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The association between the NAT2 genetic polymorphisms and risk of DILI during anti-TB treatment: a systematic review and meta-analysis

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running head: slow NAT2 genotype is a risk factor for AT-DILI.
Abstract

**Aims** To evaluate the potential association between N-acetyltransferase type 2 (NAT2) polymorphisms and drug-induced liver injury during anti-TB treatment (AT-DILI).

**Methods** We conducted a systematic review and performed a meta-analysis to clarify the role of NAT2 polymorphism in AT-DILI. PubMed, Medline and EMBASE databases were searched for studies published in English to December 31, 2017, on the association between the NAT2 polymorphism and AT-DILI risk. Outcomes were pooled with random-effects meta-analysis. Details were registered in the PROSPERO register (number: CRD42016051722).

**Results** Thirty-seven studies involving 1,527 cases and 7,184 controls were included in this meta-analysis. The overall odds ratio (OR) of AT-DILI associated with NAT2 slow acetylator phenotype was 3.15 (95%CI 2.58–3.84, I² = 51.3%, P=0.000). The OR varied between different ethnic populations, ranging from 6.42 (95%CI 2.41-17.10, I² = 2.3%) for the West Asian population to 2.32 (95%CI 0.58-9.24, I² = 80.3%) for the European population. Within the slow NAT2 genotype variation was also observed; NAT2*6/*7 was associated with the highest risk of AT-DILI (OR=1.68, 95%CI 1.09-2.59) compared to the other slow NAT2 acetylators combined.

**Conclusions** NAT2 slow acetylation was observed to increase the risk of AT-DILI in tuberculosis patients. Our results support the hypothesis that the slow NAT2 genotype is a risk factor for AT-DILI.

**Keywords.** NAT2; polymorphism; antituberculosis drug-induced liver injury; meta-analysis
What is already known about this subject (up to three bullet points)

Although a number of previous studies have evaluated the potential association between N-acetyltransferase type 2 (NAT2) polymorphisms and drug-induced liver injury during anti-TB treatment (AT-DILI), the results were inconsistent.

What this study adds (up to three bullet points)

We conducted a systematic review and performed a meta-analysis to clarify the role of NAT2 polymorphism in AT-DILI. Subgroup analyses were performed by: (i) region of origin (ii) Study type (iii) Genotyping. We evaluated the risk for specific slow NAT2 acetylators and susceptibility to AT-DILI. NAT2 slow-acetylator alleles were associated with a higher risk of AT-DILI, especially in West Asian TB patient populations, but not in European and African populations. Within the slow NAT2 acetylators, the risk was highest for NAT2*6/*7 and relatively lowest for NAT2*5/*6.
Introduction

Tuberculosis (TB) is a major public health problem in the world. In 2015, there were an estimated 10.4 million new (incident) TB cases worldwide\(^1\). The first-line multidrug combined therapy (isoniazid, rifampicin, ethambutol and pyrazinamide) is known to commonly lead to adverse drug reactions (ADRs) such as hepatotoxicity, gastrointestinal disorders, allergic reactions, arthralgia and neurological disorders\(^2,3\), the most common ADR during anti-TB treatment leading to drug discontinuation in 11% of patients\(^4\). Isoniazid is a key drug in anti-TB therapy but is also the key responsible drug for the occurrence of drug-induced liver injury during anti-TB treatment (AT-DILI). ADRs occur in 5-33% of all patients receiving oral isoniazid treatment at 300 mg once daily\(^5\). The metabolic intermediates of isoniazid appear to be the cause of hepatotoxicity\(^6\). In the liver, isoniazid is first metabolized into acetyl-isoniazid via N-acetyltransferase\(^7\). Isoniazid hydrazine and acetyl-hydrazine are two metabolites of isoniazid, which are primarily involved in the mechanism of isoniazid-induced hepatotoxicity\(^8-10\). Figure 1 shows the metabolic pathway of isoniazid.

