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Variants of NAT2 polymorphisms: Intra and inter-ethnic differences

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***N-Acetyltransferase 2* (*NAT2*) gene is known for its polymorphism. The genetic variations leads to the change in the N-acetylation activity and these differences in the acetylation activity leads to the classification of the population into various groups such as rapid, intermediate and slow acetylators. In the present study, we identified different mutations and alleles by sequencing a stretch of 927 bp which covered exon 2 of *NAT2* gene and is reported to have all the allelic variants reported till date. Previously identified mutations and some new allele sub-type were detected for *NAT2* gene in the present study. Overall, we were able to classify 94 individuals into two distinct groups as slow and intermediate acetylators based on their genotype. Out of all the reported alleles, *NAT2*4*, **5*, **6*, **7* and **12* alleles were found in the studied population (n=94) and two new allele subtype *NAT2*5P* and *NAT2*7C* were detected in the studied population which have not been reported earlier. We did not observed any gender differences in the present study based on NAT2 acetylation activity.**

Key words: N-Acetyltransferase 2 (NAT2), polymorphisms, slow acetylators, rapid acetylators.

INTRODUCTION

N-Acetyltransferase 2 (NAT2) is involved in the metabolism of arylamines and hydrazines. The substrates of NAT2 mainly include drugs, such as isoniazid (INH), as well as chemicals and carcinogens (Weber, 1997; Grant et al., 1997; Gross et al., 1999). Thus, N-acetylation activity is associated with drug effects or toxicities and susceptibility to various cancers. The ability of NAT2 to N-acetylate arylamines is subject to genetic polymorphisms in the *NAT2* gene. *NAT2* genotype and its acetylation rate are different among various populations. Acetylation polymorphism causes inter-individual variation in the biotransformation of different drugs, pro-carcinogens and other xenobiotics which have a primary aromatic or hydrazine structure. This enzyme therefore plays an important role in the detoxification and potential metabolic activation of numerous xenobiotics (Rodrigues-Lima and Dupret, 2002; Anitha and Banerjee, 2003). The *NAT2* acetylation

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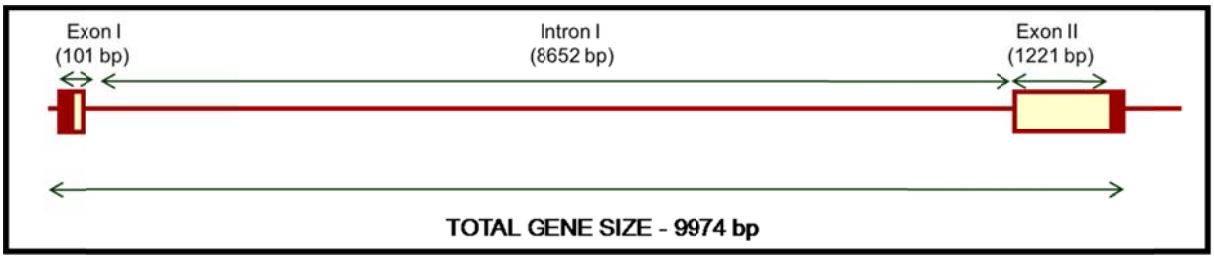

Figure 1. *NAT2* gene map (http://asia.ensembl.org/index.html).

polymorphism has been associated with higher incidences and/or severity of adverse drug reactions (ADRs) to isoniazid, hydralazine, procainamide and sulfamethoxazole (Evans, 1989). Differences in acetylation activity among individuals and in populations of diverse racio-geographic origin have led to the phenotypic classification of humans as rapid (normal activity), intermediate and slow (reduced activity) acetylators (Hein et al., 2000; Lee et al., 2002). Such phenotype/genotype variations have been observed and reported for many drugs. For example, isoniazid is one of the important drugs used in the treatment of tuberculosis and sulphamethoxazole is used in the treatment of secondary infections in AIDS patients. The homozygous occurrence of *NAT2* inactivating mutations results in a slow-inactivator phenotype. Such a phenotype, due to accumulation of the drug, leads to increased dose-dependent toxicity such as hydralazine-induced lupus, isoniazid-induced neuropathies and sulfonamide-induced hypersensitivity reactions in some ethnic groups. It is well established that slow acetylators are more likely to suffer side-effects when prescribed isoniazid, although there is also evidence that these individuals' overall response to therapy may be better as a result of being exposed to higher drug levels for a longer duration (Das et al., 1973; Evans and Relling, 1999; http://nat.mgb.duth.gr).

