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Review Article

Isoniazid-induced liver injury risk level in different variants of N-acetyltransferase 2 (NAT2) polymorphisms: A literature review

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Abstract

Individual NAT2 genotype identity data should be enriched to prevent Isoniazid-induced liver injury (IDILI) and optimize the dose of Isoniazid (INH). Therefore, this study aims to present the level of IDILI risk for specific genotype alleles. The data collection involves literature indexed by Google Scholar, Scopus, and Pubmed databases. The search uses a combination of the following keyword variants "INH" OR "INH", "liver injury" OR "hepatotoxicity", "polymorphism" OR "pharmacogenomic", and "N-acetyltransferase 2" OR "NAT2". Furthermore, the screening results of library sources were narrowed to 11 original articles that met the inclusion criteria. The IDILI risk assessment analysis due to NAT2 enzyme polymorphism following the odds ratio has a 95% confidence interval. The results showed that the IDILI risk level of the slow acetylator group was 3.11 times higher than other populations. Meanwhile, the rapid and intermediate acetylator groups were not at risk. Three variants related to *6 allele were classified as high risk; *6A/*6A risk 5.76 times, *6A/*7B (5.54 times), and *6/*7 (4 times). The three allele configurations of the *5 and *7 were also classified as a risk; *5B/*7B (5 times), *7B/*7B (3.23 times), and *5/*7 (2,74 times).

Keywords

Hepatotoxicity, Isoniazid dose adjustment, pharmacogenomic, polymorphism

Introduction

Isoniazid (INH) has been used as an anti-tuberculosis (TB) drug since 1952, and reports of INH-induced liver injury (IDILI) are ongoing and common (Erwin et al. 2019). Previously, different studies were conducted to identify IDILI susceptibility in certain populations, demographic characteristics, and its hepatoprotective ability as a follow-up solution (Zhang et al. 2020). These studies also involve the polymorphisms study of enzymes responsible for INH metabolism (Perwitasari et al. 2015; Erwin et al. 2019; Yang et al. 2019; Zhang et al. 2020).

INH metabolic activity related to IDILI are associated with polymorphisms of several genes including *N-acetyltransferase* II (*NAT2*), *Cytochrome P450 2E1* (*CYP2E1*), and *glutathione S transferases* (*GST1*) (Perwitasari et al. 2015; Erwin et al. 2019; Yang et al. 2019). Studies involving the *NAT2* genotype are very important compared to those involving enzymes triggering (Zhang et al. 2020). The single nucleotide polymorphism (SNP) in *NAT2*

affects the INH metabolism rate. In addition, it affects the variations in treatment efficacy and frequency of adverse reactions (Swaminathan and Ramachandran 2012). Populations with *NAT2* enzymes in the slow acetylator group were shown to be susceptible to IDILI exposure (Swaminathan and Ramachandran 2012; Perwitasari et al. 2015; Yang et al. 2019), and this was increased with the standard dose of INH (Shi et al. 2015).

Further studies on the genetic polymorphism of *NAT2* are expected to include different ethnic populations (Perwitasari et al. 2015). The literature enrichment guides clinicians in screening patients to predict and prevent the IDILI through optimal pharmacotherapy determination (Swaminathan and Ramachandran 2012; Wang et al. 2012; Perwitasari et al. 2015). Meanwhile, the screening for *NAT2* polymorphisms requires the identity of two haplotypes (diplotypes). This is because the identity of the individual *NAT2* genotype is very important in adjusting the INH dose (Wichukchinda et al. 2020).

Some closely related slow acetylator NAT2 alleles are identified as IDILI trigger genotypes and are related with **5*, *-*6*, and *-*7* (Cai et al. 2012; Lv et al. 2012). Previous studies have shown the increased risk of IDILI in slow *NAT2* acetylators due to the *CYP2E1**c1/c2 (Santoso et al. 2021) or its association with the GSTM1 null genotype (Cai et al. 2012).

The study priority for the causative factors of IDILI is based on the *NAT2* polymorphism, and the group with slow-acetylator was also susceptible to the exposure. Individual *NAT2* genotype identity data should be improved by clinicians to optimize INH dose. This study attempts to present the IDILI risk level for specific genotype alleles since there are limited publications that identify the related topic. Therefore, these results should be the basis for adjusting the INH dose in genotype variants susceptible to IDILI.

Methods

Literature search strategy

The study involves literature indexed by Google Scholar, Scopus, and Pubmed databases, and the search uses a combination of the following keyword variants; "isoniazid" OR "INH", "liver injury" OR "hepatotoxicity", "polymorphism" OR "pharmacogenomic", AND "N-*acetyltransferase 2*" OR "*NAT2*". This study only used *original articles* in English language which were peer-reviewed journals published between 2011 and 2020 (Fig. 1).

