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REVIEW

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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AIMS

The aim of this study is 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 1527 cases and 7184 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, I^2 = 51.3%, P = 0.000). The OR varied between different ethnic populations, ranging from 6.42 (95% CI 2.41–17.10, I^2 = 2.3%) for the West Asian population to 2.32 (95% CI 0.58–9.24, I^2 = 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.

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- 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

- 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, and (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 global public health problem. 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 drug responsible 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 [7]. 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 yet been introduced 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-analyse 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 CRD42016051722).

Two authors (M.Z. and S.W.) independently searched the PubMed, Medline and EMBASE databases for studies on the association of *NAT2* polymorphisms with risk of DILI up to 31 December 2017 using the search words: ('antituberculosis' or 'anti tuberculosis' or 'tuberculosis') and ('genetic polymorphism*' or 'polymorphism*') and ('adverse drug reaction*' or

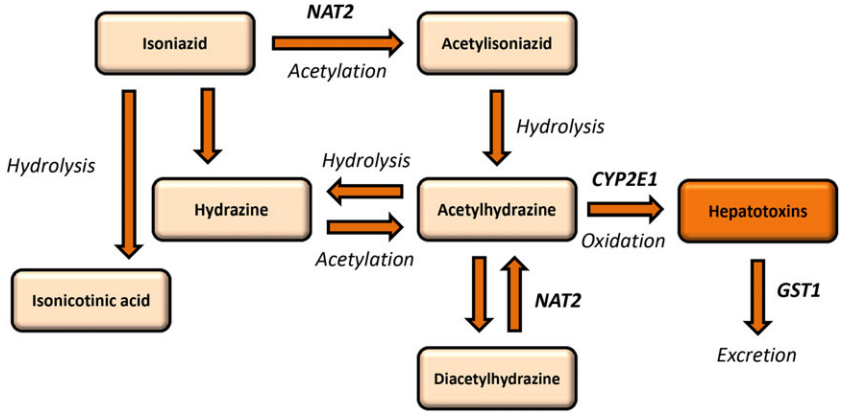

Figure 1
Pathways of metabolism of isoniazid

'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 or 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 [11–13]. The rapid acetylator phenotype and intermediate acetylator phenotypes were classified as non-slow acetylator phenotype in this review.

Inclusion and exclusion criteria

Eligible studies met the following inclusion criteria: They must (i) have 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) be original papers containing independent data, (iii) have 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 above were used to assess the quality of the included studies, and study quality was assessed according to Newcastle-Ottawa quality assessment [14]. These items included: (i) selection of study subjects, (ii) comparability of cases and

