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## Genetic polymorphisms of N-acetyltransferase 2 & susceptibility to antituberculosis drug-induced hepatotoxicity

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**Background & objectives:** The N-acetyltransferase 2 (*NAT2*) gene encodes an enzyme which both activates and deactivates arylamine and other drugs and carcinogens. This study was aimed to investigate the role of *NAT2* gene polymorphism in anti-tuberculosis drug-induced hepatotoxicity (DIH).

**Methods:** In this prospective study, polymerase chain reaction-restriction fragment length polymorphism results for *NAT2* gene were compared between 185 tuberculosis patients who did not develop DIH and 105 tuberculosis patients who developed DIH while on anti-tuberculosis drugs.

**Results:** Frequency of slow-acetylator genotype was commonly encountered and was not significantly different between DIH (82.8%) and non-DIH (77.2%) patients. However, the genotypic distribution of variant *NAT2*\*5/\*7 amongst slow-acetylator genotypes was significantly higher in DIH (56%) group as compared to non-DIH (39%) group (odds ratio 2.02; *P*=0.006).

**Interpretation & conclusions:** The present study demonstrated no association between *NAT2* genotype and DIH in the north Indian patients with tuberculosis.

**Key words** Drug-induced hepatotoxicity - genetic polymorphism - N-acetyltransferase 2 - polymerase chain reaction - restriction fragment length polymorphism - rapid acetylator - slow acetylator

N-acetyltransferase 2 (*NAT2*) is an enzyme involved in the detoxification of several carcinogenic arylamines and drugs. *NAT2* acetylates isoniazid and forms acetylisoniazid, which is hydrolyzed to monoacetylhydrazine. In case of rapid acetylators, *NAT2* rapidly acetylates this compound to form non-toxic diacetylhydrazine; however, slow acetylators encode reduced acetylation activity. Genetic polymorphisms at *NAT2* gene are known to be involved with modification of drug metabolism in the

liver and thus aid in the predisposition of an individual towards drug-induced hepatotoxicity (DIH)<sup>1</sup>. Available information in the literature regarding the role of *NAT2* polymorphism on the risk of DIH development during anti-tuberculosis (TB) treatment is conflicting.

The issue of genetic susceptibility to DIH involving acetylator status in Indian population has been addressed by only two studies<sup>2,3</sup>. The present study was designed to assess the role of *NAT2* polymorphism

in conferring susceptibility to DIH during anti-TB treatment.

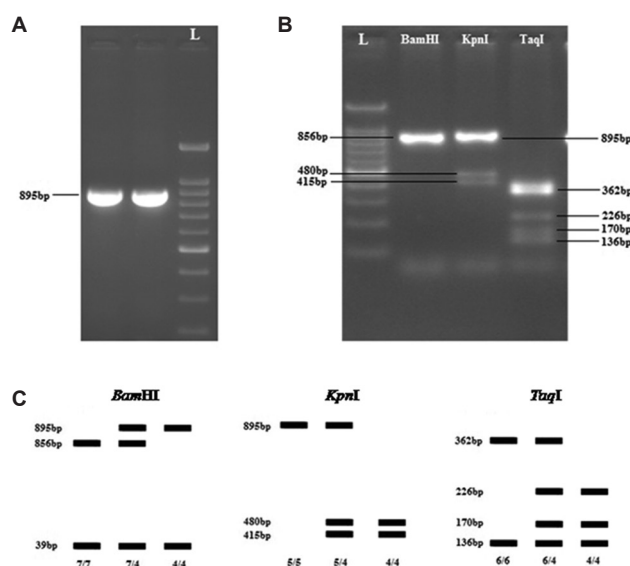
### Material & Methods

In this prospective study, patients aged 16-65 yr were recruited from the outpatient department or the inpatient facility of the department of Medicine, All India Institute of Medical Sciences (AIIMS) Hospital, New Delhi, India, between March 2007 and February 2011. A total of 113 consecutive patients who developed clinical and/or laboratory evidence of DIH, while on anti-TB drugs during the study period constituted the cases (DIH), whereas 201 patients with TB not developing DIH constituted the controls (non-DIH). Written informed consent was obtained from all patients. Institutional Ethics Committee approved the study protocol. The dose levels of anti-TB drugs administered to the TB patients were as per the earlier published study<sup>4</sup>. Briefly, the diagnostic criteria of DIH included (i) a rise of five times the upper limit of the normal levels (50 IU/l) of serum aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) on one occasion or more than three times (>150 IU/l) on three consecutive occasions; (ii) a rise in the level of serum total bilirubin level >1.5 mg/dl; (iii) an increase in serum AST and/or ALT above pre-treatment values together with anorexia, nausea, vomiting and jaundice; (iv) absence of serological evidence of infection with hepatitis viruses A, B, C or E; and (v) improvement in liver functions (serum bilirubin <1 mg/dl, AST and ALT <100 IU/l) after withdrawal of anti-TB drugs<sup>5</sup>. DIH was diagnosed if any one of the criteria i, ii or iii was present along with criteria iv and v. Non-DIH patients were observed during the same study period with regular follow up of liver function tests.