The first genetic variation in drug response ever discovered was the N-acetylation of isoniazid\(^11\). This variation was later found to be induced mainly by the polymorphisms in N- acetyltransferase 2 coding gene (NAT2), and a number of previous studies have assessed the association between NAT2 gene polymorphism and the AT-DILI. The results of the studies were inconsistent, mainly due to limited power, Therefore, personalized dosing has not been introduced yet in programmatic anti-TB treatment. However, considering the potential impact of NAT2 guided dosing on the occurrence of AT-DILI we aimed to systematically review and meta-analyze all published studies designed to assess the presence and strength of the postulated genetic associations between the NAT2 polymorphisms and susceptibility to AT-DILI.
Methods

Literature search strategy

The details of the systematic review and meta-analysis were registered in the PROSPERO register (registration number is: CRD42016051722).

Two authors (MZ and SW) independently searched the PubMed, Medline and EMBASE databases for studies on the association of NAT2 polymorphisms with risk of DILI up to December 31 2017 using the search words: ('antituberculosis' or 'anti tuberculosis' or 'tuberculosis') and ('genetic polymorphism*' or 'polymorphism*') and ('adverse drug reaction*' or 'adverse effect*' or 'adverse event*' or 'drug reaction*' or 'drug damage' or 'drug injur*' or 'drug-induced'). The search was conducted on human subjects and published in English, having no restrictions on sample size, population. The reference lists from the retrieved documents were also scanned. Through the quick reading of the title and abstract, any clearly irrelevant studies, editorials and review articles were excluded. A flow diagram summarizing the study selection process is shown in Figure 2.

NAT2 activity is divided into three main categories as slow, intermediate and rapid acetylation, with some studies combining intermediate and rapid acetylation. In this review individuals homozygous for slow NAT2 acetylator alleles (NAT2*5/*5, NAT2*5/*6, NAT2*5/*7, NAT2*6/*6, NAT2*6/*7, NAT2*7/*7) were classified as slow acetylator phenotype; individuals homozygous for rapid NAT2 acetylator alleles (NAT2*4, NAT2*11A, NAT2*12A, NAT2*12B, NAT2*12C, NAT2*13) were classified as rapid acetylator phenotype; heterozygous individuals (one rapid and one slow NAT2 allele) were classified as intermediate acetylator phenotypes \(^1\). The rapid acetylator phenotype and intermediate acetylator phenotypes were classified as non-slow acetylator phenotype in this review. The rapid acetylator phenotype and intermediate acetylator phenotype were classified as non-slow acetylator phenotype in this review.
Inclusion and exclusion criteria

Eligible studies met the following inclusion criteria: (i) evaluated the association between the NAT2 genetic polymorphisms and risk of anti-tuberculosis drug-induced DILI in humans with either case-control (including nested case-control) or prospective designs, (ii) original papers containing independent data, (iii) included sufficient data to estimate odds ratios (ORs) and their 95% confidence intervals (CIs). Studies were excluded if they met the following predetermined criteria: (i) overlapping studies, (ii) review articles, (iii) studies without complete genetic distribution data for the DILI and non-DILI groups, (iv) NEWCASTLE - OTTAWA quality assessment (NOS) <4, (v) Controls were patients without TB, (vi) not published in English.

Data extraction and assessment of study quality

The data extracted independently by the two reviewers included: name of first author, publication year, country or region of origin, study type, demographic data of age and gender, setting (clinic), stage of treatment, duration of follow-up, matching factors, treatment regimen, detailed definition of DILI, measurement method for DILI, genotyping method and genotype distribution in cases and controls. The eligibility/exclusion criteria mentioned before were used to assess the quality of the included studies, and study quality was assessed according to NEWCASTLE - OTTAWA quality assessment \(^{15}\). These items included (i) selection of study subjects, (ii) comparability of cases and controls on the basis of the design or analysis, (iii) assessment exposure or outcome studies with a score ≥ 4 estimated by the NOS were considered to be of high quality and were retained in the analysis. If any discrepancy occurred, the data were rechecked, and a third author was invited to give a final decision.

