Polymorphism of NAT2, PXR, ABCB1, and GSTT1 genes among tuberculosis patients of North Eastern States of India

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Abstract
Background: Anti-tuberculosis drug-induced liver injury (AT-DILI) in tuberculosis (TB) patients has been linked to polymorphisms in genes encoding drug metabolism enzymes and proteins.

Objective: This study aimed to monitor polymorphisms of NAT2, PXR, ABCB1, and GSTT1 genes in TB patients from three states (Manipur, Tripura, and Nagaland) in the North Eastern Region of India.

Methods: Genomic DNA was isolated from the whole blood samples of TB patients (n=219; Manipur:139; Tripura: 60; Nagaland: 20). The TaqMan allelic discrimination assay and statistical tools were used to investigate single nucleotide polymorphisms (SNP) patterns in NAT2, PXR, ABCB1, and GSTT1 genes.

Results: In the study population, ten distinct genotypes of the NAT2 gene and single variation in the PXR, ABCB1, and GSTT1 genes were identified. A strong linkage disequilibrium (LD) was observed between rs1801280 and rs1799931 of the NAT2 gene. Majority of the study populations were intermediate (~46.1%), rest were either slow acetylators (~35.6%) or fast acetylators. Interestingly, ~55% of the TB patients in Tripura were slow acetylators and majority in Manipur and Nagaland were of intermediate acetylator genotypes. For all of the markers investigated, the population had a greater prevalence of ancestral alleles and genotypes. According to a combinational study of the genotypes linked to AT-DILI, ~26.1% of the population possessed the risk genotypes.

Conclusion: These TB patients from north eastern states of India were found as carriers of the ancestral alleles and genotypes. And the risk for AT-DILI during TB treatment is low. Expanding such studies with additional markers and larger sample sizes will be useful to generate precise population-specific pharmacogenomics details for efficient TB management.
Keywords: Polymorphism, NAT2, PXR, ABCB1, GSTT1, AT-DILI.
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Introduction
Tuberculosis (TB) disease is caused by infection of Mycobacterium tuberculosis (Mtb) and leads to ~2 million annual deaths worldwide [1]. In India, the TB incidence rate is ~159/1,00,000 with a ~4% fatality rate [2]. Treatment strategy in TB patients involves multiple drugs for long duration up to 6-18 months depending on the drug susceptibility or resistance status of Mtb. Majority of these anti-TB drugs undergo further conversion by the host enzymes to become active molecules for effective killing of Mtb. This contributes to certain side effects like skin reactions, hepatitis, nausea, vomiting, purpura, lethargy, dizziness, abdominal pain, gastrointestinal and neurological disorders [3,4]. About ~1-47 % of the TB patients, during treatment, develop anti-tuberculosis drug-induced liver injury (AT-DILI) [1]. The type of prescribed drugs, race, age, genetic factors, sex, alcohol intake, TB condition, and co-infection with HIV, hepatitis B and C virus [5,6,7,8,9,10,11,12,13] influences the severity of AT-DILI. With discontinuation of the treatment, AT-DILI condition is also reversed [14]. Certain variations in the genomic regions within or near the genes encoding drug-metabolizing enzymes (NAT2, CYP2E1, GSTs), lipid metabolism (CYP7A1, BSEP, UGTs, PXR), immune adaptations (HLAs and TNF-α), transporter proteins (ABCB1) and oxidant challenges (TXNRD1, SOD1, BACH1) were reported to be associated with AT-DILI [15].
NAT2 is a cytosolic phase II conjugation enzyme responsible for the deactivation of isoniazid to acetyl isoniazid [16,17,18,19]. It further hydrolyses acetyl hydrazine, which is subsequently oxidized by cytochrome P450 2E1 (CYP2E1) to intermediates with hepatotoxic effect. NAT2 gene, present on
chromosome 8, is reported to show high polymorphisms with 36 allelic variants representing varied acetylation activities (rapid acetylators: RAs, homozygous of NAT2*4 alleles; intermediate acetylators: IAs, heterozygous of NAT2*4 alleles; and slow acetylators: SAs, homozygous/non-heterozygous of NAT2*4 alleles) [20,21,22]. Most variants (single nucleotide polymorphisms: SNPs) of the NAT2 gene are located within the 873bp intronless coding region. Four SNPs rs1801280 (341 T>C; NAT2*5), rs1799930 (590 G>A; NAT2*6), rs1799931 (857 G>A; NAT2*7), rs1801279 (191 G>A; NAT2*14) are associated with slow acetylator phenotype [23]. TB patients with low NAT2 enzyme activity show higher circulatory hydrazine levels creating a significant risk to develop AT-DILI [24, 25, 26, 27].