The *NAT2* polymorphism may also modulate risk of lung, bladder, breast and colon cancer as a result of NAT2 acetylating aromatic amines found in tobacco smoke and cooked foods. Epidemiological studies show an association between *NAT2* acetylation polymorphism and both urinary bladder and colorectal cancers (Eichholzer et al., 2012; Fontana et al., 2009; Brockton et al., 2000). Slow acetylators, who smoke tobacco, have an increased risk of development of bladder cancer compared to rapid acetylators, which are at more risk to colorectal cancer (Osian et al., 2006; Huang et al., 2007; Gu and Wu, 2011). This difference is due to their inability to detoxify aromatic amines in tobacco smoke. The slow acetylator phenotype has also been implicated as a factor in individual susceptibility to immunoglobulin E-mediated food allergy (Woosley et al., 1978; Daly, 1995).

NAT2 gene is located on chromosome 8p22 (OMIM_612182) and spans approximately 9 kb length with two exons (http://www.ensembl.org/index.html). To date, approximately 25 different alleles have been identified for *NAT2* gene (Daly, 1995; Lee et al., 2002; http://nat.mgb.duth.gr). In Caucasians, three variant alleles *NAT2*5*, *NAT2*6* and *NAT2*7*, were among the majority of slow acetylator groups which may vary in other ethnic groups. The frequency of slow acetylators also varies with ethnic origin, ranging from 90% in North Africans to less than 10% in many Asian populations (Evans, 1989). The present study was focused on detecting the known and the unknown mutations *NAT2* gene in Indian population.

MATERIALS AND METHODS

Volunteers

Healthy Indian male (65) and female (29) volunteers (n=94) of age 18 to 55 years were recruited for the present study with their consent.

DNA isolation

Peripheral blood samples (5 ml) were drawn from the volunteers, in sterile tubes containing EDTA as anticoagulant. DNA was extracted by the standard phenol-chloroform method, after digestion with Proteinase K (Sambrook et al., 1989; Sistonen et al., 2009).

Detection of NAT2 variants

NAT2 is 9974 bp gene, consisting of two exons (Figure 1) (http://asia.ensembl.org/index.html). All the alleles reported to date comprise of mutations located on exon 2 (http://nat.mgb.duth.gr). Thus, a fragment of 1393 bp was amplified from genomic DNA which covered the exon 2 (1221 bp) of *NAT2* gene. A region of 927 bp was sequenced from the pre-amplified PCR product which covered all the known alleles. Sequencing was carried out by the sequencing services provided by Polymorphic DNA Technologies, Alameda, California. A bidirectional high throughput capillary sequencing based on the Sanger's dideoxy chain-termination DNA sequencing method, was used for detection of *NAT2* variants (http://nat.mgb.duth.gr). Sequencing analysis detected the known and unknown mutations. The 927 bp sequence, located on Chromosome 8: 18257447-18258373, which was sequenced from the pre-amplified PCR product, is as follow:

CACACGAGGAAATCAAATGCTAAAGTATGATATGTTTTTATGTT
TTGTTTTTCTTGCTTAGGGGATCATGGACATTGAAGCATATTTT

Table 1. *NAT2* Allele frequency in the studied Indian population.

Allele	Frequency (%)			Enzyme activity	R/S
	Total (n=94)	Male (n=65)	Female (n=29)		
**4*	9.6	10.0	8.6	Normal	R
**5*	34.6	36.2	31.0	Almost none	S
**6*	23.4	20.0	31.0	Almost none	S
**7*	5.3	6.2	3.5	Almost none	S
**12*	27.1	27.7	25.9	Normal	R

