PHARMACOGENETICS OF TUBERCULOSIS

FARMACOGENÉTICA DA TUBERCULOSE

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ABSTRACT

Tuberculosis is a major cause of death worldwide and is especially prevalent in developing countries. It is known that the response to pharmacologic treatments can be influenced by several factors, including genetics, the focus of pharmacogenetics, and ethnicity. We conducted a review of the literature encompassing genes of pharmacogenetic interest to evaluate the data currently available in reference to treatment response alterations, allele frequencies, and interethnic differences. Through our statistical analyses, we were able to point out potential protective and risk variants for several ethnicities, especially for *NAT2* gene, indicating future paths to be pursued by further initiatives for a future of safer and more effective tuberculosis treatments.
KEYWORDS: Tuberculosis, Pharmacogenetics, Anti-TB drugs.

RESUMO

A tuberculose é uma das principais causas de morte no mundo e é particularmente prevalente em países em desenvolvimento. Sabe-se que a resposta a tratamentos farmacológicos pode ser influenciada por vários fatores, como genética, o foco da farmacogenética, e etnia. Conduzimos essa revisão de literatura englobando genes de interesse farmacogenético para avaliar os dados atualmente disponíveis no que diz respeito a alterações na resposta a tratamentos, frequências alélicas e diferenças interétnicas. Por meio de nossas análises estatísticas, conseguimos apontar variantes potencialmente protetoras e de risco para várias etnias, especialmente para o gene *NAT2*, indicando caminhos futuros a serem seguidos por iniciativas subsequentes de modo a buscar um futuro de tratamentos contra tuberculose mais seguros e eficazes.
PALAVRAS-CHAVE: Tuberculose, Farmacogenética, Drogas Anti-TB.

INTRODUCTION

Tuberculosis, a disease long known to men, with evidence indicating its presence in ancient civilizations such as Egypt more than 5000 years ago, still haunts humanity nowadays[1], especially in developing countries populations given that the World Health Organization (WHO) states that most patients with tuberculosis are from these nations[2].

According to Global Tuberculosis Report of 2022, provided by WHO, this disease was the leading cause of death from a single infectious agent until the COVID-19 pandemic, estimating that one quarter of the global population have already been infected[3]. Caused by *Mycobacterium tuberculosis* and transmitted almost exclusively by aerosol expelled from an infected individual, this malady causes necrotizing granulomatous inflammation, with most cases affecting the lungs but extrapulmonary sites can be found[4].

Although new drugs are being researched and introduced, such as bedaquiline and delamanid, tuberculosis treatment still consists mainly of four drugs: isoniazid (INH), rifampicin (RMP), pyrazinamide (PZA) and ethambutol (EMB)[5]. Anti-tuberculosis (anti-TB) treatment duration in general consists of the four aforementioned drugs for 2 months, followed by INH associated with RMP for additional 4 months, and because of its lengthiness, chances of hepatotoxicity and side effects causes significant low adherence of patients[6]. Pharmacogenetics (PGx) studies the correlation between drug response and the individual genetic information, aiming to maximize drug efficacy while minimizing the risk of adverse reactions, resulting in a personalized medicine raising the odds for a better individual outcome[7]. But, since the clinical implementation of PGx is still an ongoing process, especially in neglected diseases, more evidence is needed to support relevant information that will have its importance in the patient treatment[8]. Although individual genotyping is considered the ideal, since allele frequencies vary amongst different populations, an interesting approach for investigating the possible correlations between drug metabolism and genetic polymorphism is at an ethnicity level[9].

Clinical Pharmacogenetics Implementation Consortium[10] and The Dutch Pharmacogenetics Working Group[11] are international consortia of researchers whose goal is to guide the implementation of pharmacogenetics, and no guidelines referring to tuberculosis treatment are available until this moment. So, gathering information about PGx of tuberculosis treatment is a literature gap that needs to be filled.

The aim of this review is to gather and summarize information about the possible genes and their variants that have associations with the four main anti-TB drugs[12]. For a more meticulous approach, variants frequencies were also analyzed through an ethnical perspective.

METHODS

Firstly, PharmGKB database was searched regarding anti-tuberculosis drugs: isoniazid, rifampicin, pyrazinamide and ethambutol, retrieving candidate genes that could somehow interfere with its metabolism/transport/response, resulting in the following 14 genes: *NAT2*, *AGBL4*, *ABCB1*, *ABCC2*, *CUX2*, *GSTP1*, *NOS2*, *RIPOR2/FAM65B*, *SLCO1B1*, *TNF*, *XPO1*, *CYP2B6*, *CYP2C19* and *CYP2E1*. Afterwards, PubMed® database was searched using the terms: "<Gene name> + polymorphism + Tuberculosis". No filters were applied, and studies published until February of 2022 were considered.