Similarly, PXR in the liver, regulates the expression of many genes (CYP3A4 isoenzyme, UGT1A1, MDR1, ABCB1, glutathione S-transferase (GST), SULT2A1, UGT1A1, MRP2, OATP2) involved in hepatic drug-clearance system [28,29]. Polar groups like glucuronides are added to anti-TB drugs for detoxification by these enzymes before elimination from the host [30]. Polymorphism in the PXR gene (rs3814055; C>T) has shown an association with an increased risk of AT-DILI [31].

Glutathione S-transferases (GSTs) are phase II drug-metabolizing enzymes involved in protecting cells from oxidative stress. Xenobiotics including drugs (like isoniazid and rifampicin) and reactive oxygen species (ROS) cause liver injury and GSTs conjugate glutathione to these groups before elimination to protect the liver from damage. GSTM1 and GSTT1, members of GST, are involved in the conjugation of isoniazid. Homozygous null mutation at these loci is reported to show a higher rate of AT-DILI [32, 33].

ABCB1 (MDR1) gene, located on human chromosome 7q21.12, is reported to be highly polymorphic [34, 35]. The SNP present in exon 26 (C3435T SNP; rs1045642), alters the expression of P-gp and it affects the drug pharmacokinetics of its substrate drugs [36, 37]. Subjects with the homozygous 3435 TT genotype of ABCB1 is reported to show a higher (>3-fold) chance of developing DILI [38].

The population level genetic polymorphisms pattern varies significantly within and across countries. India harbors highest TB cases globally and presents a very diverse population with varied ethnicities. Importantly, very limited pharmacogenomics reports are available from the TB patients of the north eastern states of India that shares international boundary with Myanmar and Bangladesh. These populations show higher population diversities and higher TB incidence rates compared to the rest of the states. So, in this study we monitored the genetic variants of NAT2, PXR, GSTs, and ABCB1 genes in TB patients to generate baseline data to calculate the risk of developing AT-DILI for better TB management.

**Materials And Method**

**Subjects:** This study was approved by the ethical committees of Jawaharlal Nehru Institute of Medical Sciences (JNIMS)-Imphal, Manipur (Ref No. Ac/04/IEC/JNIMS/2017), Agartala Govt. Medical College (AGMC)-Agartala, Tripura (Ref. No. F.4(6-9)/AGMC/Academic/IEC Committee/2015/8965 dated 25th April 2018); and Naga Hospital Authority (NHAK)-Kohima, Nagaland (NHAK/HLRC-008/2012 dated 17th May 2017), Manipur University (MU), (Ref No. Ac/IHEC/MU/003/2017) and International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi (IEC/IRB No. ICGEB/IEC/2018/06). Subjects reporting with any of the symptoms (>2 weeks of cough, weight loss, night sweat, fever) of TB to the outpatient departments of JNIMS-Imphal, AGMC-Agartala, and NHAK-Kohima representing three Indian northeastern states (Manipur, Tripura and Nagaland) respectively were recruited. After receiving signed informed consent from the study participants, sputum samples were collected for the microscopy, culture and/or GeneXpert tests. And subjects with positive test results of all these tests were selected as active TB(ATB). Whole venous blood samples (~2 ml) were collected in a vial containing EDTA for genomic DNA extraction.

**Genomic DNA Extraction:** Whole blood samples were used for genomic DNA (gDNA) extraction using DNA extraction kits (QIAmp DNA Mini Kits, Qiagen) in a QIAcube system (Qiagen). Extracted
Genetic materials were quantified using a spectrophotometer (BioPhotometer, Eppendorf), and integrity was monitored by running agarose gel electrophoresis.