Statistical analysis

The NAT2 genotypes were analysed based on the genetic model of proposed risk (rapid acetylation phenotype and intermediate acetylation phenotype vs. slow acetylation phenotype) for the NAT2
polymorphisms. All of the statistical analyses were performed using STATA version 14.2 (Stata, College Station, TX, USA) and SPSS version 16.0 (SPSS, USA). Based on complete distribution data on NAT2 polymorphism in cases and controls, the pooled ORs and their 95% confidence intervals (CIs) were calculated and displayed as forest plots to assess the strength of association between NAT2 genetic polymorphisms and susceptibility to AT-DILI in TB patients. In this analysis, pre-stated ethnic subgroup analyses were performed to examine differences in the association between NAT2 genotype distribution and AT-DILI risk. Subgroup analyses were performed by: (i) region of origin (East Asian, South Asian, Southeast Asian, West Asian, Africa, Europe, South and North America), (ii) Study type (case–control study, nested case-control study, cross-sectional cohort studies, prospective cohort study), (iii) Genotyping (sequencing, HRM, RFLP, Taqman, SNP stream). Random-effects or fixed effects models were used depending on the heterogeneity among studies. Heterogeneity was assessed with the standard Q-statistic test, I² > 50% was considered to be evidence of heterogeneity.

Among all qualified studies related to NAT2 gene, we drew up the summary effects again after removing the study with the widest 95% confidence interval (CI). We also conducted a sensitivity analysis to assess the stability of the results by applying the leave-one-out method, that is repeating the meta-analysis, each time omitting one of the studies. Publication bias was assessed using Begg’s funnel plot and Egger’s test. A test P value <0.05 was considered as statistically significant.

Results

Identification and characteristics of the included studies

Using our electronic databases searches, we identified 58 articles describing the strength of the postulated genetic associations between the NAT2 polymorphisms and susceptibility to AT-DILI. Finally, a total of 37 case–control or prospective cohort design studies with 1,527 AT-DILI cases and 7,184 controls without AT-DILI were included in the meta-analysis. The main characteristics of the 37 studies are shown in Table 1. The study by An et al. (2011)\(^5\)\(^3\), Rana et al. (2012)\(^5\)\(^4\) and Rana et al. (2013)\(^5\)\(^5\) were excluded due to overlap with their other studies (we therefore selected the later
publication to analyse the distribution of the NAT2 genotype); 3 studies by Guaoua et al. (2016)\textsuperscript{56}, Ng (2014)\textsuperscript{57} and Mishra et al. (2013)\textsuperscript{58} were excluded as controls were not TB patients but healthy people; the studies by Roy et al.(2001)\textsuperscript{59} and Cavaco et al.(2003)\textsuperscript{60} were excluded due to the absence of complete NAT2 polymorphism distribution data. The study by Ohno et al.(2000)\textsuperscript{61} was excluded due to the absence of slow acetylators.

**Quantitative synthesis**

Pooling all 37 studies in the meta-analysis, comparing the slow to the non-slow NAT2 acetylators (i.e., intermediate NAT2 acetylators and fast acetylators), the overall OR for the association with AT-DILI was 3.15 (95%CI 2.58-3.84, P <0.005, Figure 3) using a random-effects model ($I^2 = 51.3\%$).

Subgroup analyses of the NAT2 polymorphism were performed. First, a subgroup analysis for region of origin was performed (Figure 3). In descending effect size the ORs for slow NAT2 genotype associated with the risk of AT-DILI were statistically significant for West Asia 6.42 (95%CI 2.41–17.10), South Asia 3.05 (95%CI 2.20–4.24), South America 3.01(95%CI 2.29–3.96), and East Asia 2.98 (95%CI 2.03–4.37), but not for North America 2.02 (95%CI 0.82–4.96) (1 study only), Africa 2.40 (95%CI 0.78–7.36) and Europe 2.32 (95%CI 0.58–9.24).

Secondly a subgroup analysis was performed across study designs (Figure 4). Of the 37 studies, 19 were case–control studies, 7 were nested case-control studies, 5 were cross-sectional cohort studies, 5 were prospective cohort studies, and 1 was a retrospective cohort study. The subgroups all showed positive effects sizes, ranging from 1.90 (94%CI 1.40-2.58) for cross-sectional cohort studies to 4.00 (95%CI 3.11-5.14) for case-control studies.