Inclusion criteria were: (1) studies comprising only human subjects; (2) articles considering anti-tuberculosis drug response; (3) articles presenting the frequency of any variant of the selected genes. Exclusion criteria were: (1) studies comprising animal models; (2) literature reviews; (3) studies that did not meet the inclusion criteria.

Ethnical groups were divided into 9 large groups: Northern Africans, Sub-Saharan Africans, Europeans, East Asians, South Asians, Southeast Asians, Middle Easterns, Admixed and Non-informed. This division was made considering genetic differences amongst populations belonging to the same continent, such as Asia and Africa[13].

Allele frequencies were calculated by a weighted average, and a chi-squared ($\chi 2$) test with correction of Yates was applied to verify frequency differences between cases and controls. All statistic tests were performed in R® language[14] with in-house scripts. Variants nomenclatures were standardized according to PharmVar[15] and NCBI-dbSNP[16] to avoid ambiguity.

RESULTS AND DISCUSSIONS

During Pubmed search, 466 articles were found, and after all screenings, 83 articles were included for data collection, as Figure 1. Data from the final 83 studies are summarized in Table 1, regarding gene and number of individuals, grouped by ethnic group, resulting in a total of 23,291 individuals.

Figure 1. Review flow diagram - Identification of studies via databases and registers.

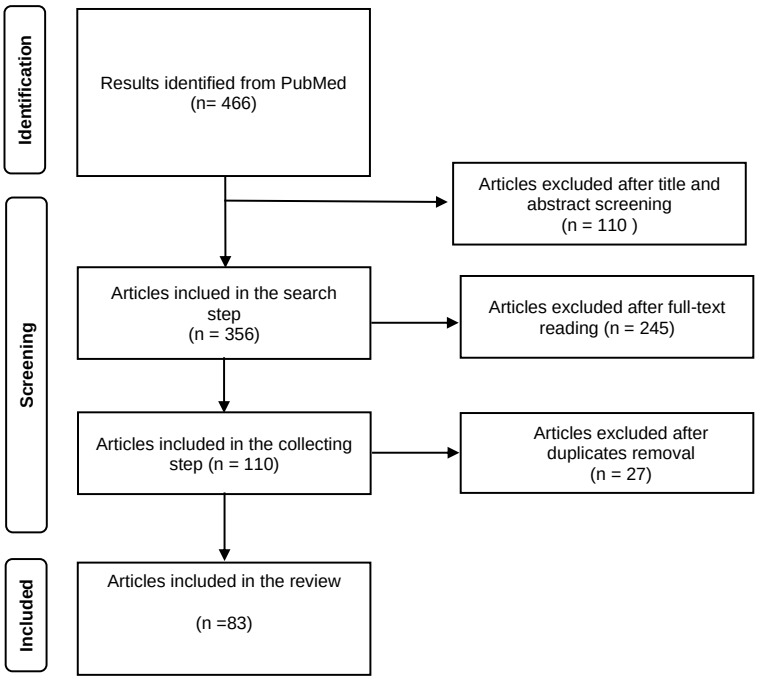
Identification
Screening
Included
Results identified from PubMed
(n= 466)
Articles excluded after title and abstract screening
(n = 110)
Articles inclued in the search step
(n = 356)
Articles excluded after full-text reading (n = 245)
Articles included in the collecting step (n = 110)
Articles excluded after duplicates removal
(n = 27)
Articles included in the review
(n =83)

Table 1: Number of individuals included in this review by gene and by ethnic group. Number of articles included by gene.

Ethnic group	***ABCB1***	***ABCC2***	***AGBL4***	***CUX2***	***CYP2B6***	***CYP2C19***	***CYP2E1***	***FAM65B***	***GSTP1***	***NAT2***	***NOS2***	***SLCO1B1***	***TNF***	***XPO1***
Admixed					220		247			1826				
Europeans										202				
East Asians	225	972	705	705	719	1037	3007	705	514	4955	1184	1786		942
South Asians							3101			2301				
Southeast Asians	50						566			1683				
Africans			402					402		761		265		
North Africans							71							
Middle Easterns									100					
Not Informed	317						1106		254	638			306	
TOTAL	592	972	1107	705	939	1037	8098	1107	768	12466	1184	2051	306	942
Number of articles	4	2	2	1	3	3	29	2	2	51	3	6	1	1