**Genotyping Analysis:** A total of six SNPs, three for NAT2, and one each for PXR, ABCB1, and GSTT1 genes were selected for the study. Details of the markers used in this study are tabulated in Table 1. Genotyping assays were performed in a qPCR (Rotor-Gene, Qiagen) using TaqMan allelic discrimination probes (Thermo Fisher, USA). The reaction mixture (10 µL) was prepared by adding gDNA (10 ng), TaqMan Universal PCR Master Mix II (5 µL) with uracil N-glycosylase (UNG2X), and Drug Metabolism Genotyping Assay Mix (20X, 0.5 µL) consisting of forward and reverse primer (18 µM) and probes specific for each allele (4 µM). The amplification process involved two steps: a hold at 95 °C for 10 minutes, followed by 50 cycles of amplification including denaturation at 92 °C for 15 seconds and annealing/extension at 60 °C for 90 seconds following the manufacturer’s protocols. The presence or absence of these alleles were analyzed and identified based on the amplification peaks of its probes (VIC/FAM fluorescent dye labeled) specific to the alleles.

**Statistical analysis:** Hardy Weinberg Equilibrium (HWE) analysis was performed for each marker in the population using SPSS software (16.0). Allelic and genotypic frequency were calculated, and a Chi-square test was performed to determine the allelic or genotypic differences among the study populations. Linkage disequilibrium (LD) between the NAT2 markers was analyzed using the Haploview program (4.1). Genetic distance between populations was estimated using Popgene32 software (1.32). Statistical significance was assumed at \( p \leq 0.05 \) at 95% confidence.

**Results:**

**Patient details:** A total of 219 TB patients (male:female, 158/61, mean age (range) in years) from three northeastern states of India (Manipur: 139; Tripura: 60; Nagaland: 20) were included in this study. The mean age of the study population was 46 years.

**Allelic and genotypic distribution of NAT2:** The NAT2 gene profile of the study population showed four alleles (NAT2*4, NAT2*5, NAT2*6, and NAT2*7) and ten different genotypes. Genotypic distributions of two of these markers confirmed homogenous distribution following HWE except for NAT2*6 marker (Table 2). Genotypes of three variants of the NAT2 gene and their corresponding phenotypic profile are tabulated in Table 3. The allelic frequencies of the three variants of NAT2 in the studied population were found to be 19.9% (95% CI, 1.32-1.48), 24.32% (95% CI, 1.40-1.56), and 18.12% (95% CI, 1.27-1.44) respectively (Table 5). The genotypic frequency of NAT2*4/*6 was highest (~20.1%), and the least was observed for NAT2*5/*7 (~3.2%) as shown in Table 3. The allelic frequencies of NAT2 markers were significantly \( (p \leq 0.05) \) different among the three sub-populations (Table 5). However, no significant difference was observed in the genotypic frequencies among the TB patients from three study sites. Manipur and Nagaland populations have higher frequencies of intermediate acetylator genotypes. NAT2*5/*6, NAT2*5/*7, NAT2*6/*6, and NAT2*6/*7 genotypes were not detected in the Nagaland population (Table 4). The majority of these study populations were found to be intermediate acetylators (~46.1 %) and the rest were either acetylators (~35.6%) or fast acetylators (~17.3 %). Majority (~55%) of Tripura ATB patients showed slow acetylator phenotype, followed by Nagaland (~30%) and Manipur (~27.4%).

**Linkage Disequilibrium (LD) analysis between NAT2 markers:** LD analysis of the three NAT2 variants, rs1801280 (341 T>C), rs1799930 (590 G>A), and rs1799931 (857 G>A) using Haploview software showed no particular block between the markers. A high LD score (D'=0.82) was observed between rs1801280 and rs1799931 (Fig. 1). The LD strength between rs1801280-rs1799930 and rs1799930-rs1799931 were comparatively lower than rs1801280-rs1799931.