R-Rapid acetylators, S-slow acetylators

GAAAGAATTGGCTATAAGAACTCTAGGAACAAATTGGACTTGG
AAACATTAACTGACATTCTTGAGCACCAGATCCGGGCTGTTCC
CTTTGAGAACCTTAACATGCATTGTGGGCAAGCCATGGAGTTG
GGCTTAGAGGCTATTTTTGATCACATTGTAAGAAGAAACCGGG
GTGGGTGGTGTCTCCAGGTCAATCAACTTCTGTACTGGGCTCT
GACCACAATCGGTTTTCAGACCACAATGTTAGGAGGGTATTTT
TACATCCCTCCAGTTAACAAATACAGCACTGGCATGGTTCACC
TTCTCCTGCAGGTGACCATTGACGGCAGGAATTACATTGTCGA
TGCTGGGTCTGGAAGCTCCTCCCAGATGTGGCAGCCTCTAGA
ATTAATTTCTGGGAAGGATCAGCCTCAGGTGCCTTGCATTTTCT
GCTTGACAGAAGAGAGAGGAATCTGGTACCTGGACCAAATCA
GGAGAGAGCAGTATATTACAAACAAAGAATTTCTTAATTCTCAT
CTCCTGCCAAAGAAGAAACACCAAAAAATATACTTATTTACGCT
TGAACCTCGAACAATTGAAGATTTTGAGTCTATGAATACATACC
TGCAGACGTCTCCAACATCTTCATTTATAACCACATCATTTTGT
TCCTTGCAGACCCCAGAAGGGGTTTACTGTTTGGTGGGCTTCA
TCCTCACCTATAGAAAATTCAATTATAAAGACAATACAGATCTG
GTCGAGTTTAAAACTCTCACTGAGGAAGAGGTTGAAGAAGTGC
TGAGAAATATATTTAAGATTTCCTTGGGGAGAAATCTCGTGCCC
AAACCTGGTGATGGATC

RESULTS

Sequence analysis of NAT2 gene

The sequencing data was analyzed for the presence of known and unknown polymorphisms. Mutations at positions 282C>T, 341T>C, 481C>T, 590G>A, 803A>G and 857G>A were detected from the sequence analysis of 94 individuals which lead to the detection of *NAT2*4*, **5*, **6*, **7* and **12* alleles. Allele not carrying any mutation was classified as *NAT2*4*, rapid acetylators (R). Alleles *NAT2*5*, **6* and **7* were classified as slow acetylators (S), while *NAT2*12* did not lead to any change in *NAT2* enzyme activity (R) (http://nat.mgb.duth.gr).

New alleles in Indian population

A mutation at position 578C>T was detected in a single individual, in the studied population (n=94), along with other known mutations at 282C>T, 341T>C, 481C>T, 590G>A, 803A>G. This mutation leads to a substitution of threonine amino acid to methionine amino acid at 193 position (T193M), which along with other mutation may lead to change in NAT2 enzyme activity. Thus, this new combination of mutations has been classified as *NAT2*5P* allele by the Arylamine N-acetyltransferase Gene Nomenclature Committee. The mutation combination leads to identification of a new allele specific to the Indian population as this combination is presently not reported in other populations. A new sub-type of *NAT2*7* allele was also found in studied population. It is being termed as *NAT2*7C*, which comprised 282C>T, 803G>A and 857G>A mutations, which may lead to the slow acetylation phenotype (http://nat.mgb.duth.gr). The frequency of this allele was found to be 8.5% (10.8% in males, 3.4% in females). The genotype observed were NAT2*6C/*7C (slow acetylation), NAT2*7C/*12A (intermediate acetylation).

NAT2 genotype and allele frequencies

The alleles *NAT2*4*, **5*, **6*, **7* and **12* were detected from the sequencing data in the studied population. The allelic frequency for these alleles of *NAT2* gene ranged from 5.3 to 34.6% (Table 1). *NAT2*5* was found in 34.6% of individuals. Individuals were classified into three categories as rapid acetylators (R), intermediate acetylators (I) and slow acetylators (S) depending on the alleles and their reported enzyme activities. Based on this classification, the allele frequency of the rapid acetylators was found to be 36.7% and slow acetylators were 63.3% in the studied population (n=94). The genotype frequency of *NAT2* is shown in Table 2. *NAT2*6/*12* genotype dominated the studied population, while no individual was found to have *NAT2*4/*4* genotype, which is wild type genotype. The genotype frequency of rapid acetylators was 5.4%, intermediate acetylators was 62.7% and slow acetylators was 31.9%. No significant difference was observed between males and females in the studied population.

