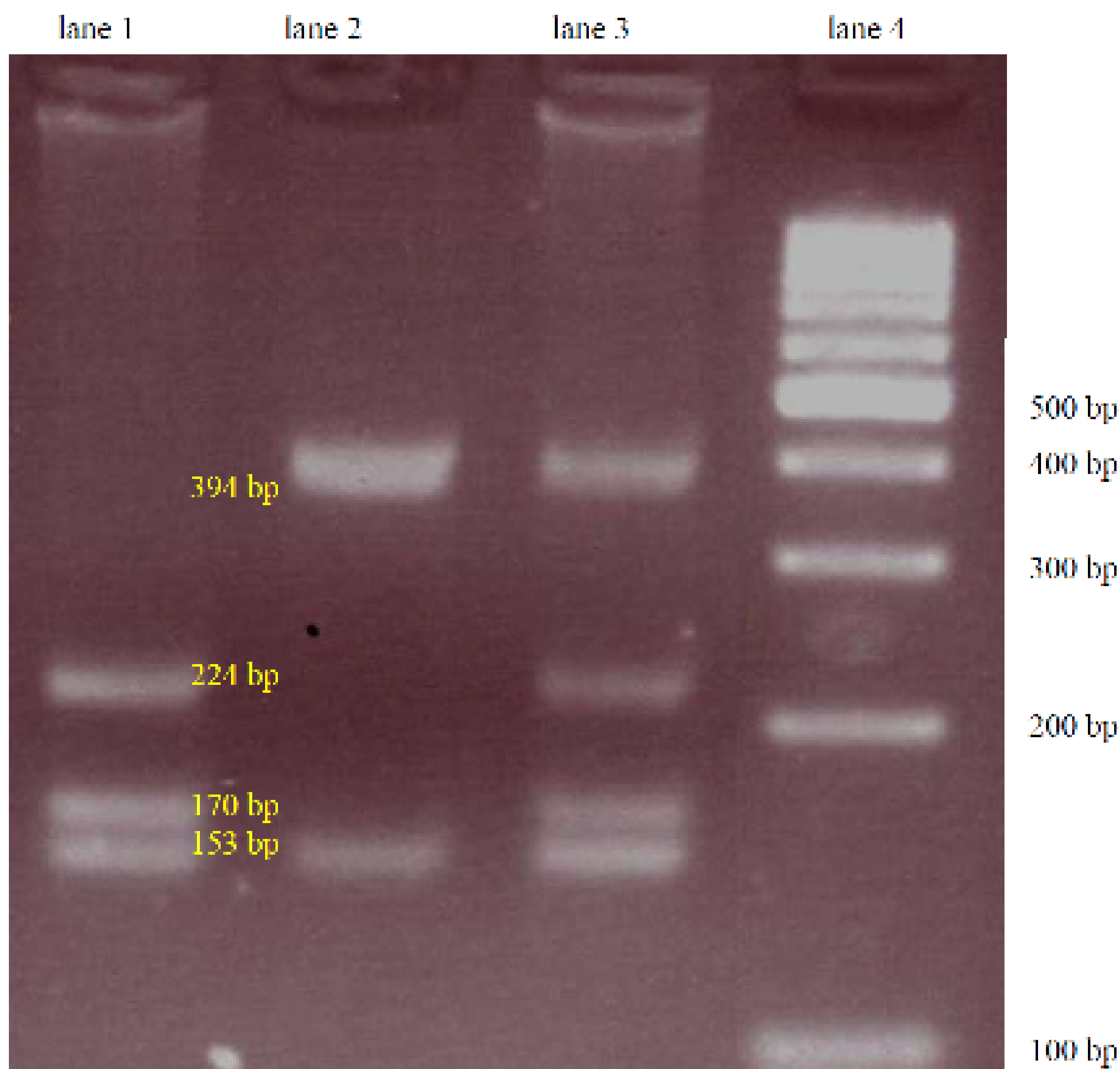

Figure 2. Detection of *NAT2*6* allele.
The analysis of three genomic DNA is shown: *NAT2* genotype with no *NAT2*6* allele (lane 1), homozygote *NAT2*6* allele (lane 2), heterozygote *NAT2*6* allele (lane 3) and DNA marker size (lane 4).