Serological testing for evidence of human immunodeficiency virus (HIV) 1, 2 infection by enzyme-linked immunosorbent assay was also done. Patients with HIV infection and chronic alcohol-dependant patients who consumed >48 g of alcohol/day for at least one year were excluded from the study. Patients receiving other potentially hepatotoxic drugs (e.g., methotrexate, phenytoin, valproate and fluconazole), pregnant women and patients who did not give written informed consent were also excluded from the study. Patients with DIH satisfying the inclusion criteria were enrolled into the study.

The DNA samples of the DIH and non-DIH patients were from the previously reported study<sup>4</sup>. Polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) results were compared in 185 non-DIH and 105 DIH patients.

*N-acetyltransferase 2 (NAT2) genotyping*: DNA was extracted from the patients' peripheral white blood cells using QIAGEN GmbH (Hilden, Germany) DNA maxi manufacturer kit. In brief, 50 µg DNA was used as the template. The primer sequences for *NAT2* were: 5'ATG GAC ATT GAA GCA TAT TTT GAA AGA ATT3' (forward) and 5'AAGGGT TTA TTT TGT TCC TTA TTC TAA AT3' (reverse). An open source Primer3 program was used for designing PCR primers (<http://bioinfo.ut.ee/primer3-0.4.0/primer3>). The oligo DNA primers were synthesized by Sigma-Aldrich, USA. The cyclic temperature profiles were followed to amplify the *NAT2* gene. To ensure complete extension, a seven minute incubation at 72°C was done. Digestion of 500 ng to 1 µg PCR product was carried out in a total volume of 50 µl using appropriate buffer and restriction enzymes<sup>3</sup>. The 895 bp PCR product of the *NAT2* gene was subjected to the restriction enzyme digestion by *KpnI*, *TaqI* and *BamHI*, (Fermentas, USA), respectively. The digested products were resolved on a three per cent agarose gel. NAT2\*4 represented the wild-type allele and the loss of restriction cutting sites, *KpnI*, *TaqI* and *BamHI* were denoted as NAT2\*5, NAT2\*6 and NAT2\*7 allele, respectively. Restriction pattern of *NAT2* genotypes is shown in the Figure. Individuals with one or two wild-type NAT2\*4 alleles were defined as rapid acetylators and those with any two polymorphic alleles as slow acetylators. Nucleotide substitutions may or may not result in an amino acid substitution: C481T,



**Figure.** Panel A represents amplification of N-acetyltransferase 2 gene, Panel B is the representative gel picture of restriction digestion, Panel C representing the restriction pattern of *BamHI*, *KpnI* and *TaqI* restriction sites which are diagnostic for mutation at nucleotide position 857, 481 and 590, respectively. The diagnostic bands for analysis of the mutation sites are shown.

**Table I.** N-acetyltransferase 2 (*NAT2*) genotype distribution in drug-induced hepatotoxicity (DIH) and non-drug-induced hepatotoxicity groups

<i>NAT2</i> genotype	DIH (n=105) (%)	Non-DIH (n=185) (%)	Unadjusted OR (95% CI)	<i>P</i> <sup>†</sup>	Adjusted OR (95% CI)	<i>P</i> <sup>††</sup>
Rapid acetylator	18 (17.1)	42 (22.7)	1		1	
Slow acetylator	87 (82.8)	143 (77.3)	1.41 (0.76-2.62)	0.25	1.69 (0.84-3.38)	0.13
<i>NAT2</i> *6/*7						
Absent	91 (86.6)	163 (88.1)	1		1	
Present	14 (13.3)	22 (11.8)	1.13 (0.55-2.33)	0.72	1.15 (0.53-2.52)	0.71
<i>NAT2</i> *5/*7						
Absent	46 (43.8)	112 (60.5)	1		1	
Present	59 (56.1)	73 (39.4)	2.02 (1.21-3.19)	0.006	1.96 (1.14-3.36)	0.01
<i>NAT2</i> *5/*6						
Absent	93 (88.5)	151 (81.6)	1		1	
Present	12 (11.4)	34 (18.3.7)	0.57 (0.28-1.16)	0.12	0.58 (0.27-1.28)	0.18
<i>NAT2</i> *5/*6/*7						
Absent	103 (98.1)	171 (92.4)	1		1	
Present	2 (1.9)	14 (7.5)	0.24 (0.03-1.07)	0.04	0.37 (0.77-1.83)	0.22
Rapid acetylator <i>NAT2</i> *4/*4						
Absent	102 (97.1)	184 (99.4)	1		1	
Present	3 (2.8)	01 (0.5)	5.4 (0.58-52.70)	0.11	3.17 (0.30-33.5)	0.33
<i>NAT2</i> *5/*4						
Absent	100 (95.2)	164 (88.6)	1		1	
Present	5 (4.7)	21 (11.3)	0.39 (0.09-1.06)	0.06	0.29 (0.09-0.94)	0.04
<i>NAT2</i> *6/*4						
Absent	99 (94.2)	173 (93.5)	1		1	
Present	6 (5.7)	12 (6.4)	0.87 (0.31-2.40)	0.74	0.76 (0.25-2.33)	0.64
<i>NAT2</i> *7/*4						
Absent	101 (96.1)	177 (95.6)	1		1	
Present	4 (3.8)	08 (4.32)	0.87 (0.25-2.98)	0.83	0.90 (0.22-3.69)	0.88

<sup>†</sup>Based on Chi-square/Fisher's exact test; <sup>††</sup>Based on multivariate logistic regression analysis with adjustment for pre-treatment serum albumin level. OR, odds ratio; CI, confidence interval

no change (*KpnI*); G590A, Arg197Gln (*TaqI*); G857A, Gly286Glu (*BamHI*).