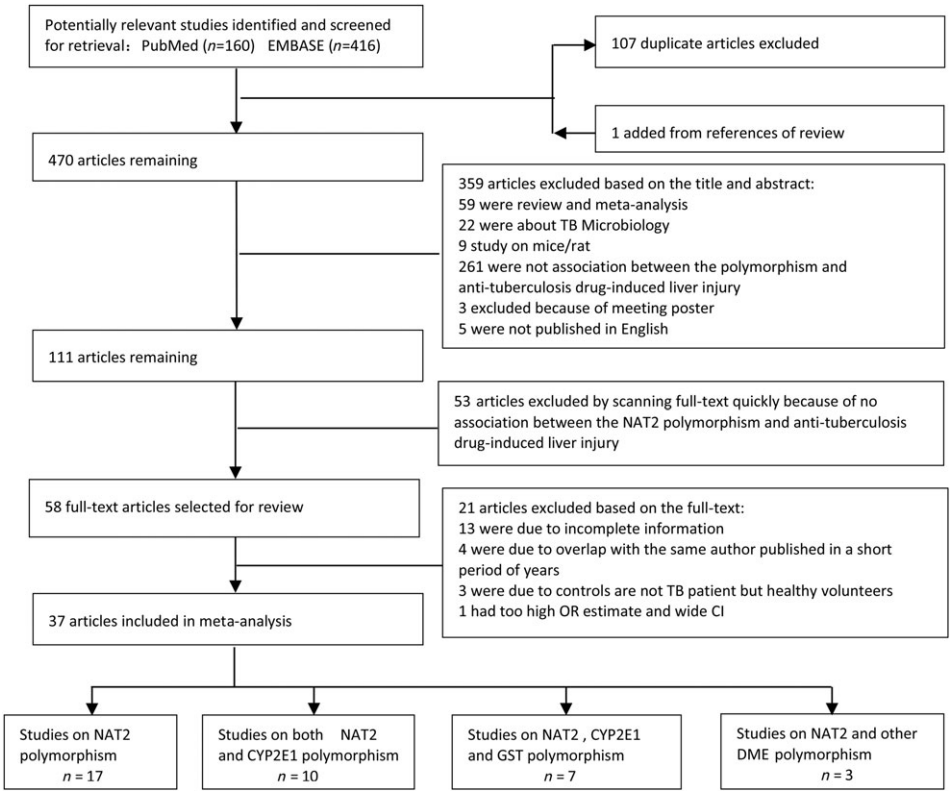

Figure 2
Flowchart for identification of studies in the meta-analysis

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 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 Asia, South Asia, Southeast Asia, West Asia, Africa, Europe, South and North America); (ii) study type (case–control study, nested case–control study, cross-sectional cohort studies, prospective cohort study); and (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 using the standard Q-statistic test, where $I^2 > 50\%$ 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 *P*-value <0.05 was considered as statistically significant.

Results

Identification and characteristics of the included studies

Using our electronic database searches, we identified 58 articles describing the strength of the postulated genetic associations between the *NAT2* polymorphisms and susceptibility to AT-DILI. A total of 37 case–control or prospective cohort design studies with 1527 AT-DILI cases and 7184 controls without AT-DILI were included in the meta-analysis. The main characteristics of the 37 studies are shown in Table 1. The studies by An *et al*. [15], Rana *et al*. [16] and Rana *et al*. [17] were excluded due to overlap with their other studies (we therefore selected the later publication to analyse the distribution of the *NAT2* genotype); three studies, by Guaoua *et al*. [18], Ng [19] and Mishra *et al*. [20], were excluded as controls were not TB patients but healthy people; the studies by Roy *et al*. [21] and Cavaco *et al*. [22] were excluded due to the absence of complete *NAT2* polymorphism distribution data. The study by Ohno *et al*. [23] 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) (one 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, seven were nested case–control studies, five were cross-sectional cohort studies, five were prospective cohort studies, and one 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, two used Taqman, one used HRM, one 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 (one 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 effects model (I^2 = 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 effects model (I^2 = 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 of slow acetylators on AT-DILI remained significant and ranged from 3.03 to 3.25 using random effects models. Heterogeneity was