**Allelic and genotypic distribution of PXR:** The PXR marker in the study population did not confirm to HWE. The allelic and genotypic frequencies of the PXR gene (rs3814055, 25385 C>T) as observed in the study population are tabulated in Table 5 and Table 6. The homozygous C and T genotypes were observed to be ~64.2% and ~4.6%, respectively and ~31.2% was of heterozygous CT genotype. The
allelic frequencies of C and T in the study population were found to be ~80% and ~20%, respectively. All three sub-populations showed a higher frequency of homozygous CC genotypes (Tripura: ~51.7%; Manipur: ~69.60%, and Nagaland: ~65%) compared to TT or CT genotypes. No significant differences were observed in the allelic and genotypic frequencies of the PXR marker among these three study sites.

Allelic and genotypic distribution of ABCB1: The ABCB1 marker distribution in the study population conforms to HWE (p=0.01, Table 2). The allelic and genotypic frequencies of the ABCB1 marker are tabulated (Tables 5 and Table 6). No significant differences in the allelic and genotypic frequencies of the marker between study sites were observed in this study. The heterozygous CT genotype was observed to be the highest (~60.3%) compared to TT (~29.2%) or CC (~10.5%) genotypes in the study population. Study populations from all three sites also show ‘T’ to be the more frequent allele (Tripura: ~60%, Manipur: ~59%, Nagaland: ~60%) compared to the ‘C’ allele.

Allelic and genotypic distribution of GSTT1: The GSTT1 marker in the study population conforms to HWE (p=0.000, Table 2). Homozygous CC genotype was observed to be more prevalent in all three sub-populations (Table 6; Tripura: ~92%, Manipur: 69%, and Nagaland: 65%). ‘C’ allele has higher frequency in the population, and a significant difference in the distribution of the allele was also observed among the populations from three study sites (p<0.00001, Table 5). The null genotype of GSTT1 marker in the study populations from Tripura, Manipur, and Nagaland were ~8.3%, ~31%, and ~35%, respectively (Table 6).

Genetic distance of ATB patients between the three study sites: A dendrogram constructed using the frequency of different alleles of NAT2, PXR, ABCB1, and GSTT1 showed that Manipur and Nagaland populations share a closer genetic similarity compared to Tripura population (Fig 2).

Combinational analysis of different genotypes: The prevalence of different combinations of the genotypes is reported to be associated with DILI. Both SA genotype of NAT2 and GSTT1 null genotype were observed in ~8.2% of the study population. Similarly, ~3.2% of the study population has both SA (NAT2) and TT (PXR) genotypes. Approximately 10.5% showed both SA (NAT2) and TT (ABCB1) genotypes. A combination of SA (NAT2), TT (ABCB1), and GSTT1 null genotypes were observed in ~3.7% of the study population. In one of the subjects (~0.5%), a combination of all the four genotypes- SA (NAT2), TT (ABCB1), TT (PXR), and GSTT1 null was detected. Overall, ~26.1% of the study population carried the risk factor genotypes for DILI. Population-wise, ~20.9% of the Manipur population carried the risk factor genotype, ~31.8% in Tripura and ~45% in the Nagaland population.

Discussion

It is well documented that host genetics plays a crucial role in metabolizing xenobiotic compounds including drugs and it significantly influence therapeutic outcomes. In case of infectious diseases like TB in which patients undertake medication for a longer duration (6-18 months) may develop severe side effects like hepatotoxicity, nephrotoxicity due to incomplete metabolism and clearance of drugs or their intermediate products. In this study, we aimed to generate baseline pharmacogenomics data of understudied TB patient populations from three different north-eastern states of India sharing international boundaries with Myanmar and Bangladesh.

It is well known that NAT2 polymorphism impacts drug metabolism and based on their patterns, the subjects could be grouped as slow-, intermediate- or fast-acetylators. The majority of this study population were found to have NAT2*4/*6 genotype, representing intermediate acetylator types. Similar observations were earlier reported from one of the neighboring state i.e. Assam [39]. NAT2*6 allele, which has been associated with a risk for DILI, was found to be the most common in this study population and corroborates earlier findings [40,14]. However, in the Tripura population, we observed the majority of the study subjects present with the slow-acetylator phenotypes. This genomics data support our earlier metabolomics related findings in which the abundance of urine anti-TB drugs and their intermediate levels were monitored using mass spectrometry and demonstrated their slow acetylation status [41]. This could be partly explained by the Indo-Aryan origin of the Tripura
population whereas the Manipur and Nagaland study population sharing a common East Asian origin. The allelic frequencies of the three variants of NAT2 in the studied population were ~19.9%, ~24.32%, and ~18.12% respectively, which is different from earlier reports [40, 41]. This could be explained by the difference in allelic frequency of the NAT2 variants with ethnicity (Table 7).