NAT2 inter-ethnic differences

A comparison of the allele frequencies of *NAT2* with other populations is shown in Table 3, and Figure 2. The majority of the studied population had *NAT2*5* (34.6%),

Table 2. *NAT2* Genotype frequency in the studied Indian population.

Genotype	Frequency (%)			Enzyme activity	R/I/S
	Total (n=94)	Male (n=65)	Female (n=29)		
**4/*5*	16.0	15.4	17.2	Decreased	I
**4/*6*	2.1	3.1	0.0	Decreased	I
**4/*12*	1.1	1.5	0.0	Normal	R
**5/*5*	14.9	13.8	17.2	Almost none	S
**5/*6*	2.1	3.1	0.0	Almost none	S
**5/*7*	2.1	1.5	3.4	Almost none	S
**5/*12*	19.1	24.6	6.9	Decreased	I
**6/*6*	9.6	7.7	13.8	Almost none	S
**6/*7*	3.2	4.6	0.0	Almost none	S
**6/*12*	20.2	13.8	34.5	Decreased	I
**7/*12*	5.3	6.2	3.4	Decreased	I
**12/*12*	4.3	4.6	3.4	Normal	R

R, Rapid acetylators; I, Intermediate acetylators; S, Slow acetylators.

Table 3. Comparison of *NAT2* allele frequency in the studied Indian population (%) with other populations (Cascorbi et al., 1995; Aynacioglu et al., 1997; Martinez et al., 1998; Jorge-Nebert et al., 2002; Loktionov et al., 2002; Anitha and Banerjee, 2003; Agúndez, 2003; Tanira et al., 2003; Belogubova et al., 2005; Deguchi et al., 2005; Srivastava and Mittal, 2005; Patin et al., 2006; Rabstein et al., 2006; Al-Yahyaee et al., 2007; Teixeira et al., 2007; Agúndez et al., 2008; Yuliwulandari et al., 2008; Sabbagh et al., 2011).

Allele	Studied population (n=94)	South Indians (n=166)	Caucasians (n=1034)	Omanis (n=127)	Japanese (n=175)	Tswana (n=101)	Korean (n=1000)	Rio de Janeiro (n=298)	Ngawbe (n=105)	Nicaraguans (n=137)	Sudanese (n=127)	Spanish (n=1312)	Turk (n=303)	German (n=844)	Russian (n=364)	Kyrgyz (n=290)	Indonesian (n=212)	R/S
**4*	9.6	11.7	22.0	17.7	68.6	13.4	66.1	17.3	72.4	41.6	8.7	22.2	23.1	22.7	23.5	39.8	37.3	R
**5*	34.6	25.9	45.4	42.4	2.0	32.2	1.6	40.1	2.4	35.8	47.3	45.6	41.7	46.4	45.6	19.3	9.0	S
**6*	23.3	36.7	26.2	26.4	19.8	18.8	20.1	26.3	0.0	17.4	28.7	26.7	30.5	27.8	27.2	26.5	36.8	S
**7*	5.3	22.3	1.2	3.9	9.6	0.0	11.5	4.2	23.3	0.0	3.1	1.2	4.5	1.3	3.1	12.1	15.3	S
**11*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	S
**12*	27.2	2.0	2.6	5.2	0.0	20.8	0.3	4.4	0.0	3.6	8.3	2.6	0.2	0.0	0.5	0.5	0.9	R
**13*	0.0	2.1	0.0	2.4	0.0	6.4	0.1	3.0	1.9	1.1	0.1	0.3	0.0	1.5	0.0	0.0	0.7	R
**14*	0.0	0.0	1.0	0.0	0.0	8.4	0.0	4.7	0.0	0.4	3.2	1.4	0.0	0.1	0.0	0.0	0.0	S

R, Rapid acetylators; S, slow acetylators.