Detection of *NAT2*7* allele
The *NAT2*7* allele was detected by PCR-RFLP assay. Homozygote *NAT2*7* genotype was represented by variant allele (547 bp length-band), while heterozygote *NAT2*7* genotype was represented by variant allele (547 bp length-band) and invariant allele (490 and 57 bp length bands) on 2% agarose gel electrophoresis (Figure 3).

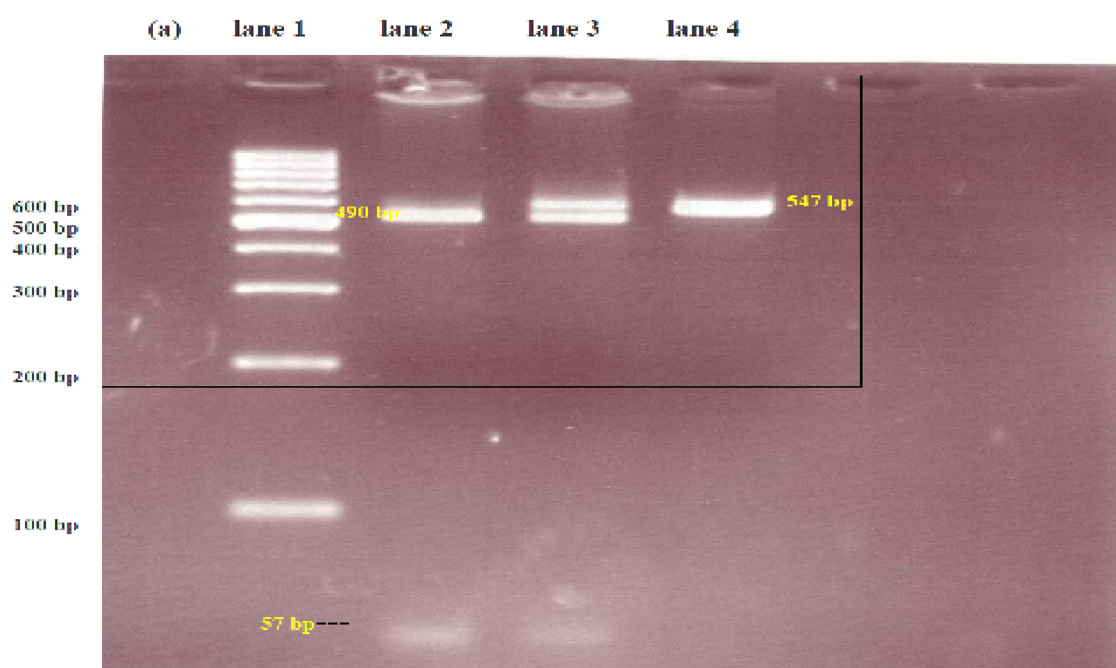

Figure 3. Detection of *NAT2*7* allele.
DNA size marker (lane 1), *NAT2* genotype with no *NAT2*7* allele (lane 2), heterozygote *NAT2*7* genotype (lane 3) and homozygote *NAT2*7* genotype (lane 4).

Chi-squared (χ 2) analysis was used as a statistical tool for comparison of NAT2 genotypes and alleles among both genders (p-value<0.05).

Results and Discussion

One hundred and fifty DNA samples (78 males and 72 females) were analyzed by PCR-RFLP assay. Data analysis by using Chi-squared (χ 2) with (p-value<0.05) showed that there was no difference between *NAT2* genotypes and alleles with gender (Table 1 and 2).

Table 1. Gender distribution of *NAT2* genotypes.