In the study population, we observed higher ancestral C alleles of the PXR gene and homozygous CC genotypes than the variant T alleles. In the case of the ABCB1 (MDR-1) gene, T alleles are more prevalent than the C allele in the studied population. A higher prevalence of the heterozygous CT genotypes was observed than the homozygous TT genotype corroborating earlier reports (Table 8). In an earlier study on Asian population (n=298, from Singapore), Balram et al., reported a C and T allele frequencies of ~38% and ~62% and similar observations were observed in our study population (C: 41% , T: 59%) [47, 52, 54]. However, the earlier reported study population had a higher prevalence of the homozygous TT genotype than the heterozygous CT genotype and may be due to the differences in ethnicity.

A higher prevalence of homozygous CC than the null genotypes for GSTT1 was observed in this study population. Homozygous null mutation at this locus in Manipur (~31%) and Nagaland (~35%) populations were higher than Tripura (~8.3%) population. Overall, ~75% of the population in the study has a homozygous CC genotype, exhibiting limited loss of Glutathione S-transferase activity in this study population. From the genetic distance analysis, the population from Manipur and Nagaland were found to be closer to each other compared to the Tripura population (Fig. 2).

Analysis of different combinations of the genotypes associated with DILI such as slow acetylator (SA) genotype of NAT2, GSTT1 null genotype, homozygous TT genotype of PXR and homozygous TT genotype of ABCB1 (Table 9) showed that ~26.1% of the study population has two or more combination of these risk genotypes. Earlier reports demonstrated combinations of SA genotype of NAT2 and null genotypes of GSTs were significantly associated with AT-DILI. A single study subject (~0.5%) from Manipur, exhibited all the four genotypes associated with DILI. In the Manipur population, ~20.9% showed different combinations of risk genotypes. Nagaland (~45%) and Tripura (~31.9%) populations showed different combinations of risk genotypes. With this relatively small sample size, the baseline data was generated from three study sites, on which limited or no prior data on pharmacogenomics was available. Further focused expansion of this study will bring additional clarity on these understudied populations. Overall, the prevalence of the risk factor genotypes of the studied genes is ~ 26.1%, which indicates a low risk to develop DILI during TB treatment in these patients.

In conclusion, this pilot genetic polymorphism study including NAT2, PXR, GSTs, and ABCB1 genes in TB patients from the three north-eastern states of India showed that majority of these patients were carriers of ancestral allele and genotypes with low risk to DILI development during the treatment. This baseline data could be expanded further with additional marker profiles and population size to develop appropriate policy interventions for these under-studied areas.

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Table 1: Details of the markers used in this study.

| Name of Gene | Markers | Alleles | Global MAF | Position | Location (Chromosome) | Assay ID |
|--------------|---------|---------|------------|----------|-----------------------|---------|
| NAT2         | rs1801280 | T>C     | 29         | 341      | 8:18400344            | C_1204093_20 |
|              | rs1799930 | G>A     | 27         | 590      | 8:18400593            | C_1204091_10 |
|              | rs1799931 | G>A     | 8          | 857      | 8:18400860            | C_572770_20  |
| PXR (NR1I2)  | rs3814055 | C>T     | 32         | 1135     | 3:119781188           | C_27504984_30 |
| ABCB1 (MDR1) | rs1045642 | C>T     | 40         | 3435     | 7:87509329            | C_7586657_20  |
| GSTT1        | rs2234953 | C>T     | NA         | NA       | NA                    | C_11486658_20 |

NA- Not available

Table 2: Hardy Weinberg’s Equilibrium analyses of the population in the study.