Literature eligibility and research data extraction

This study only involves results that meet all of the following criteria; (a) Study on subjects with tuberculosis without other comorbidities, (b) Study on subjects receiving INH as part of a standard regimen of 5 mg/Kg/day (maximum dose 300 mg/day), (c) Case study-control on subjects exposed to IDILI, (d) Study outcome involving the distribution of single nucleotide polymorphism allele identity and acetylator phenotype.

Each literature was extracted by identifying the name of the publication journal, the author's name, year, title, antituberculosis drugs combination, population settings, number of respondents, and study outcomes in the total number of cases and controls. It also includes the identity frequency of the single nucleotide allele polymorphism and acetylator phenotype.

Statistic analysis

The main analysis aims to measure the level of IDILI risk in each genotype. Meanwhile, the risk assessment due

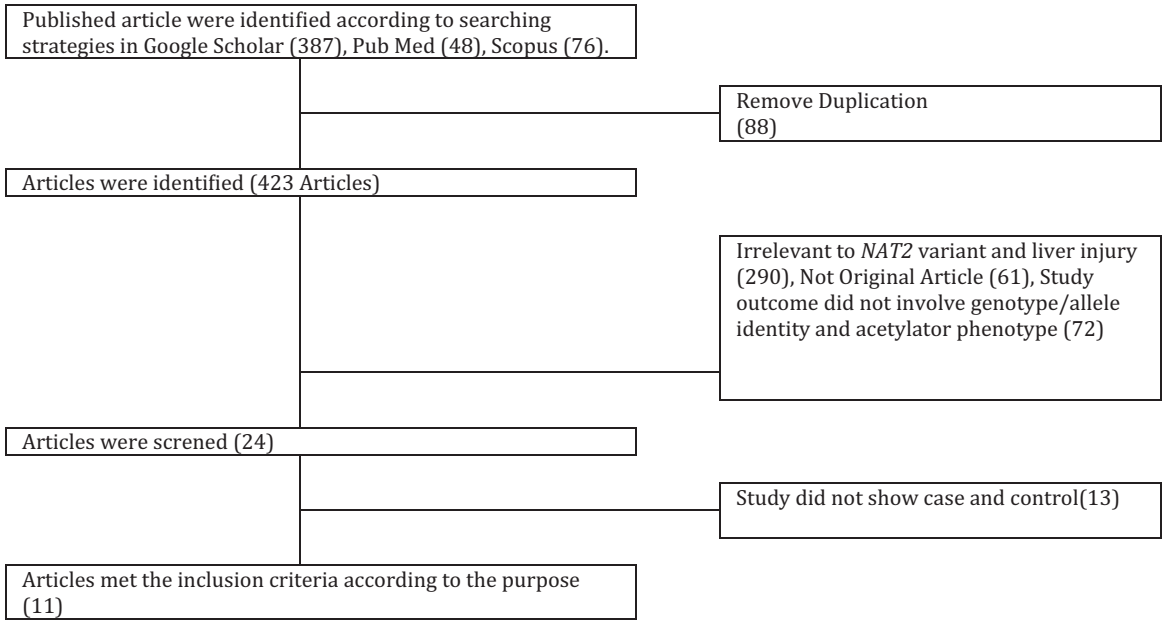

Figure 1. Literature study of workflow.

to *NAT2* polymorphism was based on the odds ratio of 95% confidence interval. The distribution of IDILI cases is compared against the controls, with individual case and control comparisons across all other genotypes combined for each allele. Furthermore, the analysis uses Stata MP software edition 14.

Results and discussion

Characteristics of research subjects

The literature search strategy obtained 423 articles, and a total of 11 articles met the inclusion criteria (Fig. 1). A series of studies conducted across diverse regions, including Indonesia, Tunisia, Japan, Brazil, Spain, China, and India, aimed to unravel the intricate relationship between genetic factors and the risk of Isoniazid-Induced Liver Injury (IDILI), a concerning side effect of tuberculosis treatment. These investigations primarily focused on NAT2 gene variants and acetylator status, shedding light on their pivotal role in IDILI susceptibility. Notably, NAT2 slow acetylators consistently emerged as a high-risk group for IDILI, with some studies highlighting ultra-slow acetylators as even more susceptible, while fast and intermediate acetylators showed reduced risk. Furthermore, certain studies explored the influence of additional genetic factors, such as CYP2E1 and GST genes, in hepatotoxicity linked to isoniazid use. Collectively, these findings underscore the importance of genetic profiling in tailoring tuberculosis treatment regimens and enhancing patient safety (Table 1).