Gender	Genotype									Total
	*NAT2*4/4*	*NAT2*4/5*	*NAT2*4/6*	*NAT2*4/7*	*NAT*5/5*	*NAT2*5/6*	*NAT2*6/6*	*NAT2*6/7*	*NAT2*7/7*	
Female	3	11	10	5	10	23	8	1	1	
Male	9	15	8	1	11	21	12	1	0	
Total	**12**	**26**	**18**	**6**	**21**	**44**	**20**	**2**	**1**	**150**

Table 2. Gender distribution of *NAT2* alleles among Caucasians

	Female	Male
*NAT2*4*	32	42
*NAT2*5*	54	58
*NAT2*6*	50	54
*NAT2*7*	8	2
Total	144	156

The most common alleles among both genders were *NAT2*5* and *NAT2*6* followed by *NAT2**4 allele. The *NAT2**7 allele frequency was the lowest frequency where its proportion was slightly higher among female than males. However, this difference is not statistically significant. It was reported that *NAT2*4* is predominant among male colon cancer patient [10]. The results of the present study showed that *NAT2*4* frequency is almost similar among males and females in Caucasian populations. The most common genotypes and their frequencies were *NAT2*5/6*, *NAT2*4/5, NAT2*5/5* and *NAT2*6/6* with a genetic variations among both gender. *NAT2*4/7* genotype showed in a higher frequency among females than males. In addition, *NAT2*7/7* genotype was shown in a single female genomic sample. On the other hand, *NAT2*5/7* and *NAT2*6/7* genotypes frequency were equal in the proportion among both genders. This slight increasing in *NAT2*7*-containing genotype among females needs further investigation.

Also, the present study showed neither differences in the frequency of heterozygote *NAT2* genotypes frequency nor homozygote ones. This in line with the previous studies were gender based differences were not reported for acetylation phenotype and genotype.

The present study concludes that NAT2 genotype isn't associate with the gender among Caucasian volunteers.

References

1. Dupre JM, Rodrigues-Lima F. Structure and regulation of the drugmetabolizing enzymes arylamine N-acetyltransferases. Curr Med Chem 2005,12: 311-8.
2. Harron DW, Brogden, RN. Acecainide (N-acetylprocainamide). A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in cardiac arrhythmias. Drugs 1990 39:720-40.
3. Kita T, Tanigawara Y, Chikazawa S, Hatanaka H, Sakaeda T, Komada F, Iwakawa S, Okumura K. N-Acetyltransferase2 genotype correlated with isoniazid acetylation in Japanese tuberculous patients. Biol Pharm Bull 2001, 24:544-9.
4. García-Martín E. Interethnic and intraethnic variability of NAT2 single nucleotide polymorphisms. Curr Drug Metab 2008, 9:487-97.
5. Lin HJ, Han CY, Lin BK, Hardy S. Ethnic distribution of slow-acetylator mutations in the polymorphic N-acetyltransferase2 (NAT2) gene. Pharmacogenetics 1994, 4:125-134.

6. Tadmouri GO, Al-Haj-Ali S. Isoniazid inactivation . The Catalogue for Transmission Genetics in Arabs, Centre for Arab Genomic Studies 2005.
7. Hein DW, Doll MA, Fretland AJ, Leff MA, Webb SJ, Xiao GH, Devanaboyina US, Nangju NA, Feng Y. Molecular genetics and epidemiology of the NAT1 and NAT2 acetylation polymorphisms. Cancer Epidemiol Biomarkers Prev 2000; 9:29-42.
8. Zang Y, Doll MA, Zhao S, States JC, Hein DW. Functional characterization of single-nucleotide polymorphisms and haplotypes of human Nacetyltransferase 2. Carcinogenesis 2007, 28:1665-71.
9. Gross M, Kruisselbrink T, Anderson K, Lang N, McGovern P, Delongchamp R, Kadlubar F. Distribution and concordance of N-acetyltransferase genotype and phenotype in an American population. Cancer Epidemiol Biomarkers Prev 1999, 8:683-692.
10. Huang C C, Chien W P, Wong R H, Cheng Y W, Chen M C, Chou M C, Lee, H. NAT2 fast acetylator genotype is associated with an increased risk of colorectal cancer in Taiwan. Dis Colon Rectum 2009 50 :981-9.