| Marker   | Genotype | Chi-square value | P-value |
|----------|----------|------------------|---------|
| NAT2*5   | TT       | 6.771            | 0.034*  |
|          | TC       |                  |         |
|          | CC       |                  |         |
| NAT2*6   | GG       | 0.198            | 0.906   |
|          | GA       |                  |         |
|          | AA       |                  |         |
| NAT2*7   | GG       | 24.760           | 0.000*  |
|          | GA       |                  |         |
|          | AA       |                  |         |
| PXR      | CC       | 0.237            | 0.888   |
|          | CT       |                  |         |
|          | TT       |                  |         |
| ABCB1    | CC       | 13.607           | 0.01*   |
|          | CT       |                  |         |
|          | TT       |                  |         |
| GSTT1    | CC       | 56.260           | 0.000*  |
|          | NULL     |                  |         |
Table 3: Genotypic frequency of NAT2 gene among the TB patients of Manipur, Nagaland and Tripura.

| Genotypes   | Genotype frequencies | Phenotypes | Percentage of Phenotypes |
|-------------|----------------------|------------|--------------------------|
| NAT2*4/*4   | 0.173                | RA         | 17.3                     |
| NAT2*4/*5   | 0.137                | IA         |                          |
| NAT2*4/*6   | 0.201                | IA         |                          |
| NAT2*4/*7   | 0.123                | IA         |                          |
| NAT2*5/*5   | 0.059                | SA         |                          |
| NAT2*5/*6   | 0.096                | SA         | 35.6                     |
| NAT2*5/*7   | 0.032                | SA         |                          |
| NAT2*6/*6   | 0.05                 | SA         |                          |
| NAT2*6/*7   | 0.041                | SA         |                          |
| NAT2*7/*7   | 0.078                | SA         |                          |

RA for Rapid acetylator, IA for Intermediate acetylator, and SA for Slow acetylator.

Table 4: Acetylator status of the three study populations.

| Acetylator Status | Fast | Intermediate | Slow |
|-------------------|------|--------------|------|
|                   | NAT2*4/*4 | NAT2*4/*5 | NAT2*4/*6 | NAT2*4/*7 | NAT2*5/*5 | NAT2*5/*6 | NAT2*5/*7 | NAT2*6/*6 | NAT2*6/*7 | NAT2*7/*7 |
| Tripura           | 7 (11.7)  | 7(11.7)   | 12(20)   | 01(1.7)   | 7(11.7)   | 13(21.7)  | 02(3.3)   | 5(8.3)    | 02(3.3)   | 4(6.7)    |
| Total % of phenotypes | 11.7  | 33.4       |         | 55         |
| Manipur           | 26(18.7) | 22(15.8) | 29(20.9) | 23(16.5) | 4(2.9)   | 8(5.8)   | 5(3.6)   | 6(4.3)    | 6(4.3)    | 9(6.5)    |
| Total % of phenotypes | 18.7  | 53.2       |         | 27.4       |
| Nagaland          | 6(30)   | 1(5)       | 4(20)    | 3(15)     | 2(10)    | 0        | 0        | 0        | 0        | 4(20)     |
| Total % of phenotypes | 30    | 40         |         | 30         |

Data shown as: frequency (%)
Table 5: Allelic frequencies of \(\text{NAT2}, \text{PXR}, \text{ABCB1}\) and \(\text{GSTT1}\) among the studied populations.