Subgroup analysis for different methods of genotyping was performed (Figure 5). Of the 37 studies, 15 used sequencing, 18 used RFLP, 2 used Taqman, 1 used HRM, 1 used SNP stream. The subgroups all showed positive effects sizes, ranging from 2.06 (95%CI 0.93-4.57) for Taqman to 8.82 (95%CI 3.26-23.89) for HRM (1 study only).
This meta-analysis also evaluated the risk for specific slow NAT2 acetylators and susceptibility to AT-DILI. There were statistically significant associations between NAT2*5/*5, NAT2*5/*6, NAT2*5/*7, NAT2*6/*6, NAT2*6/*7, NAT2*7/*7 and the risk of AT-DILI. Within the slow NAT2 acetylators, we found a relatively lower risk of AT-DILI with NAT2*5/*6. The ORs for NAT2*5/*6 slow NAT2 acetylators compared with other slow NAT2 acetylators combined was 0.43 (95% CI 0.27-0.68) (Figure 6) using a fixed-effect model (I² = 12.8%, P=0.328). In contrast, NAT2*6/*7 was associated with a relative increased risk of AT-DILI compared to the other slow NAT2 acetylators combined (OR=1.68, 95% CI 1.09-2.59) using a fixed-effect model (I² = 44.0%, P=0.075) (Figure 7).

Sensitivity analyses and publication bias

The sensitivity analysis was conducted via sequential analysis after omitting one study at a time to assess the effects of individual studies on the overall meta-analysis estimate. This analysis shows that the results of the meta-analysis are statistically robust as the ORs for the overall association between slow acetylators on AT-DILI remained significant and ranged from 3.03 to 3.25 using random-effects models. Heterogeneity was specifically decreased (I² = 41.3%), when the study by Lv et al. (2012) (22) was removed.

A funnel plot of these 37 studies suggested a possibility of the preferential publication of positive findings (Figure 8). The Egger test provided evidence that there was no small-study publication bias among the studies included (p < 0.001). The Begg’s test gave the same result.

Discussion

This meta-analysis examined well-characterized polymorphisms of NAT2 gene in the relationship to AT-DILI susceptibility. It determined that NAT2 slow-acetylator alleles were associated with a higher risk of AT-DILI, especially in West Asian TB patient populations. Significant results were also found in South Asian, East Asian and American populations, but not in European and African populations.

The previous meta-analyses (62-64) did not include data from the African population which has the largest incidence of TB in the world. Compared with the previous meta-analyses, the present study is...
much larger, with more than 1.5 to 2 times as many cases. It also adjusts classification in the study of Yimer et al. (2011) that categorized Ethiopian patients together with European patients. In contrast to our meta-analysis the previous meta-analysis did not include data from Indonesian populations which has the fifth largest incidence of TB in the world. Therefore, this meta-analysis is more comprehensive and powerful, especially because it contains Asian countries listed in the top 30 TB “high burden country” in the 2016 latest global TB report. We performed a subgroup analysis for different study designs and methods of genotyping to investigate whether the NAT2 gene polymorphism was associated differently with AT-DILI risk when using different designs and genotyping methods. Our results on the role of the polymorphism of NAT2 in different ethnicities were consistent across study design and genotyping method. Furthermore, we evaluated the risk for specific slow NAT2 acetylators and susceptibility to AT-DILI, which previous meta-analyses never reported.