**Statistical analysis:** The genotypic distribution was compared between cases and controls using Chi-square/Fisher's exact test. The odds ratio (OR) of DIH associated with *NAT2* genotype was calculated using logistic regression.

### Results & Discussion

Table I shows the *NAT2* genotypes in 185 non-DIH and 105 DIH patients with TB. The frequency of a

slow-acetylator variant *NAT2*\*5/\*7 of *NAT2* genotype in DIH (56%) group was significantly higher as compared with the non-DIH (39%) group (OR=2.02, *P*=0.006). However, the combined frequency of all slow-acetylator variants namely, *NAT2*\*6/\*7, *NAT2*\*5/\*7, *NAT2*\*5/\*6 and *NAT2*\*5/\*6/\*7 together with an OR of 1.41 [95% confidence interval (CI), 0.76-2.62] was not significant (*P*=0.25) (Table I). After adjustment with baseline serum albumin, the results for slow-acetylator variant *NAT2*\*5/\*7 of *NAT2* gene still remained significant with an OR of 1.96 (95% CI,

**Table II.** Comparison of studies in different ethnicity for N-acetyltransferase 2 polymorphisms in drug-induced hepatotoxicity (DIH) patients

Study	Country	Cases versus controls (n)	Association
Gurumurthy <i>et al</i> <sup>6†</sup>	India	DIH (850) versus non-DIH (2150)	No association
Roy <i>et al</i> <sup>2</sup>	India	DIH (33) versus non-DIH (33)	No association
Huang <i>et al</i> <sup>1</sup>	Taiwan	DIH (33) versus non-DIH (191)	Slow-acetylator
Bose <i>et al</i> <sup>3</sup>	India	DIH (41) versus non-DIH (177)	Slow-acetylator
Lv <i>et al</i> <sup>7</sup>	China	DIH (89) versus non-DIH (356)	No association
Present study	India	DIH (105) versus non-DIH (185)	No association

<sup>†</sup>Authors had studied acetylator-phenotype in 3000 south Indian patients and reported no association with the development of DIH

1.14-3.36),  $P=0.01$  (Table I). The overall *NAT2* slow-acetylator genotype after adjustment with baseline serum albumin remained non-significant with an OR of 1.69 (95% CI, 0.84-3.38),  $P=0.13$ .

The *NAT2* polymorphisms have been considered as a major risk factor for ATT-induced hepatotoxicity<sup>1</sup>. However, there is no consensus as to whether *NAT2* acetylator status could be used as a marker to identify patients at higher risk of developing DIH (Table II). The genotype frequency of *NAT2* slow acetylator in south Indian population was reported to be 60 per cent<sup>6</sup>. Roy *et al*<sup>2</sup> compared the *NAT2* gene polymorphism in 33 patients with pulmonary TB who developed DIH, and 33 TB patients who did not develop DIH. Their study did not reveal the significant differences in the *NAT2* slow-acetylator allele frequencies between cases and controls, and our findings were in agreement with this study. Bose *et al*<sup>3</sup> reported a significantly higher frequency of slow-acetylator genotypes in DIH patients (70.73%) when compared to non-DIH patients (44.63%), with OR of 2.99 (95% CI, 1.4-6.2),  $P=0.0045$ . A stratified analysis indicated that the slow-acetylator status was associated with DIH amongst the patients with poor nutritional status<sup>3</sup>. However, the overall effect of slow acetylator adjusted for nutritional status was not reported. Further, the study was conducted in only 41 patients who developed DIH. Another study by Lv *et al*<sup>7</sup> in Chinese population included 89 DIH and 356 non-DIH patients. They also did not find a significant association between *NAT2* genotype and DIH. Gurumurthy *et al*<sup>6</sup> studied a large prospective cohort (850 DIH vs. 2150 non-DIH patients) to determine the association between acetylator phenotype and susceptibility to DIH in south Indian population and found no association of DIH with acetylator status. In our study, DIH developed mostly in those patients who were slow acetylators (82.8%). Amongst the

slow-acetylator variants, *NAT2*\*5/\*7 was present in high frequency, suggesting that this variant was common in the north Indian population. However, the overall *NAT2* genotype was not associated with the development of DIH.

In conclusion, our results showed that *NAT2* genotype was not a risk factor for the development of DIH in north Indian TB patients. The discordance between findings of the present study with that of the other studies conducted in Asian populations could be due to the difference in statistical analysis approach to evaluate the risk factor, and different selection criteria used for cases and controls.

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**Conflicts of Interest:** None.

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