| Markers (Gene) | Allele | Allelic count in three populations | Total Population (%) | P-value (95% significance) |
|---------------|--------|-----------------------------------|----------------------|---------------------------|
|               |        | Manipur (freq) | Tripura (freq) | Nagaland (freq) |                  |
| rs1801280 (NAT2*5) | T      | 234(0.84) | 81(0.675) | 35(0.875) | 80.10 | *0.000 |
|               |        | C        | 44(0.16) | 39(0.325) | 5(0.125) | 19.90 |
| rs1799930 (NAT2*6) | G      | 221(0.79) | 76(0.64) | 35(0.875) | 75.68 | *0.000 |
|               |        | A        | 57(0.21) | 44(0.36) | 5(0.125) | 24.32 |
| rs1799931 (NAT2*7) | G      | 231(0.83) | 106(0.88) | 28(0.7) | 81.88 | *0.000 |
|               |        | A        | 47(0.17) | 14(0.12) | 12(0.3) | 18.12 |
| rs3814055 (PXR) | C      | 231(0.83) | 87(0.725) | 32(0.8) | 80.0 | 0.053 |
|               |        | T        | 47(0.17) | 33(0.275) | 8(0.2) | 20.0 |
| rs1045642 (ABCB1) | C      | 114(0.41) | 48(0.4) | 16(0.4) | 41.0 | 0.979 |
|               |        | T        | 164(0.59) | 72(0.6) | 24(0.6) | 59.0 |
| rs2234953 (GSTT1) | C      | 194(0.69) | 110(0.92) | 26(0.65) | 75.3 | *0.000 |
|               |        | Null     | 84(0.31) | 10(0.08) | 14(0.35) | 24.7 |

Table 6: Genotypic frequencies of \(\text{NAT2}, \text{PXR}, \text{ABCB1}\) and \(\text{GSTT1}\) among TB patients in three populations of North East India.

| Markers (Gene) | Genotypes | Genotypic count in three populations | Total population (%) | p-value (95% significance) |
|---------------|-----------|-------------------------------------|----------------------|---------------------------|
|               |           | Manipur (freq) | Tripura (freq) | Nagaland (freq) |                  |
| rs1801280 (NAT2*5) | TT      | 99(0.72) | 30(0.5) | 17(0.85) | 67.0 | 0.084 |
|               |           | TC       | 36(0.26) | 21(0.35) | 1(0.05) | 26.1 |
|               |           | CC       | 4(0.029) | 9(0.15) | 2(0.1) | 6.9 |
| rs1799930 (NAT2*6) | GG      | 88(0.62) | 24(0.4) | 15(0.75) | 57.8 | 0.196 |
|               |           | GA       | 45(0.33) | 28(0.47) | 5(0.25) | 35.8 |
|               |           | AA       | 6(0.043) | 8(0.14) | 0(0) | 6.4 |
| rs1799931 (NAT2*7) | GG      | 99(0.72) | 50(0.83) | 12(0.6) | 72.0 | 0.373 |
|               |           | GA       | 33(0.23) | 6(0.1) | 4(0.2) | 19.7 |
|               |           | AA       | 10(0.071) | 4(0.07) | 4(0.2) | 8.3 |
| rs3814055 (PXR) | CC       | 97(0.69) | 31(0.52) | 13(0.65) | 64.2 | 0.068 |
|               |           | CT       | 37(0.27) | 25(0.42) | 6(0.3) | 31.2 |
|               |           | TT       | 5(0.036) | 4(0.07) | 1(0.05) | 4.6 |
| rs1045642 (ABCB1) | CC      | 12(0.086) | 9(0.15) | 2(0.1) | 10.5 | 0.449 |
|               |           | CT       | 90(0.65) | 30(0.5) | 12(0.6) | 60.3 |
|               |           | TT       | 37(0.27) | 21(0.35) | 6(0.3) | 29.2 |
| rs2234953 (GSTT1) | CC      | 97(0.69) | 55(0.92) | 13(0.65) | 75.3 | 0.104 |
|               |           | Null     | 42(0.31) | 5(0.083) | 7(0.35) | 24.7 |
Table 7: NAT2 allele frequencies among Indian population and other ethnic groups.

| Populations          | Frequency (%) |         |         |         |
|----------------------|---------------|---------|---------|---------|
|                      | n 341 T>C     | 590 G>A | 857 G>A | references |
| Indian               | 250 15.6      | 42.8    | 8.4     | [39]     |
| Indians              | 61 33.0       | 38      | 3.0     | [40]     |
| Japanese             | 79 1.9        | 23      | 1.1     | [40]     |
| Caucasians           | 3531 46.0     | 28.5    | 2.9     | [42]     |
| Southern Korean      | 288 1.0       | 22.4    | 13.2    | [43]     |
| Americans            | 387 4.37      | 26.6    | 1.9     | [43]     |
| Southern Brazil      | 254 28.9      | 10.4    | 2.1     | [44]     |
| South Africa         | 97 36.1       | 17.0    | 6.7     | [43]     |
| Spanish              | 258 47        | 25.0    | 0.6     | [43]     |
| Egyptian             | 199 49.7      | 26      | 2.8     | [45]     |
| Tunisians            | 100 31.5      | 17.5    | 15      | [46]     |
| North East Indian    | 219 19.9      | 24.32   | 18.12   | Current study |