It came to our attention that although association of NAT2 slow acetylators with AT-DILI was not observed for Europeans and Africans, it was observed in the Brazilian study of Teixeira, which is interesting since Brazilian population has in it’s formation the contribution of Africans, Europeans and Amerindians. Considering the ethnic diversity of Brazilian population, a more consistent comparison of the results found among these populations would be of importance and could contribute even more to the definition of such association in different populations. At present, there is still a lack of research data on different groups of people in Brazil, and such research should be encouraged in the future. To our knowledge, this is the first systematic review and meta-analysis to evaluate the association between specific slow NAT2 acetylators and the susceptibility to AT-DILI. Previous studies only showed that the NAT2*6 allele significantly predicts predisposition to AT-DILI in Taiwanese, Japanese and Chinese. Of the 37 studies included in our meta-analysis, 9 studies investigated the association between slow NAT2 acetylators and susceptibility to AT-DILI and when combined, showed a relatively higher risk of AT-DILI with NAT2*6/*7, which is in accordance with previous studies in Taiwanese, Japanese and Chinese populations.
The World Health Organization reported that over 95% of TB deaths occur in low- and middle-income countries. Six countries account for 60% of the total, with India leading the count, followed by Indonesia, China, Nigeria, Pakistan and South Africa\(^1\). In Figure 3, we can see that two-thirds of included studies were conducted in East Asian, South Asian and Southeast Asian populations, from India, Indonesia, China, Taiwan, Iran, Japan, and Korea. The pharmakokinetic profiles of INH and its metabolites differ significantly between individuals. Patients can be categorized according to their number of functional NAT2 alleles into slow, intermediate, and fast acetylator phenotypes. Therefore, it should be feasible and would be useful to help guide programmatic TB drug therapy through pharmacogenomics, to reduce the occurrence of adverse reactions in individual patients.

To provide a rational dosing design to balance the inherent trade-off between treatment efficacy and toxicity in INH-based chemotherapy, it should be considered that there are several polymorphisms in NAT2 leading to altered catalytic activities for INH acetylation\(^{65-68}\). Some authors suggested an adaptation of administered INH dosages according to patient acetylator status may benefit patients\(^{69-71}\). In one clinical trial a INH QD dose of 5 mg · kg\(^{-1}\) of body weight was modified to doses of 2.5 mg · kg\(^{-1}\) for slow acetylators, 5mg · kg\(^{-1}\) for intermediate acetylators, and 7.5 mg · kg\(^{-1}\) for fast acetylators, resulting in reduced adverse effects in fast acetylators while maintaining overall treatment efficacy in all acetylator phenotypes\(^{70}\).

In the past five years, personalized dosing therapy based on drug metabolizing enzymes and transporter genomes has become one of the focuses of personalized medicine. If the association between the genetic polymorphisms and risk of AT-DILI is determined, maybe a personalized clinical drug-dosage model can be developed for the treatment of tuberculosis taking into account other well-known factors that influence drug exposure\(^{72,73}\). The personalized clinical drug-dosage model is especially important for the population of South and East Asia with high incidence of AT-DILI. It could effectively reduce the incidence of ADRs in the treatment of tuberculosis, especially for the treatment interruption caused by AT-DILI. For the TB high burden countries, reducing the incidence of ADRs may...
be cost-effective because the cost of treating AT-DILI is often higher than the treatment of TB\textsuperscript{[1]}. The WHO "End TB Strategy", approved by the World Health Assembly in 2014, calls for a 90% reduction in TB deaths and an 80% reduction in the TB incidence rate by 2030, compared with 2015\textsuperscript{[74]}. This clinical model of tuberculosis drug therapy could play a role in the realization of this goal.

Although we included a large number of studies with a considerable overall sample size and performed subgroup analyses to explore differences in effects of the NAT2 polymorphisms on AT-DILI risk, several potential limitations should be taken into consideration when interpreting our results. Firstly, the NAT2 polymorphism has a higher minor allele frequency in different populations, so the lack of information about polymorphisms distributions in the target populations does not allow for estimating the attributable fraction of NAT2 polymorphisms on AT-DILI occurrence. Secondly, the lack of information on other potential causative/protective factors, in particular age, sex, dietary habits, nutrition status, body mass index (BMI), drinking and smoking habits were available for only a limited number of the studies and as such, we were not able to adjust effect sizes. Thirdly, not all studies provided information on the definitions applied for AT-DILI and hepatotoxicity. Lastly, only some studies provided information on synergism of the TB drugs used and the Hardy-Weinberg equilibrium test, which may have impacted the effect size, and simultaneously hindered an adequate exploration of a potential source of heterogeneity. Despite these limitations, our review and met-analysis provided important new information that was statistically robust in sensitivity analyses and has yielded relevant and reliable results that were robust in sensitivity analyses.