Table 1

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

Genotype/ Author	Year	Country	Study	NOS score	Genotyping	Sample size		Slow acetylators	
						Case	Control	Case	Control
NAT2									
Chan [44]	2017	Singapore	Case–control study	6	Sequencing	24	79	18	17
Wattanapokayakit [45]	2016	Thailand	Case–control study	5	Sequencing	53	85	39	21
Mushiroda [46]	2016	Japan	Case–control study	6	Sequencing	73	293	13	14
Yuliwulandari [47]	2016	Indonesia	Case–control study	5	Sequencing	50	191	32	65
Wang [48]	2015	China	Cross-sectional cohort study	7	Sequencing	70	285	23	62
Ho [49]	2013	China	Nested case–control study	6	Sequencing	19	329	12	67
Lv [24]	2012	China	Nested case–control study	6	RFLP	89	356	18	74
Ben Mahmoud [50]	2012	Tunisia	Nested case–control study	6	RFLP	14	52	11	22
Rana [16]	2012	Indian	Case–control study	6	RFLP	50	201	19	30
Leiro-Fernandez [51]	2011	Spain.	Nested case–control study	7	RFLP	50	67	36	44
Sistanizad [52]	2011	Iran	Cross-sectional cohort study	6	RFLP	14	36	9	11
Khalili [53]	2011	Iran	Case–control study	6	RFLP	14	36	9	5
Bozok [54]	2008	Turkey	Case–control study	6	HRM	30	70	23	19
Higuchi [30]	2008	Japan	Nested case–control study	6	RFLP	18	82	6	4
Possuelo [55]	2008	Brazil	Prospective cohort study	8	Sequencing	14	240	9	60
Shimizu [56]	2005	Japan	Case–control study	5	RFLP	10	32	4	1
Huang [31]	2002	China	Nested case–control study	6	RFLP	33	191	14	39
NAT2, CYP2E1									
Rana [17]	2014	India	Prospective cohort study	7	RFLP	55	245	21	36
Chamorro [57]	2013	Argentina.	Cross-sectional cohort study	6	RFLP	47	128	28	48
Gupta [58]	2013	India	Nested case–control study	7	RFLP	50	165	28	63
Santos [59]	2013	Brazil	Case–control study	6	Sequencing	18	252	11	75
An [60]	2012	China	Case–control study	6	Sequencing	101	107	40	13
Bose [61]	2011	India	Case–control study	7	RFLP	41	177	29	79
Lee [62]	2010	China	Case–control study	7	Taqman	45	95	21	20
Yamada [63]	2009	Canada	Case–control study	5	Sequencing	23	147	14	64
Cho [64]	2007	Korean	Case–control study	6	Sequencing	18	114	7	12
Vuilleumier [65]	2006	Switzerland	Case–control study	7	RFLP	8	81	3	32
NAT2, CYP2E1, GST									
Chamorro [66]	2017	Argentina	Prospective cohort study	6	RFLP	96	249	64	102
Heinrich [67]	2016	Brazil	Cross-sectional cohort study	7	RFLP	20	88	15	44
Singla [68]	2014	India	Case–control study	6	RFLP	17	391	15	213
Xiang [69]	2014	China	Cross-sectional cohort study	6	Taqman	71	1614	28	501
Costa [70]	2012	Brazil	Prospective cohort study	5	Sequencing	54	75	22	13
Teixeira [29]	2011	Brazil	Case–control study	6	Sequencing	26	141	18	64
Sotsuka [71]	2011	Japan	Case–control study	6	RFLP	52	92	8	5
NAT2, CYP2E1, CYP3A4									
Zaverucha-do-Valle [72]	2014	Brazil	Retrospective cohort study	7	Sequencing	52	79	37	36

(continues)

Table 1

(Continued)

Genotype/ Author	Year	Country	Study	NOS score	Genotyping	Sample size		Slow acetylators	
						Case	Control	Case	Control
NAT2, CYP2B6, CYP3A5, ABCB1, UGT2B7, SLCO1B1									
Yimer [28]	2011	Ethiopian	Prospective cohort study	5	Sequencing	41	160	31	107
NAT2, CYP2E1, CYP2C9, CYP2C19, CYP2D6									
Kim [73]	2009	Korean	Case–control study	6	SNP stream	67	159	21	28

specifically decreased (I^2 = 41.3%), when the study by Lv *et al*. [24] 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 [25–27] 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 one-and-a-half to two times as many cases. It also adjusts the classification used in the study by Yimer *et al*. [28], which 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 countries" in the 2016 latest global TB report [1].

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 [29], which is interesting as the Brazilian population includes contributions from Africans, Europeans and Amerindians in its heritage. Considering the ethnic diversity of the 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 individuals [24, 30, 31]. Of the 37 studies included in our meta-analysis, nine 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 pharmacokinetic 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 ADRs 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 [32–35]. Some authors suggested that an adaptation of administered INH dosages according to patient acetylator status may benefit patients [36–38]. In one clinical trial an INH QD dose of 5 mg kg^{-1} of body weight was modified to doses of 2.5 mg kg^{-1} for slow acetylators, 5 mg kg^{-1} for intermediate acetylators and