Table 8: Genotype and allele frequencies of ABCB1 (MDR-1) gene C3435T polymorphism in various ethnic groups.

| Populations          | n  | CC   | CT   | TT   | C    | T    | references |
|----------------------|----|------|------|------|------|------|------------|
| Indians              | 93 | 18.0 | 39.0 | 43.0 | 0.380| 0.620| [47]       |
| Jordanians           | 100| 17.0 | 50.0 | 33.0 | 0.420| 0.580| [48]       |
| Iranians             | 200| 20.0 | 52.5 | 27.5 | 0.463| 0.537| [49]       |
| Turkish              | 150| 20.0 | 53.0 | 27.0 | 0.470| 0.530| [50]       |
| Spanish              | 408| 26.0 | 52.0 | 22.0 | 0.520| 0.480| [51]       |
| British              | 190| 24.0 | 48.0 | 28.0 | 0.480| 0.520| [52]       |
| Chinese              | 132| 32.0 | 42.0 | 26.0 | 0.530| 0.470| [52]       |
| German               | 188| 28.0 | 48.0 | 24.0 | 0.520| 0.480| [36]       |
| Japanese             | 154| 35.7 | 47.4 | 16.9 | 0.594| 0.406| [53]       |
| Ghanaians            | 206| 66.0 | 34.0 | 0    | 0.830| 0.170| [52]       |
| French               | 81 | 36.0 | 42.0 | 22.0 | 0.570| 0.430| [54]       |
| Current study        | 219| 10.5 | 60.3 | 29.2 | 0.410| 0.590| Current study |
Table 9: Analysis of different combinations of risk genotypes of \textit{NAT2}, \textit{PXR}, \textit{GSTT1}, and \textit{ABCB1} genes associated with TB- DILI in the three populations of the study.

| Genotype combinations                  | Manipur (n=139) | Tripura (n=60) | Nagaland (n=20) | Total (n=219) |
|----------------------------------------|-----------------|----------------|-----------------|---------------|
| SA+ \textit{GSTT1} null                | 10(7.2%)        | 4(6.7%)        | 4(20%)          | 18(8.2%)      |
| SA+TT (\textit{PXR})                  | 5(3.6%)         | 1(1.7%)        | 1(5%)           | 7(3.2%)       |
| SA+TT (\textit{ABCB1})                | 9(6.5%)         | 12(20%)        | 2(10%)          | 23(10.5%)     |
| SA+\textit{GSTT1} null+TT (\textit{ABCB1}) | 4(2.9%)        | 2(3.4%)        | 2(10)           | 8(3.7%)       |
| SA+\textit{GSTT1} null+TT (\textit{ABCB1})+TT (\textit{PXR}) | 1(0.7%)         | 0              | 0               | 1(0.5%)       |
| Total                                  | 29(20.9%)       | 19(31.8%)      | 9(45%)          | 56(26.1%)     |

\textbf{Fig. 1 (A)} Graphical representation of pairwise linkage disequilibrium (LD) of \textit{NAT2} gene markers in TB patients. The numerical values in the boxes represent the normalized linkage disequilibrium coefficient D' values. The color intensities of the boxes is dependent on the D'/LOD value. The higher the intensity of the red colour, higher is the D' value. \textbf{(B)} LD analysis between marker pairs of \textit{NAT2} gene in TB patients indicating the ratio of D' to correlation coefficient (D'/r^2).
Fig 2: UPGMA dendrogram based Nei’s (1972) unbiased measure of genetic distance among the three study populations representing different north eastern states of India (MN- Manipur; NL-Nagaland; AG- Tripura).