**Conclusion**

In summary, this meta-analysis not only demonstrated that the NAT2 slow acetylation phenotype was significantly associated with increased risk of AT-DILI depending on the population studied, it also suggests that there is variation within the slow NAT2 acetylator group: the risk was highest for NAT2*6/*7 and relatively lowest for NAT2*5/*6. In March 2016, the United States Clinical Pharmacogenetics Implementation Consortium updated 33 pharmacogenomic drug application
guidelines, 25 of which relate to drug metabolism and transport. NAT2 was not yet included in these guidelines but based on our results, may have a place in future updates. Considering the complex mechanisms involved in the development of AT-DILI, and limitations of the available observational studies on the impact NAT2 polymorphisms, we recommend to design a randomized controlled trial with adequate sample size to assess the true effect of NAT2. Also evaluating gene-to-gene interactions (between human genetic polymorphisms and risk of AT-DILI, such as CYP2E1, GST, CYP3A4, CYP2C19) should be encouraged. Additional evidence from such well-designed trials would support guideline development and would aid development of a clinical tool for INH dosage adjustment based on genetic and clinical risk factors, in order to reduce hepatotoxicity and improve TB treatment outcomes.

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Costa GN, Magno LA, Santana CV, Konstantinovas C, Saito ST, Machado M, et al. Genetic interaction between NAT2, GSTM1, GSTT1, CYP2E1, and environmental factors is associated with adverse reactions to anti-tuberculosis drugs. Mol Diagn Ther 2012 Aug 1;16(4):241-250.


Tables (each table complete with title and footnotes)

Table 1  Studies investigating the association between the NAT2 polymorphisms and AT-DILI risk

<table>
<thead>
<tr>
<th>Genotype/Author</th>
<th>Year</th>
<th>Country</th>
<th>Study</th>
<th>NOS score</th>
<th>Genotyping</th>
<th>Sample size</th>
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Figure 1. Pathways of metabolism of isoniazid
Figure 2. Flowchart for identification of studies in the meta-analysis.
Figure 3 Forest plot of the association of the NAT2 polymorphism with risk of AT-DILI (subgroup analyses were performed by region of origin). For each effect measure, the forest plot indicates the pooled treatment effect estimate with its 95%CI, the weight measure and the $I^2$ heterogeneity measure among the studies included. OR = odds ratio; CI = confidence interval.
Figure 4  Forest plot of the association of the NAT2 polymorphism with risk of AT-DILI (subgroup analyses were performed by type of study). For each effect measure, the forest plot indicates the pooled treatment effect estimate with its 95%CI, the weight measure and the I² heterogeneity measure among the studies included. OR = odds ratio; CI = confidence interval.
Figure 5  Forest plot of the association of the NAT2 polymorphism with risk of AT-DILI (subgroup analyses were performed by method of genotyping). For each effect measure, the forest plot indicates the pooled treatment effect estimate with its 95% CI, the weight measure and the $I^2$ heterogeneity measure among the studies included. OR = odds ratio; CI = confidence interval.
Figure 6  Forest plot of the association of the NAT2*5/*6 slow NAT2 acetylators compared with other slow NAT2 acetylators combined with risk of AT-DILI. For each effect measure, the forest plot indicates the pooled treatment effect estimate with its 95%CI, the $I^2$ heterogeneity measure among the studies included. OR = odds ratio; CI = confidence interval.
Figure 7  Forest plot of the association of the NAT2*6/*7 slow NAT2 acetylators compared with other slow NAT2 acetylators combined with risk of AT-DILI. For each effect measure, the forest plot indicates the pooled treatment effect estimate with its 95%CI, the $I^2$ heterogeneity measure among the studies included. OR = odds ratio; CI = confidence interval.
Figure 8. Begg’s funnel plot to detect publication bias for the NAT2 polymorphism