Research conducted in Indonesia found a significant association between NAT2 slow and ultra-slow acetylators, which increased the risk of IDILI, while fast and intermediate acetylators decreased the risk (Yuliwulandari et al. 2019). A similar study in Indonesia identified NAT2*5, *6, and *7 polymorphisms, revealing distinct genotypes and genetic susceptibility (Suhuyanly et al. 2017). Similarly, in Thailand, slow NAT2 acetylators were significantly associated with an increased risk of IDILI in tuberculosis patients. These findings underscore the crucial role of NAT2 gene variants and acetylator status in understanding and predicting IDILI risk across diverse populations (Wattanapokayakit et al. 2016).

Japan developed a predictive IDILI risk system, showing a significant association between NAT2 slow acetylators and increased risk. A logistic regression model with age and NAT2 genotype predicted effectively (Mushiroda et al. 2016). In Tunisia, high isoniazid serum levels, combined with NAT2/CYP2E1 gene polymorphisms, posed an IDILI risk (Ben Fredj et al. 2017). Slow acetylators in Tunisia had a higher hepatotoxicity risk, with specific NAT2 variants increasing the risk (Ben Mahmoud et al. 2012).

China identified NAT2 slow acetylator genotypes, like NAT26A/7B and NAT26A/6A, as hepatotoxicity risk factors, with combined NAT2/CYP2E1 genotypes amplifying this risk (An et al. 2012). In India, slow acetylators and specific NAT2 genotypes were linked to higher hepatotoxicity risk, with the NAT24 haplotype providing protection protection (Gupta et al. 2013). Additionally, India found specific NAT2 genotypes more prevalent among hepatotoxicity patients, along with lower C1/C1 allele occurrence in the CYP2E1 gene and higher GSTM1 levels (Rana et al. 2014).

Table 1. Summary of literature objectives and results.

No	Study	Setting	Objectives	Results
1.	(Yuliwulandari et al. 2019)	Indonesia	Investigating NAT2 variants and acetylator status in the severity of IDILI.	NAT2 slow acetylators had a significant association with IDILI risk. Ultra-slow acetylators had an even stronger association, while fast and intermediate acetylators were associated with decreased IDILI risk.
2.	(Suhuyanly et al. 2017)	Indonesia	Investigating NAT2 polymorphisms in IDILI	NAT2*5, *6, and *7 polymorphisms were associated with specific genotypes, providing insights into genetic susceptibility.
3.	(Ben Fredj et al. 2017)	Tunisia	Assessing the relationship between isoniazid serum concentration and the incidence of IDILI.	High serum concentration of isoniazid was a risk factor for IDILI. Combined NAT2/CYP2E1 gene polymorphisms increased the risk of IDILI
4.	(Mushiroda et al. 2016)	Japan	Developing a predictive system for IDILI risk associated with anti-tuberculosis agents.	NAT2 slow acetylators were significantly associated with IDILI risk. A logistic regression model using age and NAT2 genotype showed good predictive ability.
5.	(Santos et al. 2013)	Brazil	Investigating the role of NAT2 and CYP2E1 in hepatotoxicity..	Slow NAT2 acetylators, particularly allele *5, had a strong association with hepatotoxicity risk.
6.	(Leiro-Fernandez et al. 2011)	Spain	Analyzing NAT2 polymorphisms for their association with IDILI.	Slow NAT2 genotypes were more prevalent in cases, suggesting an increased risk of hepatotoxicity.
7.	(An et al. 2012)	China	Investigating NAT2 and CYP2E1 genetic polymorphisms in IDILI.	NAT2 slow acetylator genotypes, especially NAT26A/7B and NAT26A/6A, were hepatotoxicity risk factors. Combined NAT2/CYP2E1 genotypes increased risk.
8.	(Ben Mahmoud et al. 2012)	Tunisia	Evaluating NAT2 gene polymorphisms in IDILI.	Slow acetylators had a higher risk of hepatotoxicity. Specific NAT2 variant diplotypes were associated with increased risk.
9.	(Gupta et al. 2013)	India	Assessing NAT2 and CYP2E1 gene polymorphisms in IDILI.	Slow acetylators and specific NAT2 genotypes were associated with a higher risk of hepatotoxicity. NAT2*4 haplotype provided protection.
10.	(Rana et al. 2014)	India	Elucidating NAT2, CYP2E1, and GST gene polymorphisms in IDILI.	Specific NAT2 genotypes were significantly higher in hepatotoxicity patients. C1/C1 allele of CYP2E1 gene was lower in hepatotoxicity patients. GSTM1 was significantly higher in hepatotoxicity patients.
11.	(Wattanapokayakit et al. 2016)	Thailand	Investigating NAT2 genotype status in TB patients with IDILI	Slow NAT2 acetylators had a significant association with IDILI risk.

Spain observed a higher prevalence of slow NAT2 genotypes among hepatotoxicity cases (Leiro-Fernandez et al. 2011). In Brazil, slow NAT2 acetylators, especially allele 5, had a higher hepatotoxicity risk (Santos et al. 2013).

