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By ENDANG DARMAWAN

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Original Article

Polymorphisms of NAT2, CYP2E1, GST, and HLA Related to Drug-Induced Liver Injury in Indonesian Tuberculosis Patients

Dyah Aryani Perwitasari[1], Endang Darmawan[1], Ully Adhi Mulyani[2], Pieter Van Der Vlies[3], Jan-Willem C. Alffenaar[4], Jarir Atthobar[5], Bob Wilffert[6]

[1]Unit of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, [2]Center of Health Technology and Clinical Epidemiology, National Health Institute, Jakarta, [5]Unit of Pharmacology and Therapy, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia, Departments of [3]Genetics and [4]Clinical Pharmacy and Pharmacology, University of Groningen, University Medical Center Groningen, [6]Unit of PharmacoTherapy, Epidemiology and Economics, University of Groningen, Groningen Research Institute of Pharmacy, Groningen, The Netherlands

Abstract

Background: Gene polymorphisms have been associated with drug-induced liver injury (DILI). This study aimed to elucidate the association between polymorphisms of *NAT2*, *CYP2E1*, *GSTT1*, *GSTM1*, and *HLA* genes with isoniazid plasma concentration and DILI. **Methods:** This study was a prospective cohort study recruiting adult newly diagnosed tuberculosis (TB) patients who met the inclusion criteria from the Public Health Centers in Yogyakarta and Lampung. Defined single-nucleotide polymorphisms were rs1799929, rs1799930, rs1799931, rs1801280, and rs1041983 of NAT2; rs2031920, rs8192775, and rs2515641 of CYP2E1; rs1041981, rs1063355, and rs6906021 of *HLA*. *GSTT1* and *GSTM1* were defined as *GSTT1*, *GSTM1*, and *GSTT1* deletion and *GSTM1* deletion. The DNA was taken from the patient saliva. Data of anti-TB drug plasma concentration on the weeks 4–8 of treatment were retrieved from the patients' medical report. Statistical analysis was performed using Chi-square test, Student's *t*-test, and multinomial logistic regression. **Results:** Over the 207 patients, up to 1.9% of them experienced DILI. The percentage of slow acetylators of *NAT2* was 69.5%. Patients with extensive acetylator phenotype did not experience DILI (odds ratio [OR]: 0.46; 95% confidence interval [CI]: 0.23–0.94). The G carriership of *HLA* rs1063355 could protect the patients from the DILI (OR: 0.39; 95% CI: 0.14–0.9). Furthermore, the C carriership of *HLA* rs1041981 can protect the patients from DILI (OR: 0.38; 95% CI: 0.15–0.50). The genotype of *HLA-DQB**0302 significantly affects the isoniazid concentration. **Conclusion:** The *NAT2* genotype was significantly associated with DILI. Furthermore, the absence of G carriership of *HLA-DQA**0102 could protect the patients from DILI without being associated with an effect on the isoniazid concentration.

Keywords: Antituberculosis, CYP2E1, drug-induced liver injury, GST, HLA, Indonesia, NAT2

Introduction

The World Health Organization (WHO) reported in 2017, that in 2016, the mortality of tuberculosis (TB) cases in Indonesia reached 110,000 with 42/100,000 persons. Indonesia is in the second rank of TB burden after India.[1] Measures to improve the success of programmatic treatment of TB are urgently needed, including programs for drug resistance, duration of treatment, adverse drug reactions (ADRs), relapse, and prolonged infections.[2]

Several genetic polymorphisms have been associated with hepatotoxicity but have not been studied together and combined with isoniazid drug concentrations. The aim of this study was to elucidate the association between the polymorphisms of *NAT2*, *CYP2E1*, *GSTT1*, *GSTM1*, and *HLA* genes in combination with isoniazid plasma concentration and hepatotoxicity.

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Methods

Ethic

This study was approved by the National Ethics Committee on Health Research, Jakarta, Indonesia, Number: KE 01.06/EC/531/2012. Written informed consent was obtained from all patients.