*NAT2*6* (23.3%) and *NAT2*12* (27.2%) allele while, most of the other populations mentioned in Table 3, were predominated by *NAT2*5*, *NAT2*6* or *NAT2*7* alleles. Significant differences were observed when the allele frequency of slow acetylators from the present study were compared

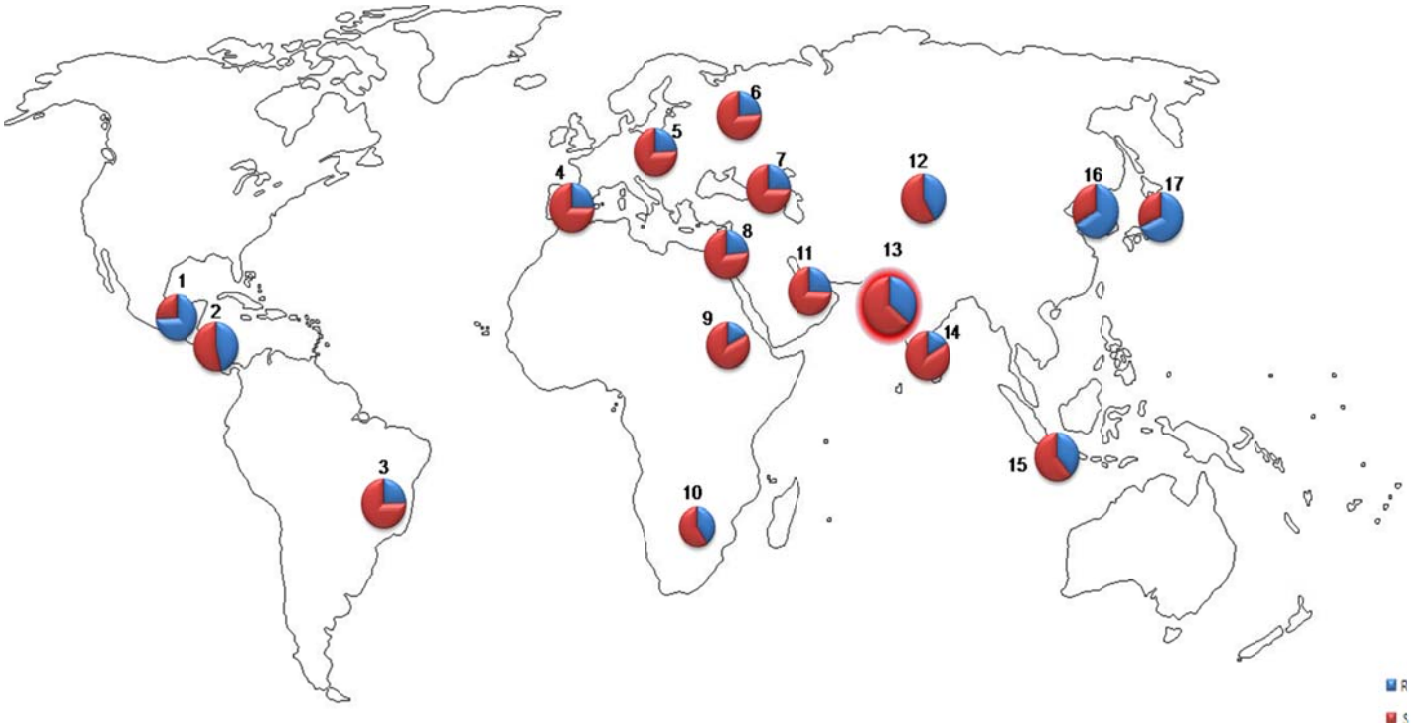

Figure 2. Comparison of *NAT2* allele frequency in different populations (R-Rapid acetylators, S-Slow acetylators): 1, Nicaraguans (n=137); 2, Ngawbe (n=105); 3, Rio de Janeiro (n=298); 4, Spanish (n=1312); 5, German (n=844); 6, Russian (n=364); 7, Caucasians (n=1034); 8, Turk (n=303); 9, Sudanese (n=127); 10, Tswana (n=101); 11, Omanis (n=127); 12, Kyrgyz (n=290); 13, Studied population (n=94) ; 14, South Indians (n=166); 15, Indonesia (n=212); 16, Korean (n=1000); 17, Japanese (n=175) (Cascorbi et al., 1995; Aynacioglu et al., 1997; Martinez et al., 1998; Jorge-Nebert et al., 2002; Loktionov et al., 2002; Anitha and Banerjee, 2003; Agúndez, 2003; Tanira et al., 2003; Belogubova et al., 2005; Deguchi et al., 2005; Srivastava and Mittal, 2005; Patin et al., 2006; Rabstein et al., 2006; Al-Yahyaee et al., 2007; Teixeira et al., 2007; Agúndez et al., 2008; Yuliwulandari et al., 2008; Sabbagh et al., 2011).