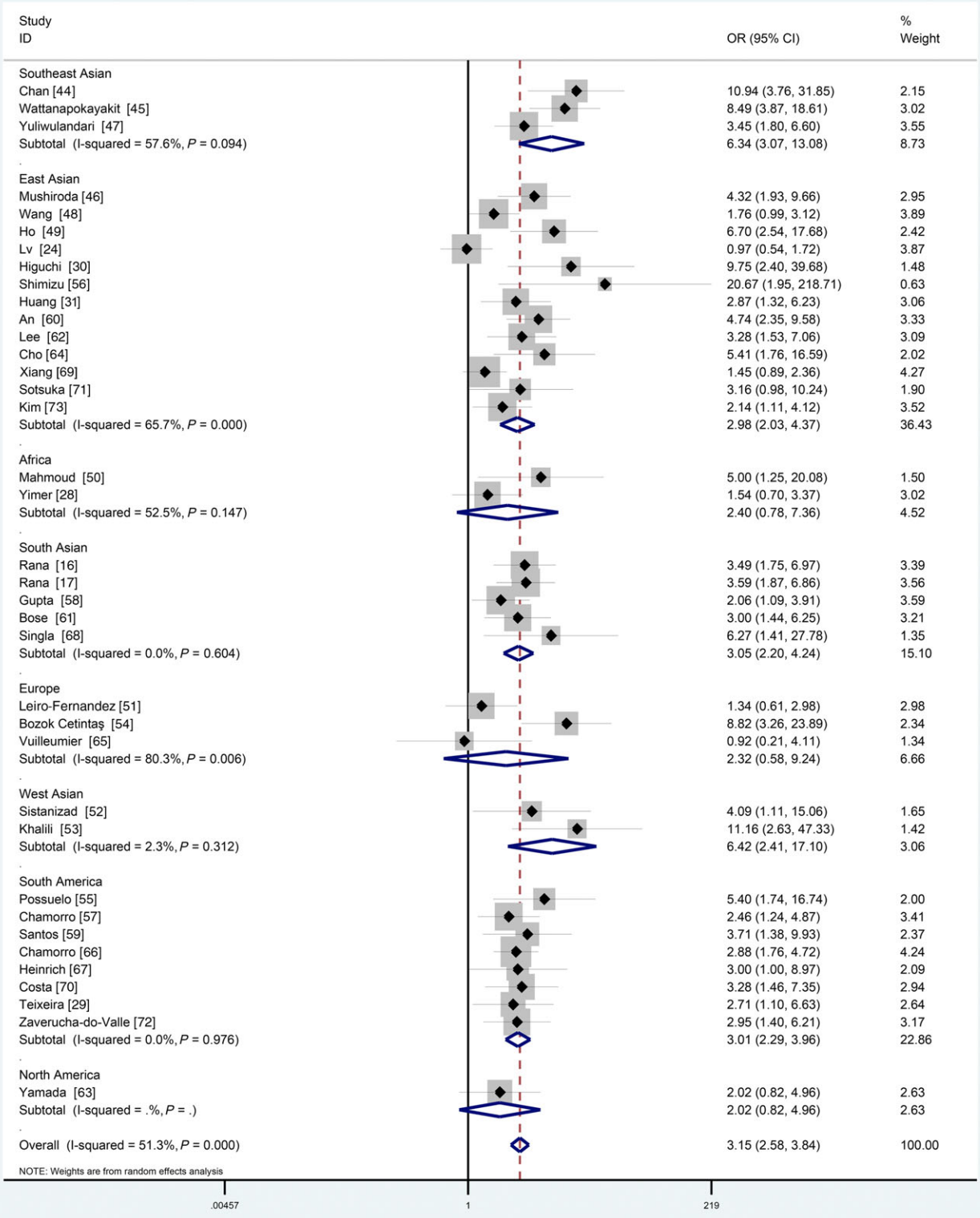

Figure 3

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. CI = confidence interval; OR = odds ratio