The study population came from 8 countries representing 6 regions of East Asia; Japan (17%) and China (10%), South Asia; India (24%), Southeast Asia; Indonesia (18%), and Thailand (6%), South America; Brazil (13%), Africa; Tunisia (6%), and Europe; Spain (5%). The 563 (26%) cases and 1577 (74%) controls were recapitulated, and based on the phenotype of the *NAT2* enzyme, the subjects included rapid, intermediate, and slow acetylators of 538 (25%), 917 (43%), and 685 (32%) respectively (Tables 2–4).

IDILI risk in the rapid acetylator of NAT2 enzyme group

Generally, the rapid acetylator *NAT2* group has OR (CI) value of 0.61 (0.48–0.77) with a significance of <0.0001, and the identification obtained one type of genotype with significant analysis results. Meanwhile, allele **4/*4* has OR (CI) value; 0.66 (0.52–0.85), and this result showed almost no risk of IDILI due to INH (Table 2).

IDILI risk in the intermediate acetylator of NAT2 enzyme group

The intermediate *NAT2* acetylator group has OR (CI) value of 0.48 (0.39–0.59) with a significance of <0.0001, and the identification obtained 2 types of genotypes with significant analysis. Each of the identification has OR (CI) values; **4/*5*; 0.31 (0.21–0.47), **4/*7B*; 0.64 (0.42–0.98), and these results indicate the absence of IDILI due to INH (Table 3).

IDILI risk in the slow acetylator of NAT2 enzyme group

The slow *NAT2* acetylator group has OR (CI) group of 3.11 (2.55–3.80) with a significance of <0.0001, and the identification obtained 6 types of genotypes with significant analysis results. Each of the group has OR (CI) values with the smallest to the largest including; **5/*7*: 2.74 (1.49–5.02), **7B/*7B*: 3.23 (1.17–8.96), **6/*7*: 4.00 (1.76–9.05), **5B/*7B*: 5.00 (2.09–11.99), **6A/*7B*: 5.54 (3.56–8.64), **6A/*6A*: 5.76 (3.71–8.95). Meanwhile, the IDILI risk due to INH ranged from 2.76 to 5.76 times that of other populations in selected genotypes (Table 4).

Significant frequency of cases and controls of six variants triggers IDILI

The *NAT2*6A/*6A* group had an IDILI risk of 5.76 times that of other populations, and are spread over five countries, with a total frequency of 4%. Furthermore, the doubled cases from controls occurred in the populations of China, Thailand, Indonesia, and Japan. The distribution of cases and controls in Tunisia was balanced, and the *NAT2*6A/*7B* group was exposed to 5.54 times. They were spread over four countries, with a total frequency of 4%. In addition, the ratio of the total cases in China and Thailand was higher than the controls. On the contrary, the case reports in Indonesia and Japan were lower than the controls even though the numbers were almost equal. The *NAT2*6/*7* group had an IDILI risk of 4 times that of the other population, and they were spread over five countries with a total frequency of 1%. More cases than controls were found in India, Indonesia, and Spain. Meanwhile, all populations in Tunisia and Brazil acted as the controls (Fig. 2).

Discussion

The results confirmed the IDILI risk level of the slow acetylator group to be 3.11 times higher than in other populations. These data complement previous publications, which suggested that the risk ranged from 3.18 to 4.7 times (Cho et al. 2007; Cai et al. 2012; Wang et al. 2012). Moreover, the 6 variants of the *NAT2* polymorphism that significantly trigger IDILI were highlighted. Three variants were related to *6 allele and were classified as high risk; **6A/*6A* risk 5.76 times, **6A/*7B* (5.54 times), and **6/*7* (4 times). Furthermore, three alleles configurations of the *5 and *7 were classified as a significant risk; **5B/*7B* (5 times), *7B/*7B (3.23 times), and **5/*7* (2.74 times) (Fig. 2).

Table 2. IDILI risk level in the *NAT2* phenotype of the rapid acetylator group.