with reported frequency for South Indian, Japanese, Korean, Ngawbe and Spanish ($p<0.0001$); Sudanese ($p<0.005$), whereas the difference between our data, Caucasians, Tswana, Rio de Janeiro and Omanis ($p<0.1$); Nicaraguans, Turk, German, Russian, Kyrgyz and Indonesian ($p>0.1$) was not significant (Cascorbi et al., 1995; Aynacioglu et al., 1997; Martinez et al., 1998; Jorge-Nebert et al., 2002; Loktionov et al., 2002; Anitha and Banerjee, 2003; Agúndez, 2003; Tanira et al., 2003; Belogubova et al., 2005; Deguchi et al., 2005; Srivastava and Mittal, 2005; Patin et al., 2006; Rabstein et al., 2006; Al-Yahyaee et al., 2007; Teixeira et al., 2007; Agúndez et al., 2008; Yuliwulandari et al., 2008; Sabbagh et al., 2011).

Comparison of the genotype frequencies of *NAT2* among Asians is shown in Table 4 and Figure 3. *NAT2*6/*12* genotype predominated the studied population, while this combination was not found in South Indians, Omanis and Japanese. The frequency of *NAT2*5/*6* was found as low as 2.1% in the present study but among the South Indians and Omanis, was somewhere around 19% and was not detected in Japanese population (Anitha and Banerjee, 2003; Agúndez, 2003; Tanira et al., 2003; Deguchi et al., 2005).

DISCUSSION

NAT2 previously identified mutations and new allele subtypes were detected in the present study, by sequencing a stretch of 927 bp covering exon 2, which is reported to have all the allelic variants of *NAT2* gene (Evans, 1989; http://asia.ensembl.org/index.html). *NAT2*4*, **5*, **6*, **7* and **12* alleles were found in the studied population (n=94) in which alleles *NAT2*5*, **6* and **7* are classified as slow acetylators (S) (http://nat.mgb.duth.gr). The wild type allele which did not have any mutation was classified as *NAT2*4*, the rapid acetylator (R). Individuals grouped as slow acetylators usually do not respond to drugs like isoniazid and may show signs of ADRs.

Furthermore, NAT2 is also responsible for detoxification of many aromatic amines found in tobacco smoke, etc which, on accumulation in the body might develop ADRs or diseases like cancer (Hirvonen and Vineis, 1999; Anitha and Banerjee, 2003; Chauhan et al., 2007; Sistonen et al., 2009). Thus, as observed in the present study, 31.9% of the total population might be at a risk of developing such ADRs or may be susceptible to certain diseases. Individuals classified as intermediate acetylators were found to be 62.7% in the studied

Table 4. Comparison of *NAT2* genotype frequency in the studied Indian population (%) with other populations (Anitha and Banerjee, 2003; Tanira et al., 2003; Deguchi et al., 2005).

Genotype	Studied population (n=94)	South Indians (n=166)	Omanis (n=127)	Japanese (n=175)	R/I/S
*4/*4	0.0	0.0	2.4	46.5	R
*4/*5	16.0	2.0	12.6	1.7	I
*4/*6	2.1	10.2	11.8	30.2	I
*4/*12	1.1	1.0	1.6	0.0	R
*4/*7	0.0	9.1	2.4	12.2	I
*4/*13	0.0	1.0	0.8	0.0	R
*5/*5	14.9	9.2	22.9	0.6	S
*5/*6	2.1	19.3	19.7	0.0	S
*5/*7	2.1	11.1	3.1	1.2	S
*5/*12	19.2	1.0	8.7	0.0	I
*6/*6	9.5	13.3	10.3	3.5	S
*6/*7	3.2	17.2	0.0	2.3	S
*6/*12	20.2	0.0	0.0	0.0	I
*6/*13	0.0	0.0	1.6	0.0	I
*7/*12	5.3	2.0	0.0	0.0	I
*7/*13	0.0	3.1	0.8	0.0	I
*7/*7	0.0	1.0	0.0	1.7	S
*12/*12	4.3	0.0	0.0	0.0	R
*13/*13	0.0	0.0	0.8	0.0	R

R, Rapid acetylators; I, intermediate acetylators; S, slow acetylators.