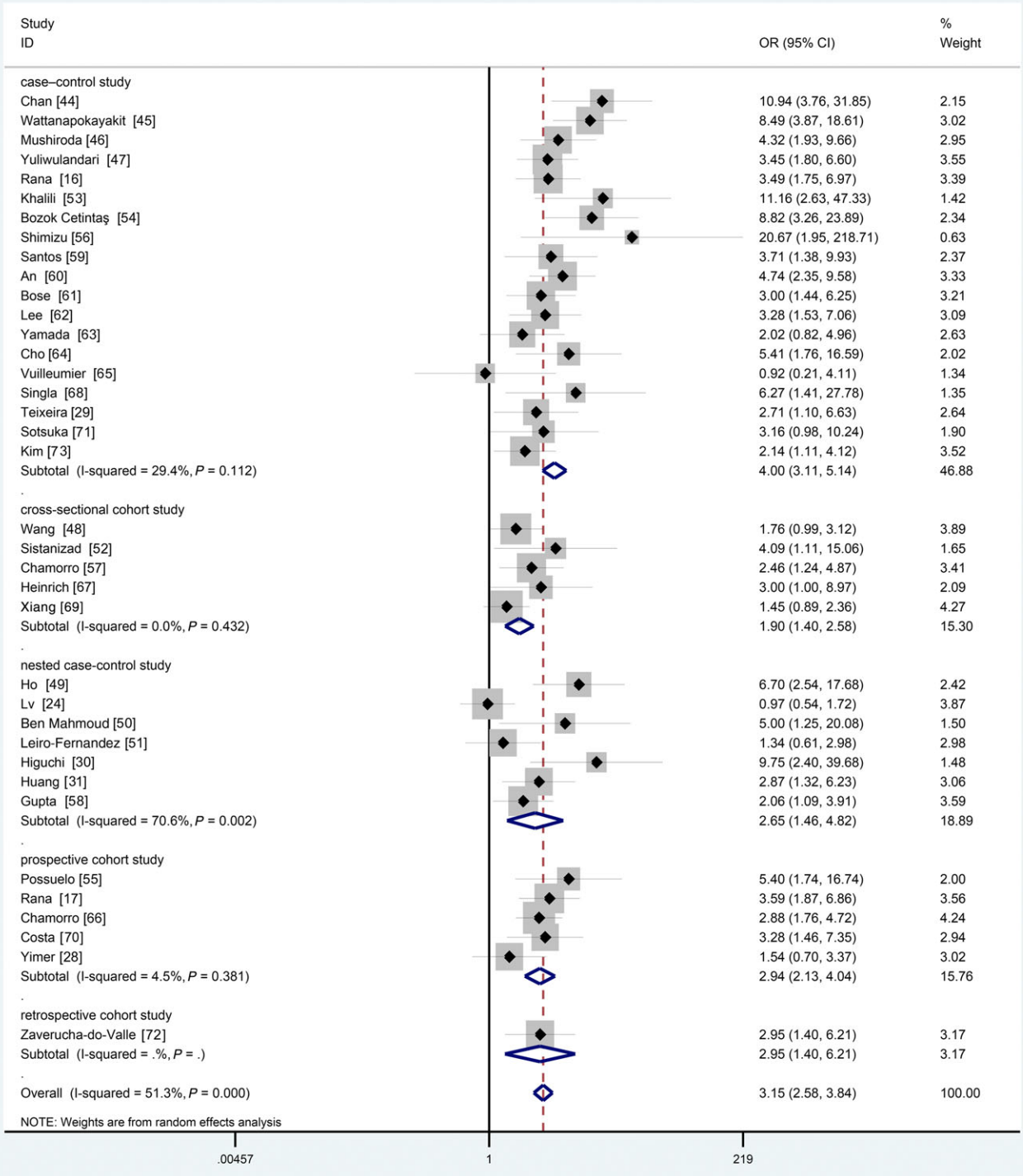

Figure 4

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 I^2 heterogeneity measure among the studies included. CI = confidence interval; OR = odds ratio