Num	SNP	N (Case/Control)	OR	CI 95%	Z Statistic	Sig	References
1	*4/*4	97/377	0.6626	0.5173–0.8486	3.2600	0.0011**	(An et al. 2012; Ben Fredj et al. 2017; Ben Mahmoud et al. 2012; Gupta et al. 2013; Leiro-Fernandez et al. 2011; Mushiroda et al. 2016; Rana et al. 2014; Santos et al. 2013; Suhuyanly et al. 2017; Wattanapokayakit et al. 2016; Yuliwulandari et al. 2019)
2	*4/*11	0/1	0.9326	0.0379–22.9265	0.0430	0.9659	(Santos et al. 2013)
3	*4/*12	1/13	0.2141	0.0279–1.6402	1.4840	0.1379	(An et al. 2012; Ben Fredj et al. 2017; Santos et al. 2013)
4	*4/*12A	0/5	0.2528	0.0140–4.5802	0.9300	0.3522	(Yuliwulandari et al. 2019)
5	*4/*13	0/2	0.5592	0.0268–11.6658	0.3750	0.7076	(Santos et al. 2013; Wattanapokayakit et al. 2016)
6	*4/*13A	4/4	2.8140	0.7014–11.2895	1.4600	0.1444	(An et al. 2012; Yuliwulandari et al. 2019)
7	*11/*11	2/23	0.2409	0.0566–1.0250	1.9270	0.0540	(Santos et al. 2013)
8	*11/*12	0/1	0.9326	0.0379–22.9265	0.0430	0.9659	(Santos et al. 2013)
9	*12/*12	0/2	0.5592	0.0268–11.6658	0.3750	0.7076	(Santos et al. 2013)
10	*12/*13	0/3	0.3992	0.0206–7.7401	0.6070	0.5438	(Santos et al. 2013)
11	*12A/*13A	0/1	0.9326	0.0379–22.9265	0.0430	0.9659	(Yuliwulandari et al. 2019)
12	*13/*13	1/1	2.8043	0.1751–44.9106	0.7290	0.4662	(Santos et al. 2013; Wattanapokayakit et al. 2016)
Overall		**105/433**	**0.6057**	**0.4769–0.7694**	**4.1090**	**0.0001****	

Table 3. IDILI risk level in the *NAT2* phenotype of the intermediate acetylator group.

Num	SNP	N (Case/ Control)	OR	CI 95%	Z Statistic	Sig	References
1	*4/*5	27/218	0.3140	0.2079–0.4742	5.508	<0.0001**	(Leiro-Fernandez et al. 2011; Gupta et al. 2013; Santos et al. 2013; Rana et al. 2014; Ben Fredj et al. 2017; Suhuyanly et al. 2017)
2	*4/*5A	1/0	8.4133	0.3422–206.8371	1.304	0.1924	(Yuliwulandari et al. 2019)
3	*4/*5B	9/39	0.6407	0.3083–1.3311	1.193	0.2327	(An et al. 2012; Ben Mahmoud et al. 2012; Mushiroda et al. 2016; Wattanapokayakit et al. 2016; Yuliwulandari et al. 2019)
4	*4/*6	20/64	0.8707	0.5220–1.4523	0.530	0.5959	(Leiro-Fernandez et al. 2011; Gupta et al. 2013; Rana et al. 2014; Ben Fredj et al. 2017; Suhuyanly et al. 2017)
5	*4/*6A	69/191	1.0136	0.7555–1.3598	0.090	0.9284	(An et al. 2012; Ben Mahmoud et al. 2012; Mushiroda et al. 2016; Wattanapokayakit et al. 2016; Yuliwulandari et al. 2019)
6	*4/*6J	1/1	2.8043	0.1751–44.9106	0.729	0.4662	(An et al. 2012)
7	*4/*7	12/37	0.9065	0.4693–1.7509	0.292	0.7700	(Leiro-Fernandez et al. 2011; Santos et al. 2013; Rana et al. 2014; Suhuyanly et al. 2017)
8	*4/*7A	0/1	0.9326	0.0379–22.9265	0.043	0.9659	(Yuliwulandari et al. 2019)
9	*4/*7B	28/119	0.6412	0.4199–0.9793	2.057	0.0397*	(An et al. 2012; Ben Mahmoud et al. 2012; Mushiroda et al. 2016; Wattanapokayakit et al. 2016; Yuliwulandari et al. 2019)
10	*4/*10	0/2	0.5592	0.0268–11.6658	0.375	0.7076	(An et al. 2012)
11	*4/*19	0/1	0.9326	0.0379–22.9265	0.043	0.9659	(An et al. 2012)
12	*5/*11	2/26	0.2127	0.0503–0.8989	2.105	0.0353	(Santos et al. 2013)
13	*5/*12	0/22	0.0613	0.0037–1.0129	1.951	0.0511	(Santos et al. 2013; Ben Fredj et al. 2017)
14	*5/*13	0/3	0.3992	0.0206–7.7401	0.607	0.5438	(Santos et al. 2013)
15	*5B/*12A	0/2	0.5592	0.0268–11.6658	0.375	0.7076	(Yuliwulandari et al. 2019)
16	*6/*12	0/4	0.3103	0.0167–5.7722	0.785	0.4327	(Ben Fredj et al. 2017)
17	*6A/*12A	0/8	0.1638	0.0094–2.8433	1.242	0.2141	(Yuliwulandari et al. 2019)
18	*6A/*13	0/2	0.5592	0.0268–11.6658	0.375	0.7076	(Wattanapokayakit et al. 2016)
19	*6A/*13A	0/2	0.5592	0.0268–11.6658	0.375	0.7076	(Yuliwulandari et al. 2019)
20	*7B/*12A	0/4	0.3103	0.0167–5.7722	0.785	0.4327	(Yuliwulandari et al. 2019)
21	*13A/*7A	1/1	2.8043	0.1751–44.9106	0.729	0.4662	(An et al. 2012)
Overall		**170/747**	**0.4806**	**0.3914–0.5902**	**6.995**	**<0,0001****	