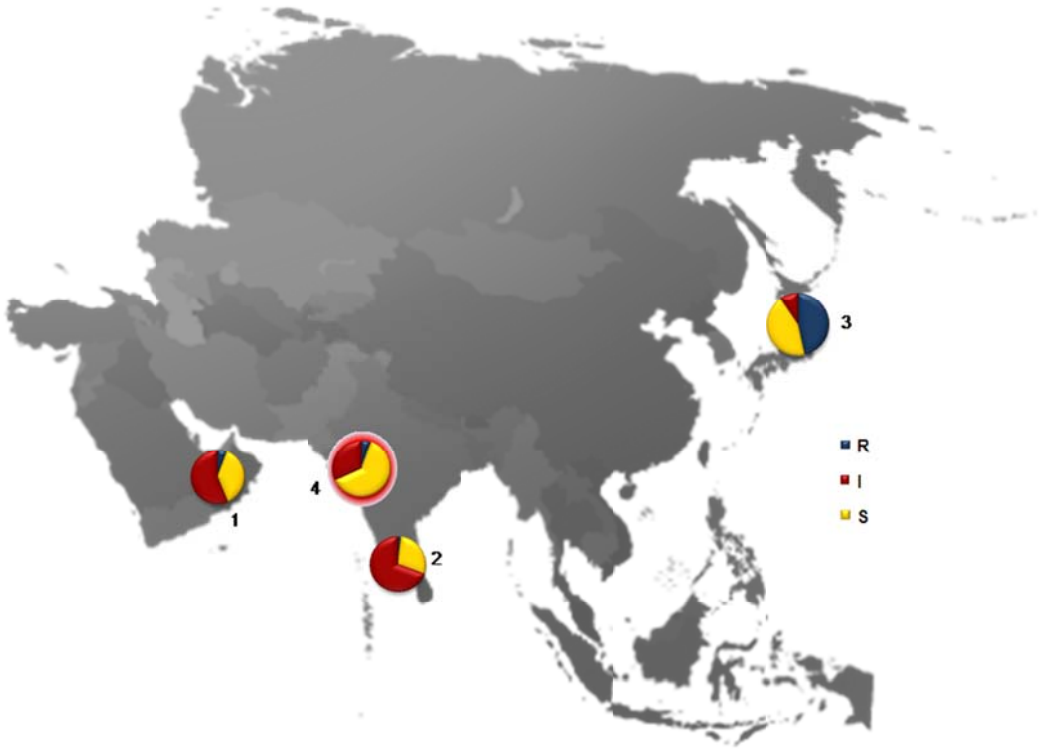

Figure 3. Comparison of *NAT2* genotype frequency among Asians (R-Rapid acetylators, I-Intermediate acetylators, S-Slow acetylators): 1- Omanis (n=127), 2- South Indians (n=166), 3- Japanese (n=175), 4- Studied population (n=94) (Anitha and Banerjee, 2003; Tanira et al., 2003; Deguchi et al., 2005).

population. The present study, thus reflects that the entire population can be divided into slow or intermediate acetylators which might display partial or very low acetylation activity (Table 4) (Anitha and Banerjee, 2003; Tanira et al., 2003; Deguchi et al., 2005). A new sub-type of *NAT2*5* and *NAT2*7* allele were detected in the studied population which has not been reported earlier. *NAT2*5* sub-type consisting of 282C>T, 341T>C, 481C>T, 578C>T, 590G>A, 803A>G mutation has been named as *NAT2*5P* allele by the Arylamine N-acetyltransferase Gene Nomenclature Committee. Whereas, *NAT2*7* allele sub-type has been termed as *NAT2*7C* allele having mutations at position 282C>T, 803G>A and 857G>A (http://nat.mgb.duth.gr). Among the studied population, 31.9% individuals were estimated to be slow acetylators for *NAT2* from the genotype frequency; whereas 71.1% South Indians and 9.3% Japanese were reported as individuals showing slow acetylation phenotype for *NAT2* (Table 4) (Anitha and Banerjee, 2003; Tanira et al., 2003; Deguchi et al., 2005). All these differences suggest diversity among various ethnic groups and within Indian population for *NAT2* gene.

Conflict of Interests

The author(s) have not declared any conflict of interest.

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