Address for correspondence: Dr. Bob Wilffert, Unit of Pharmaco Therapy, Epidemiology and Economics, University of Groningen, Groningen Research Institute of Pharmacy, Antonius Deusinglaan 1, 9713 Av, Groningen, The Netherland. E-mail: b.wilffert@rug.nl

ORCID: Bob Wilffert: https://orcid.org/0000-0002-8759-5697

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Subjects

This study was a prospective cohort study recruiting adult newly diagnosed TB patients from Public Health Centers in Yogyakarta and Lampung. The inclusion criteria were newly diagnosed of TB patients. The diagnose of TB used the result of sputum test which showed the positive result for Acis-Fast Bacili (AFB) and X-ray results, were treated with fixed-dose combination of anti-TB drugs dosed according to the WHO guidelines,[3] and had normal function of the kidney and liver at the baseline measurement. The exclusion criteria were participants fulfilled the inclusion criteria who are having human immunodeficiency virus and/or diabetes mellitus history, liver abnormality history, abnormality of the renal and/or liver function, and reactive results of hepatitis B surface antigen test.

According to the WHO, DILI was defined as a level of alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) of at least 3.0 the upper limited number.[4,5] The normal ranges of ALT and AST in the Indonesian population are <31 U/L and 3–45 U/L, respectively, using the kinetic method of the International Federation of Clinical Chemistry assay.[6,7]

DNA collection

The DNA was taken from the patients' saliva using an Oragene DNA collection kit (DNA Genotek, Ottawa, Canada) and all the samples were purified using GlycoBlue Coprecipitant (Thermo Fisher Scientific, Waltham, USA). The quality of DNA was verified by NanoDrop™ spectrophotometer measurement.

Single-nucleotide polymorphisms identification

This study defined the following single-nucleotide polymorphisms (SNPs): rs1799929, rs1799930, rs1799931, rs1801280, and rs1041983 of *NAT2*; rs2031920, rs8192775, and rs2515641 of *CYP2E1*; rs1041981, rs1063355, and rs6906021 of *HLA*, according to the previous studies.[8-10] Genotyping was performed with the MassARRAY iPlex pro system (Agena BioScience, San Diego, USA). The *NAT2* was phenotyped into extensive, intermediate, and slow acetylators according to the function of the allele of the SNPs used in this study.[11,12]

GSTT1 and *GSTM1* were defined as *GSTT1*, *GSTM1*, and *GSTT1* deletion and *GSTM1* deletion. PCR was carried out according to the literature,[13] using PrimeSTAR Polymerase (TAKARA BIO INC., Shiga, Japan). PCR products were analyzed by 0.8% agarose gel electrophoresis. The presence of the deletion and the presence of the gene were tested separately because of their difference in fragments length (625 and 969 bp for the genes and 3106 and 4748 bp for the deletions, respectively).

Antituberculosis drug plasma concentration

Data of anti-TB drug plasma concentration on the weeks 4–8 of the treatment were retrieved from the patients' medical report. Plasma samples had been collected in 57 TB patients, 2 h after administration of the drug in fasted condition.[6,7,14,15] The samples were processed after sample collection and subsequently stored in the frozen condition until routine analysis. The method has been validated previously.[16,17] The mixed solution was vortexed for 15 s and was centrifuged for 10 min at 10,000 rpm. The supernatant was taken from the solution and was used for the next process. Ether (3 ml) was added to 200 µL of the supernatant, and the water phase was taken. The 20 µL water phase was injected into the high-performance liquid chromatography system. The accuracy and the intraday–interday precisions met the criteria from the US Department of Health and Human Services Food and Drug Administration.[18] We defined the normal range of Isoniazid, Rifampicin, Pirazinamide, Ethambutol (HRZE) as 3–6 µg/ml, 8–24 µg/ml, 20-50 µg/ml, and 2–6 µg/ml, respectively.[14]

Data analysis

Data were analyzed using Chi-square test, Student's *t*-test, and multivariate analysis. Multinomial logistic regression was performed among phenotype of *NAT2*, *GSTT1*, *GSTM1*, rs2031920 (*CYP2E1*), rs2515641 *(CYP2E1)*, rs8192775 (*CYP2E1*), rs1041981 (*HLA*), rs1063355 (*HLA)*, and rs6906021 as independent variables and DILI as a dependent variable.

Results

Patients' characteristics

In total, 232 newly diagnosed adult TB patients from Yogyakarta and Lampung were screened and 207 met inclusion criteria and were recruited. Patient characteristics are shown in Table 1. Most of the patients were female (60.6%), with a mean age of 40.7 (standard deviation: 14.8). Most of the patients had a positive result of the AFB test (85%) and around 57% of them were underweight. The average of body mass index was also in underweight condition (18.3). Only around 8% of the patients had comorbidities or disease history and Type 2 diabetes mellitus was the most prevalent comorbidity in this study (4.0%).