Figure 2. Frequency distribution of IDILI cases and controls of six *NAT2* variants in various countries. Description of the country code abbreviation as follows; Brazil (BR), China (CN), Japan (JP), India (IN), Indonesia (ID), Thailand (TH), Tunisia (TN), Spain (ES). The country code with the suffix IDILI indicates the case population in that country while without affix indicates control population (Leiro-Fernandez et al. 2011; An et al. 2012; Ben Mahmoud et al. 2012; Gupta et al. 2013; Santos et al. 2013; Rana et al. 2014; Mushiroda et al. 2016; Wattanapokayakit et al. 2016; Ben Fredj et al. 2017; Suhuyanly et al. 2017; Yuliwulandari et al. 2019).

Table 4. IDILI risk level in the *NAT2* phenotype of the slow acetylator group.

Num	SNP	N (Case/ Control)	OR	CI 95%	Z Statistic	Sig	References
1	*5/*5	28/97	0.7985	0.5184–1.2300	1.021	0.3073	(Leiro-Fernandez et al. 2011; Gupta et al. 2013; Santos et al. 2013; Rana et al. 2014; Ben Fredj et al. 2017; Suhuyanly et al. 2017)
2	*5B/*5B	9/16	1.5850	0.6964–3.6073	1.098	0.2724	(Ben Mahmoud et al. 2012; Mushiroda et al. 2016; Wattanapokayakit et al. 2016; Yuliwulandari et al. 2019)
3	*5/*6	40/86	1.3260	0.8995–1.9548	1.425	0.1542	(Leiro-Fernandez et al. 2011; Gupta et al. 2013; Santos et al. 2013; Rana et al. 2014; Ben Fredj et al. 2017; Suhuyanly et al. 2017)
4	*5B/*6A	14/33	1.1931	0.6337–2.2463	0.547	0.5844	(Ben Mahmoud et al. 2012; Mushiroda et al. 2016; Wattanapokayakit et al. 2016; Yuliwulandari et al. 2019)
5	*5/*7	21/22	2.7386	1.4941 – 5.0196	3.259	0.0011*	(Leiro-Fernandez et al. 2011; Santos et al. 2013; Rana et al. 2014; Ben Fredj et al. 2017; Suhuyanly et al. 2017)
6	*5B/*7B	14/8	5.0014	2.0868–11.9868	3.609	0.0003**	(An et al. 2012; Ben Mahmoud et al. 2012; Mushiroda et al. 2016; Wattanapokayakit et al. 2016; Yuliwulandari et al. 2019)
7	*6/*6	19/35	1.5388	0.8728 – 2.7128	1.490	0.1363	(Leiro-Fernandez et al. 2011; Gupta et al. 2013; Santos et al. 2013; Rana et al. 2014; Ben Fredj et al. 2017; Suhuyanly et al. 2017)
8	*6A/*6A	60/32	5.7592	3.7066 – 8.9484	7.787	<0.0001**	(An et al. 2012; Ben Mahmoud et al. 2012; Mushiroda et al. 2016; Wattanapokayakit et al. 2016; Yuliwulandari et al. 2019)
9	*6A/*19	0/1	0.9326	0.0379 – 22.9265	0.043	0.9659	(An et al. 2012)
10	*6/*7	14/10	3.9960	1.7647–9.0486	3.322	0.0009**	(Leiro-Fernandez et al. 2011; Santos et al. 2013; Rana et al. 2014; Ben Fredj et al. 2017; Suhuyanly et al. 2017)
11	*6A/*7B	58/32	5.5452	3.5601–8.6372	7.576	<0.0001**	(An et al. 2012; Mushiroda et al. 2016; Wattanapokayakit et al. 2016; Yuliwulandari et al. 2019)
12	*6J/*7B	1/0	8.4133	0.3422 – 206.8371	1.304	0.1924	(An et al. 2012)
13	*7/*7	1/6	0.4659	0.0560–3.8785	0.706	0.4799	(Rana et al. 2014; Suhuyanly et al. 2017)
14	*7/*11	0/6	0.2145	0.0121–3.8144	1.048	0.2945	(Santos et al. 2013)
15	*7/*12	0/2	0.5592	0.0268–11.6658	0.375	0.7076	(Santos et al. 2013)
16	*7A/*7B	0/4	0.3103	0.0167 – 5.7722	0.785	0.4327	(An et al. 2012)
17	*7B/*7B	8/7	3.2329	1.1669 – 8.9567	2.257	0.0240*	(An et al. 2012; Mushiroda et al. 2016; Wattanapokayakit et al. 2016; Yuliwulandari et al. 2019)
18	*7B/*13	1/0	8.4133	0.3422–206.8371	1.304	0.1924	(Wattanapokayakit et al. 2016)
Overall		**288/397**	**3.1128**	**2.5470–3.8043**	**11.095**	**<0.0001****	