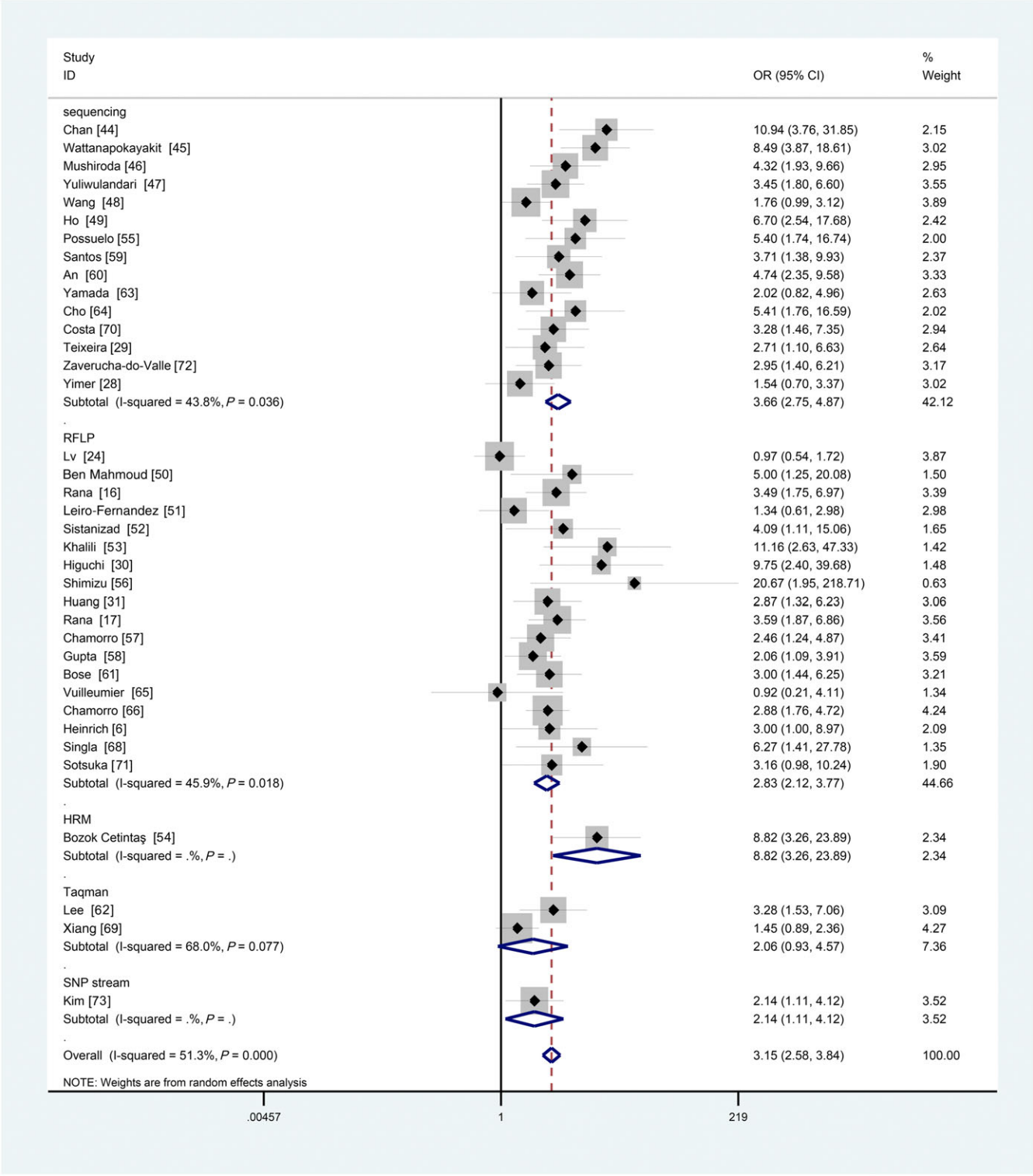

Figure 5

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. CI = confidence interval; OR = odds ratio

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 [37].