Hepatotoxicity

Increased ALT and AST was predominantly observed in the 2nd month of TB treatment [Table 2]. In total, 1.0%, 1.9%, and 1.0% of the TB patients experienced mild DILI based on increased ALT, AST, and ALT-AST, respectively. The occurrence of DILI was not associated with any of the patients' characteristics (data were not shown: $P > 0.05$) [Table 1].

Single-nucleotide polymorphisms characteristics and association with hepatotoxicity

The *NAT2* genes are phenotyped into extensive (25.0%), intermediate (5.6%), and slow acetylators (69.5%). In *CYP2E1*, the CC of rs2031920, GG of rs8192775, and CC of rs2515641 had the highest percentages of the genotypes (72.2%, 72.4%, and 62.4%, respectively). The percentages of *GSTT1* homozygous deletion and *GSTM1* homozygous deletion are 9.4% and 21.6%, respectively. The percentages of GT of *HLA-DQA**0102 and CC of *HLA-DQB*0302* are the highest compared to the other genotypes (44% and 29%, respectively).

The results of association analysis between the genes polymorphisms and DILI are presented in Table 3. The highest percentages of genotypes which experienced DILI were 48.9% of slow acetylators of *NAT2*, 50% CC of rs2031920, 41.7% CC of rs2515641, and 66.4% G carrier of rs8192775 of *CYP2E1*. Among the *GSTT1* and *GSTM1*, the highest percentages of DILI patients were seen in the homozygous wild-type and heterozygous, which are 62.6% and 51.4%, respectively. Moreover, among the *HLA*, the highest percentages of patients who experienced DILI were a presence on the G carriers of *HLA-DQA*0102* (53.6%), CC and CT genotypes of *HLA-DQB*0302* (41.7%), and C carriers of rs1041981 (35.4%).

Table 1: Tuberculosis patients' characteristics (*n*=207)

Patients' characteristics	*n* (%)
Age, mean (SD)	40.7 (14.8)
Gender, *n* (%)	
Male	82 (39.4)
Female	125 (60.6)
City of origin, *n* (%)	
Yogyakarta	77 (37.2)
Lampung	130 (62.8)
Type of TB, *n* (%)	
Pulmonary	203 (98.1)
Extrapulmonary	4 (1.9)
Diagnosis test, *n* (%)	
Positive AFB	177 (85.6)
Positive X-ray	18 (8.7)
Positive AFB X-ray	12 (5.8)
BMI (baseline), mean (SD)	18.13 (2.90)
Underweight, *n* (%)	118 (57.5)
Normal, *n* (%)	71 (34.2)
Overweight, *n* (%)	18 (8.7)
Smoking status, *n* (%)	
Never	89 (43.0)
Currently stop	89 (43.0)
Smoking	20 (9.7)
Disease history in the last 6 months and comorbidities, *n* (%)	
Typhoid	4 (1.9)
Malaria	1 (0.5)
Type 2 diabetes mellitus	11 (5.3)
Hypertension	2 (1.0)
Gastritis	3 (1.5)
HNP	1 (0.5)
Chest pain	1 (0.5)

TB: Tuberculosis, SD: Standard deviation, AFB: Acid-fast bacilli, BMI: Body mass index, HNP: Hernia nucleus purposus

There were no significant associations between gene polymorphisms of *CYP2E1*, *GSTT1*, and *GSTM1* and the risk of DILI. A significant association was found between the *NAT2* phenotype and patients experiencing DILI. Patients with extensive acetylators phenotype did not experience DILI (odds ratio [OR]: 0.46; 95% confidence interval [CI]: 0.23-0.94). The authors find that T carriers of rs2515641, homozygous deletion of *GSTM1*, and TC carriers of *HLA* rs6906021 tend to be a predictor of DILI. The significant associations are also shown by G carriers of *HLA* rs1063355 which could protect the patients from the DILI (OR: 0.39; 95% CI: 0.14–0.9) and the C carriership of *HLA* rs1041981 which can also protect the patients from DILI (OR: 0.38; 95% CI: 0.15–0.50).

Antituberculosis drug plasma concentration and association with single-nucleotide polymorphisms

Table 4 shows that only 13.9% of the patients had an isoniazid plasma concentration in the normal range; furthermore, 0.66%, 41.9%, and 2.90% of the patients had plasma concentration of ethambutol, pyrazinamide, and rifampicin, respectively, in the normal range. According to the therapeutic range, 99.3% and 70.2% of the patients had ethambutol and rifampicin concentrations under the normal range. About 79% of the patients had high isoniazid concentration (above the therapeutic range) and 53% had high pyrazinamide concentrations. According to the acetylator category, 86.3% slow acetylators had Isoniazid (INH) concentration higher than upper range concentration. This pattern also can be seen in the extensive acetylators patients, even though the proportion of patients is lower than slow acetylators.