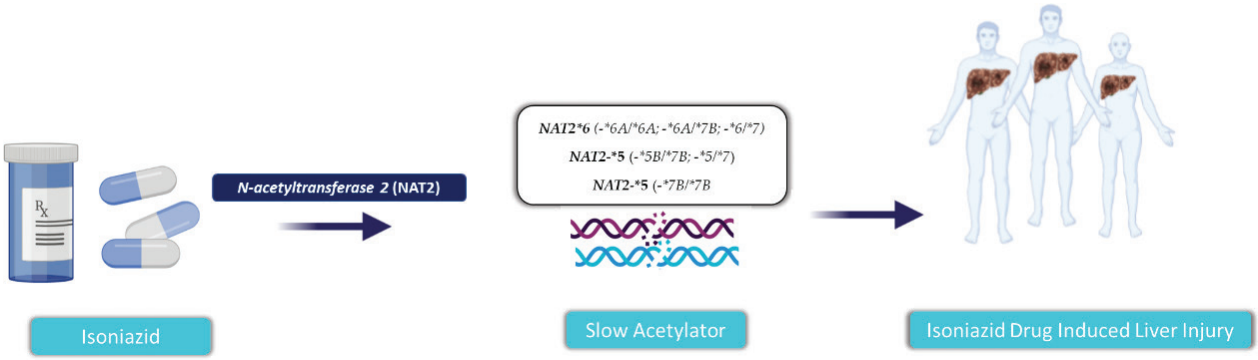

Figure 3. The role of slow acetylator of N-acetyltransferase 2 (NAT-2) polymorphism induce drug liver injury.

The six variants were the combinations of alleles **5*, **5B*, **6*, **6A*, **7*, and **7B* encoding a slow metabolic phenotype (Boukouvala et al. 2016; Igumnova et al. 2016; Mthiyane et al. 2020). The numbers of *NAT2*6A* and **7B* were spread across many Asian and Caucasian populations (Mthiyane et al. 2020). Meanwhile, the highest IDILI risk involved were found in the population having the **6* alleles, *NAT2*6A/*6A* risk 5.76 times, *-*6A/*7B* (5.54 times), *NAT2*6/*7* (4 times). This confirmed the predictions of other reports that the *NAT2*6* group is highly susceptible to IDILI. The combinations that form the **6/*6* genotype is an ultra-slow metabolic phenotype (Igumnova et al. 2016).

Previous reports suggested the IDILI risk for both Asian and non-Asian is evenly distributed across alleles and ethnic variants of the population (Wang et al. 2012). Moreover, other reports suggested that Asian races are more susceptible to IDILI cases, unlike Caucasians (Cho et al. 2007; Cai et al. 2012; Swaminathan and Ramachandran 2012). However, the development of pharmacogenomic research related to tuberculosis is more involved in the countries of Asia compared to others. Similarly, the cases predominance of this study involved reports from the Asian region (Leiro-Fernandez et al. 2011; An et al. 2012; Ben Mahmoud et al. 2012; Gupta et al. 2013; Santos et al. 2013; Rana et al. 2014; Mushiroda et al. 2016; Wattanapokayakit et al. 2016; Ben Fredj et al. 2017; Suhuyanly et al. 2017; Yuliwulandari et al. 2019), even though Tunisian population caught attention. Zero cases were recorded when various populations showed high cases of *NAT2*5/*7*, *-*6/*7*, and *-*5B/*7B* variants. In the ultra-slow *NAT2*6A/*6A* variant, the number of cases and controls were balanced. Further studies should be conducted to show that the incidence of IDILI in the slow acetylator variant is not greater than the control (Ben Mahmoud et al. 2012; Ben Fredj et al. 2017).

The identity variants of the *NAT2* genotype have consistently coded for the INH metabolic phenotype (Zabost et al. 2013; Seng et al. 2015; Huerta-García et al. 2020). This is because genotype identity is not a single trigger for IDILI. The risk also depends on the configuration of cooperation with other enzymes related to the metabolic activity of INH. Furthermore, it encodes the late phenotype, such as *CYP2E1*c1/c2*, and the null genotype *GSTM1* (Cai et al. 2012; Santoso et al. 2021) to consider the variance of the population weight factor (Huerta-García et al. 2020). For every 10 kg increase in body weight, the concentration of INH is reduced by 1 mg/L [12]–[15], [17](Jung et al. 2015). Therefore, these variants should be considered in the new era of INH dose personalization.