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

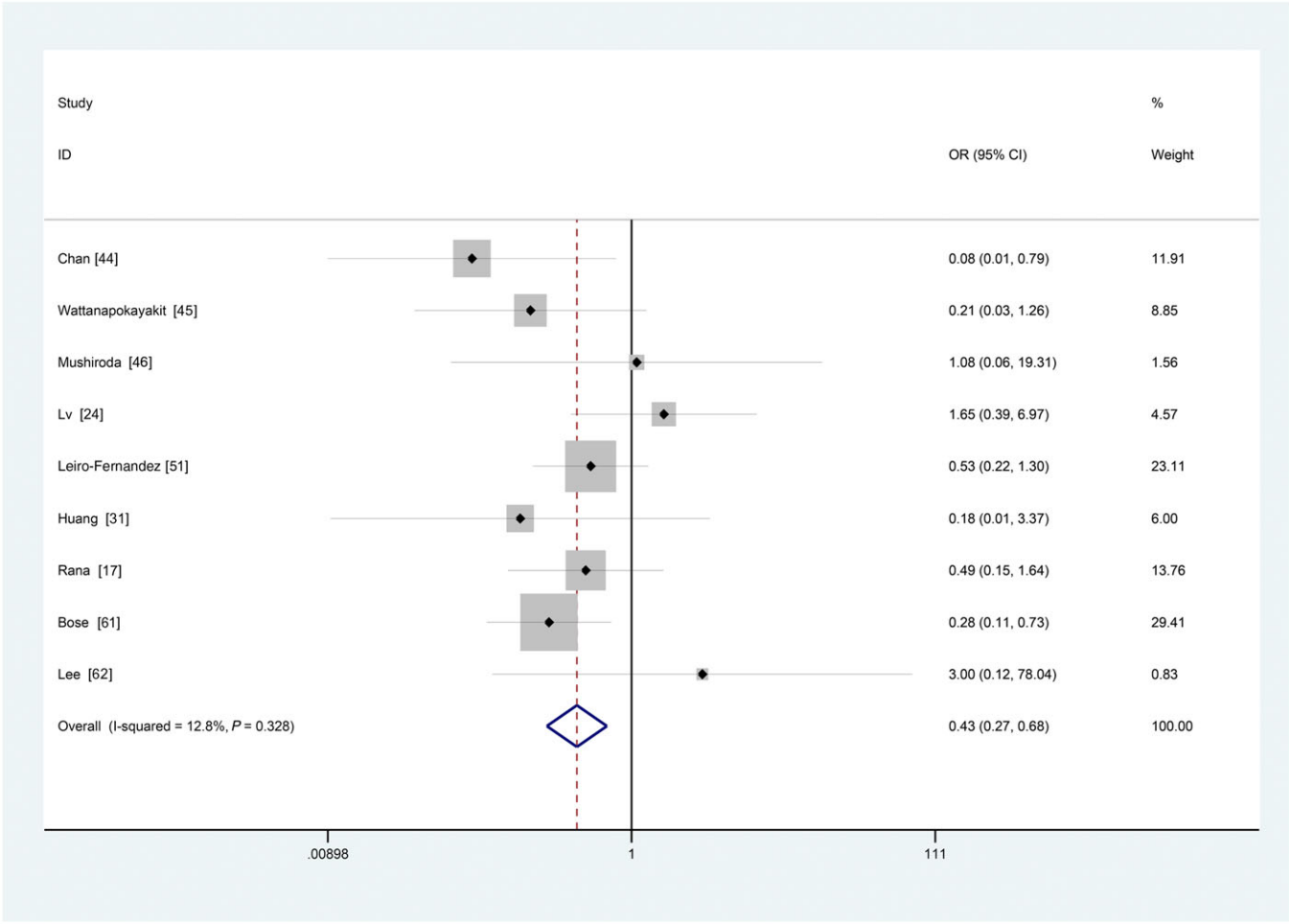

Figure 6

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. CI = confidence interval; OR = odds ratio

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 [39, 40]. 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 high-burden TB 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 [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 [41]. 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 polymorphism distributions in the target populations does not allow us to estimate 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 meta-