According to the 57 data of isoniazid plasma concentration, only 37 patients experienced DILI. Table 5 presents the association between gene polymorphisms and isoniazid plasma concentration. Patients with high isoniazid plasma concentration have 2.6 times higher risk of experiencing DILI (95% CI; 0.5–13.1). The slow acetylators of *NAT2* and homozygous deletion of *GSTT1* and *GSTM1* have a risk of having high isoniazid plasma concentration as amounting to 3.0, 1.1, and 1.36, respectively (95% CI: 0.7–20.7; 1.0–1.3; 0.2–9.1).

Table 6 shows the significant difference in isoniazid concentration among genotypes of *HLA-DQB**0302. However, there is no significant difference in isoniazid concentration among the genotypes of *HLA-DQA**0102 and rs1041981. The TC genotype has the highest isoniazid plasma concentration compared to CC and TT (P = 0.017). The isoniazid plasma concentrations in the TC genotype are above

Table 2: Liver test results and category of hepatotoxicity (*n*=207)

	Baseline, mean (SD) (μ/L)	2nd month, mean (SD) (μ/L)	*n* (%)	4th month, mean (SD) (μ/L)	*n* (%)
AST	19.9 (11.6)	23.3 (21.0)	123 (59.4)	16.9 (8.8)	79 (38.1)
ALT	21.8 (9.7)	25.8 (17.6)	92 (44.4)	23.3 (21.0)	72 (34.7)
AST and/or ALT			136 (65.7)		

AST: Aspartate aminotranferase, ALT: Alanine aminotransferase, SD: Standard deviation

Table 3: Association between gene polymorphisms and drug-induced liver injury

	Percent of patients experienced DILI	Percent of patients did not experience DILI	OR (CI: 95%), *P*
NAT2 acetylator			
Extensive	31 (15.9)	22 (14.7)	0.5 (0.23-0.94)*, 0.02*
Slow	86 (48.9)	33 (20.6)	
CYP2E1			
rs2031920			
T carrier	30 (18.5)	15 (9.3)	0.9 (0.4-1.8), 0.45
CC	81 (50.0)	36 (22.2)	
rs2515641			
T carrier	38 (27.2)	16 (11.4)	1.2 (0.5-2.5), 0.37
CC	57 (41.7)	29 (20.7)	
rs8192775			
G carrier	95 (66.4)	46 (32.2)	0.7 (0.6-0.8), 0.46
AA	2 (1.4)	0 (0.)	
GSTT1			
Homozygous deletion	11 (6.1)	6 (3.3)	0.8 (0.3-2.3), 0.45
Homozygous wild-type and heterozygous	114 (62.6)	51 (28.0)	
GSTM1			
Homozygous deletion	30 (16.2)	10 (5.4)	1.6 (0.7-3.5), 0.17
Homozygous wildtype and heterozygous	95 (51.4)	50 (27.0)	
HLA			
rs1063355 (*HLA-DQA**0102)			
Carrier G	101 (53.6)	56 (31.3)	0.39 (0.14-0.9), 0.048*
TT	23 (12.4)	5 (2.7)	
rs6906021 (*HLA-DQB**0302)			
TC	41 (23.6)	19 (10.0)	1.2 (0.6-2.3), 0.35
CC and TT	77 (41.7)	43 (24.7)	
rs1041981			
AA	17 (9.1)	6 (3.3)	0.380 (0.15-0.5), 0.033*
C carrier	105 (57.1)	56 (30.4)	

*Significant result. DILI: Drug-induced liver injury, OR: Odds ratio, CI: Confidence interval

Table 4: Results of therapeutic drug monitoring measured by high-performance liquid chromatography (*n*=57)

Drug name (range of concentration μg/ml)	Dose (mg), mean (range)	Concentration μg/ml, geometric mean (range)	Percentage concentration less than the lower range	Percentage concentration more than the upper range
Isoniazid (3-6)	228.7 (150-375)	10.24 (1.06-27.98)	7.1	79.0
Slow acetylators	228.7 (150-375)	11.97 (11.67-20.62)	6.9	86.3
Extensive acetylators	225 (150-300)	10.63 (1.06-25.28)	15.4	69.2
Ethambutol (2-6)	840.7 (550-1375)	0.60 (0.02-1.20)	99.3	0
Pirazinamid (20-50)	1222.9 (800-2000)	37.04 (2.35-65.30)	5.3	53.1
Rifampicin (8-24)	455.2 (300-750)	1.58 (0.26-25.47)	70.2	26.9

the normal range (3–6 μg/ml). This data support the previous data that patients with the TC genotype had 1- and 2-time risk to experience DILI (95% CI: 0.6–2.3).