Several investigations consistently affirm the association between NAT2 slow acetylator status and an elevated susceptibility to (Leiro-Fernandez et al. 2011; Ben Mahmoud et al. 2012; Gupta et al. 2013; Mushiroda et al. 2016; Wattanapokayakit et al. 2016; Ben Fredj et al. 2017; Yuliwulandari et al. 2019). Consequently, when prescribing anti-tuberculosis medications, this genetic factor should be taken into consideration. Research also suggests that NAT2 genotyping has the potential to personalize drug dosing, particularly for isoniazid, based on genetic information, which could optimize treatment outcomes and reduce the risk of hepatotoxicity (Ben Mahmoud et al. 2012; Santos et al. 2013; Suhuyanly et al. 2017). Furthermore, combining NAT2 and CYP2E1 genotype analysis can provide valuable insights into the risk assessment of IILI. It has been observed that slow NAT2 acetylator status, especially when coupled with specific CYP2E1 genotypes, may heighten susceptibility to IILI (An et al. 2012; Ben Fredj et al. 2017). Therefore, monitoring isoniazid serum concentrations is vital, as elevated levels are associated with a greater risk of IDILI (An et al. 2012). Therapeutic drug monitoring (TDM) for isoniazid can help identify individuals at risk and facilitate necessary treatment adjustments.

Moreover, there is a call to develop predictive models that integrate both genetic and clinical risk factors to evaluate the risk of IDILI (Mushiroda et al. 2016). Prospective clinical trials are essential to validate the clinical utility of these models and guide INH dosage adjustments. Large-scale screening for NAT2 and CYP2E1 genotypes, as proposed by studies (Santos et al. 2013; Rana et al. 2014), can effectively predict adverse effects and enhance clinical management. Routine genotyping tests may be warranted for populations at risk of IILI. Recognizing population-specific genetic variations in NAT2 is crucial (Santos et al. 2013; Wattanapokayakit et al. 2016). Clinicians should consider the genetic diversity within their patient populations when making treatment decisions. Finally, we should highlight the feasibility and cost-effectiveness of genetic testing for preventing severe adverse drug reactions within healthcare systems (Wattanapokayakit et al. 2016).

Recently, some reports have followed up on the *NAT2* variant phenotype impact by offering a pharmacokinetic model, to establish a new era of INH dose personalization (Huerta-García et al. 2020; Mthiyane et al. 2020). The studies included diverse TB and healthy populations (Chen et al. 2009; Rodriguez et al. 2019; Huerta-García et al. 2020; Jing et al. 2020) co-infected with the human immunodeficiency virus (Bhatt et al. 2014) (Kubota et al. 2011; Seng et al. 2015). However, the development of a pharmacokinetic study involving the identity of the specific genotype is still limited.

The results showed that the genotypes *NAT2*5/*7*, *-*7B/*7B*, *-*6/*7*, *-*5B/*7B*, *-*6A/*7B*, and *-*6A/*6A*, have a risk range of IDILI 2.74 to 5.76 times. Meanwhile, the case finding is dominant in the Asian population, due to the pharmacogenomic study development related to tuberculosis involving more populations from Asian countries. Furthermore, personalization of INH dosage is a solution to reduce the IDILI rate. However, the pharmacokinetic development model should consider the synergistic configuration factor of slow acetylator *NAT2* with other enzymes related to the INH metabolic activity. These enzymes also encode the slow and null phenotype, such as *CYP2E1*c1/c2* and GSTM1 respectively. The six variants are expected to become priorities for future studies, given the limited database on the IDILI susceptible types.

Conclusions

Three variants related to the NAT2*6 allele were classified as high risk; -*6A/*6A risk 5.76 times, -*6A/*7B (5.54 times), and -*6/*7 (4 times). The three allele configurations of the -*5 and -*7 were also classified as a significant risk; -*5B/*7B (5 times), -*7B/*7B (3.23 times), and -*5/*7 (2.74 times). Based on these findings, incorporating NAT2 genotyping into anti-tuberculosis drug prescriptions, in conjunction with CYP2E1 genotype analysis and predictive models, offers promising prospects for personalized dosing, risk assessment, and treatment monitoring. This approach has the potential to enhance treatment efficacy and reduce the risk of liver toxicity in tuberculosis patients. Therefore, further pharmacokinetic studies to establish INH dosage adjustments for these six variants are warranted.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SBS, PP; data collection: SBS, PP; analysis and interpretation of results: SBS, PP, LMI; draft manuscript preparation: SBS, PP, LMI. All authors reviewed the results and approved the final version of the manuscript.

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