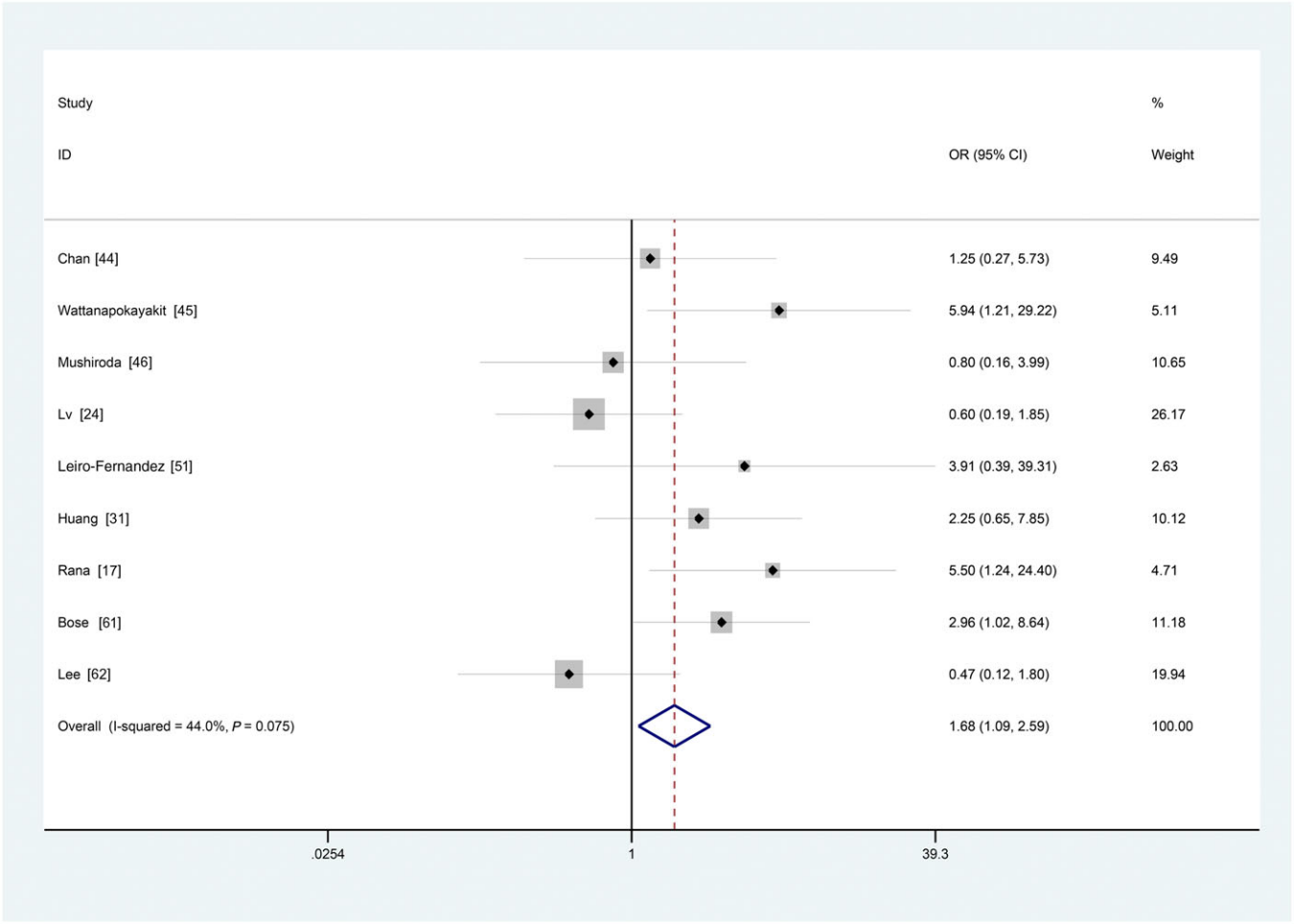

Figure 7

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. CI = confidence interval; OR = odds ratio

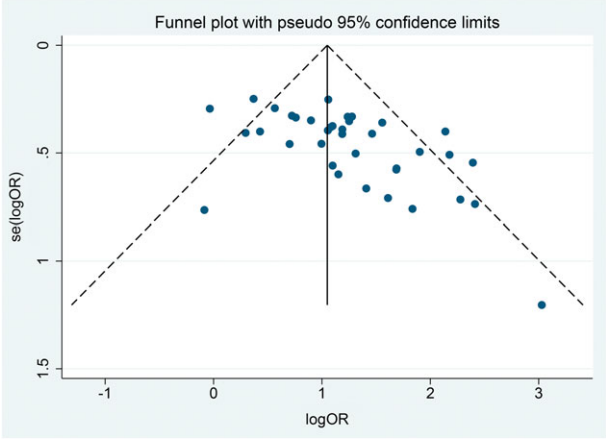

Figure 8

Begg's funnel plot to detect publication bias for the *NAT2* polymorphism

analysis provides important new information that is statistically robust in sensitivity analyses and has yielded relevant and reliable results.

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* has not yet been 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 of *NAT2* polymorphisms, we recommend a randomized controlled trial be designed 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 hepatoxicity and improve TB treatment outcomes.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [42], and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 [43].

Competing Interests

There are no competing interests to declare.

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