Multinomial logistic regression was performed among phenotype of *NAT2*, *GSTT1, GSTM1*, rs2031920 (*CYP2E1*), rs2515641 *(CYP2E1*), rs1041981 (*HLA*), rs1063355 (*HLA)*, and rs6906021 as independent variables and DILI as a dependent variable. The authors found that G carriers of HLA-DQA*0102 have protective effect against DILI compared to T carriers (OR: 0.38; 95% CI: 0.15–0.95).

Discussion

Our study showed that slow acetylators of the *NAT2* phenotype are highly prevalent in this cohort of Indonesian patients. There are some variations in the genotypes of *CYP2E1* in rs2031920, rs8192775, and rs2515641. Furthermore, the *GSTM1* homozygous deletion is predominant in the study population. The variations of *HLA-DQA**0102 and *HLA**DQB0302* are also available.

A statistically significant association with genetic variants was found between DILI and *NAT2* phenotype and G carriers

Table 5: Association between isoniazid plasma concentration and genes polymorphisms (*n*=55)

Genotypes	Mean of isoniazid concentration (μg/ml)	Isoniazid, *n* (%)		OR (CI: 95%), *P*
		High concentration	Normal range concentration	
NAT2 acetylator				
Extensive	11.8	8 (19.0)	3 (7.1)	0.3 (0.05-1.84), 0.12
Slow	14.4	26 (61.9)	3 (7.1)	
CYP2E1				
rs2031920				
T carrier	12.2	7 (17.9)	1 (2.6)	1.35 (0.1-13.5), 0.64
CC	14.1	26 (66.7)	5 (12.8)	
rs2515641				
T carrier	15.5	12 (36.4)	1 (3.0)	3.0 (0.2-30.4), 0.33
CC	12.3	16 (48.5)	4 (12.1)	
rs8192775				
G carrier	14.0	28 (82.4)	5 (14.7)	0.5 (0.5-1.8), 0.85
AA	6.5	1 (2.9)	0 (0.0)	
GSTT1				
Homozygous deletion	21.9	1 (2.2)	0 (0.0)	1.1 (1.0-1.3), 0.84
Homozygous wild-type and heterozygous	13.2	37 (82.2)	7 (15.6)	
GSTM1				
Homozygous deletion	16.7	4 (8.7)	1 (2.2)	1.36 (0.2-9.1), 0.58
Homozygous wild-type and heterozygous	13.2	35 (76.1)	6 (13.0)	

Table 6: INH concentration among the *HLA-DQB0302 genotypes (rs6906021) and *HLA DQA**0102 (rs1063355)**

Genotype	Mean of isoniazid concentration (range) μg/ml	*P*
*HLA-DQB**0302		
CC	13.11 (1.06-25.28)	0.017*
TC	16.92 (6.53-27.98)	
TT	11.55 (9.35-13.75)	
*HLA-DQB**0102		
CC	14.27 (1.34-25.38)	0.761
TC	14.88 (1.06-27.98)	
TT	11.92 (6.52-15.81)	
rs1041981		0.691
CC	13.8 (1.34-25.88)	
AC	13.6 (1.06-27.98)	
AA	16.9 (13.33-22.76)	

*Significant. INH: Isoniazid

of *HLA-DQA**0102. Furthermore, a significant association between H-concentration and C carriers of *HLA-DQB**0302 was found, but not between DILI and C carriers of *HLA-DQB**0302. Extensive acetylators and G carriers of *HLA-DQA**0102 may protect against DILI. Trends were found for the slow acetylators of *NAT2*, T carrier of *CYP2E1* rs2516451, and homozygous deletion of *GSTM1* which are related to a higher number of patients with DILI.

This finding is also supported by the previous meta-analysis of 13 randomized studies with rapid and slow acetylators. The rapid acetylators may predict the treatment failure, the absence of ADR, and relapse possibility. This study also suggested that the treatment failure and ADR were significantly associated with the pharmacokinetics of this single drug in the combination.[19]

Even though the significant associations were only presented between the *NAT2* and DILI, our study showed that some variations of the gene may predict the incidence of DILI, such as the slow acetylators of *NAT2*, T carrier of CYP2E1 rs2516451, and homozygous deletion of *GSTM1*, which are related to the higher number of patients with DILI. These results are also supported by the high plasma concentrations of INH in the slow acetylators of *NAT2*, T carrier of *CYP2E1* rs2516451, and homozygous deletion of *GSTM1*. The previous study in Japan about the *NAT2* genotype-guided regimen presented that 78% patients with standard treatment of TB experienced DILI; however, none of the patients with pharmacogenetic screening-guided treatment experienced DILI or failure of the treatment.[20]

Regarding the INH concentration, both slow and extensive acetylators showed the high concentration exceeds the upper limit of INH's range concentration. Remarkably, the proportion of patients with the high concentration of INH in the slow acetylators group was higher than the extensive acetylators group. These results were also supported by the previous study conducted in India. TB patients with slow acetylators had higher concentrations of INH than patients with intermediate and rapid acetylators and the INH concentration among the three groups was significantly different. India's study also presented that patients with slow, intermediate, and rapid acetylators had lower INH concentration, which was <3 μg/ml.[21]

The presence of slow acetylators of NAT2 (37%) in Colombian Caribbean Coast region which may predict the DILI was

consistent with our study results.[22] The Chinese population also showed similar results of slow acetylators of *NAT2* which had higher risk of DILI.[23] Our study is also supported by another previous study in Thai populations. The slow acetylators had a higher frequency of DILI than the immediate and rapid acetylators in the group of patients. However, in the control group, the intermediate acetylators had the highest frequency.[24] This confirmed earlier results showing that slow acetylators of *NAT2* both in Asian and non-Asian population had higher risk of DILI.[25] In the Moroccan population, the frequency of c1/c1 of *CYP2E1* rs2031920 was around 98%, also in the other populations such as Turkish, Germanians, Serbians, French, English, and Brazilians. However, the frequency of c1/c1 of *CYP2E1* rs2031920 in Taiwanese and Chinese was around 50%. These results are different from our study which shows that the percentage of the CC genotype of *CYP2E1* rs2031920 reaches 70% and the T carriers of this SNP had a risk of having high isoniazid plasma concentration. Contradictorily, according to the meta-analysis of the *CYP2E1* rs2031920, the c1/c1 genotype was related to DILI, especially in the Chinese and Korean population.[26] In the Indian population, the risk of DILI did not associate with the polymorphisms of rs2031920 of CYP2E1.[8]

Our current study shows that the homozygous deletion of *GSTM1* needs to be explored further to predict the DILI in the Indonesian population. This result is supported by some previous studies, such as in children in the Chinese Han population; the *GSTT1* and *GSTM1* did not correlate with DILI and the age was more associated with DILI.[27] According to a meta-analysis, the East Asian, including Chinese population with the null genotypes of *GSTM1* experienced an increase of DILI risk. However, the null genotypes of *GSTT1* did not have an association with the DILI in patients receiving isoniazid, rifampicin, pyrazinamide, and ethambutol.[10,28]

The *HLA* gene was supposed to have a correlation with the incidence of pulmonary TB due to the immunity mechanism. In some specific areas in India, some haplotypes of *HLA* were found in pulmonary TB patients.[9,29] Our study found that the G carriers of *HLA-DQA**0102 had more protection to the DILI, although the H-concentrations were not associated with the genotype. This finding is supported by a previous study in North Indian which stated that the absence of *HLA-DQA**0102 and the presence of *HLA-DQB1**0201 became the independent factor of ATDH.[9] To the best of the authors' knowledge, the significant difference of isoniazid plasma concentration among the genotype of *HLA-DQB**0302, although not accompanied by associations with DILI is a new finding in the Indonesian population.

The limited sample size and limited pharmacokinetic assessment by measuring only C2 concentrations are important limitations of our study. An increased sample size number in combination with a full pharmacokinetic curve allowing correlation with actual Cmax and AUC will likely increase the statistical power.

Conclusion

We conclude that in the Indonesian population, the *NAT2* genotype, but not the *CYP2E1*, *GSTM*, and *GSTT1* genotype, is significantly associated with DILI. Furthermore, the absence *of HLA-DQA*0102* could protect the patients from DILI without being associated with an effect on the H-concentration in contrast to the *NAT2* genotype. C carriership of HLA-*DQB*0302* is significantly associated with increased H-concentrations, but not with DILI.

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Conflicts of interest

There are no conflicts of interest.

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