* Numbers of individuals may not correspond to the p-value tables because not all polymorphisms had sufficient information to perform chi-squared

A.F.= Allele frequency

Ethnic representation, in a pharmacogenetics context, has a considerable importance because the frequency of notable polymorphisms varies among populations[17]. Thus, to analyze and comprehend this subject is important to translate research findings into clinical practice. In a continental perspective, Asia was the most represented in number of individuals and encompassed all genes proposed. In contrast to the number of

studies regarding pharmacogenetics, Europe had a low number of individuals analyzed, only 202 which represents 0.87% overall, when a higher representation was to be expected[18]. However, those numbers are coherent with the tuberculosis incidence rate reported by the WHO in their "Global Tuberculosis Report 2022", indicating that Asia is a high incidence continent, while Europe rates are low.

Allele frequencies were statistically compared between cases and controls when a satisfactory number of individuals was presented, and the allele/genotype frequency values were available. As *RIPOR2/FAM65B, TNF, SLCO1B1, ABCB1, XPO1, CYP2E1, CYP2B6, AGBL4* and *CUX2* genes presented no significant differences, their results are available in Table S1.

The significant results along with the discussions of *ABCC2, CYP2C19/CYP2C9, GSTP1, NOS2* and *NAT2* genes are presented below:

ABCC2

ATP Binding Cassete Subfamily C Member 2, also known as *ABCC2, cMOAT* or *MRP2*, is a member of the subfamily of *ABCC* genes, alongside other 12 members, which encode an active cellular membrane transporter of the same name[19]. Due to its expression in the apical membrane of various polarized cells, such as hepatocytes, kidney proximal tubules cells and epithelium cells of the intestines, ABCC2 protein is responsible for controlling efflux of various substrates, especially conjugated endogenous substances and xenobiotics in the final phases of detoxification[20]. Mutations in the *ABCC2* gene can lead to a rare hereditary disease called Dubin-Johnson syndrome, which is expressed by a hindered capability to eliminate conjugated bilirubin[21]. Due to these characteristics, especially for being an efflux pump, added to the fact that anti tuberculosis drugs can lead to adverse side effects when accumulated in the organism, *ABCC2* might be a candidate for further investigation[22,23].

Due to high incidence rates of anti-TB induced hepatotoxicity, reaching numbers higher than 10%, Bai et al. investigated the possible correlation between *ABCC* genes and hepatotoxicity due to anti-TB treatment in the Western region of China[24]. 746 patients met the inclusion criteria, with 118 of them manifesting symptoms coherent with anti-TB induced hepatotoxicity. Thirty-nine single nucleotide variants were analyzed, distributed amongst the 13 *ABCC* genes. After Bonferroni correction, *ABCC2* rs3740065 was the only variant with a significant p-value of 0.049 (95% CI) and Odds Ratio of 0.6 for the minor allele (G), thus relating to a protective factor against anti-TB induced hepatotoxicity. Also, the GG and GA genotypes were related to a decreased risk of anti-

TB drug induced hepatotoxicity, in a dominant model (OR, 0.46; 95%CI, 0.31-0.69; p=0.005 after Bonferroni correction). The authors discussed their efforts in isolating genotyping from other variables, such as age, sex or linkage disequilibrium with other genes known to have impacts on anti-TB drugs efficiency.

CYP2C19/CYP2C9 haplotype

Cytochrome P450 (CYP) enzymes have many substrates, and regarding drug metabolism, they are related to oxidation and reduction reactions[25], aiming to detoxify the organism. CYP enzymes are codified by homonymous genes, which are known to be very polymorphic[15]. Among them, *CYP2C19* and *CYP2C9* are two of the most variable and studied genes, and their variants are associated with drug response of many classes of treatments[12].

A study conducted by Kim and collaborators in South Korea enrolled 221 patients diagnosed with pulmonary and/or pleuritis tuberculosis that underwent first line anti-TB drugs (isoniazid, rifampicin, ethambutol and pyrazinamide) from seven university hospitals, and analyzed the possible correlation between anti-TB drugs induced maculopapular eruption (MPE) and polymorphisms in genes of interest, including *CYP2C19* and *CYP2C9*[26]. *CYP2C9* -1565 C>T (p=0.022, OR=0.23) and *CYP2C19* W212X (*3) (p=0.042, OR=0.270) had a significant p-value at 95% level of confidence, and both showed a protective factor for the minor allele. As the authors presented the genotype frequencies together for both SNPs (e.g. GG and GA+AA), we were not able to recalculate allele frequency to insert on our table. Furthering their investigation, the authors took notice that the *CYP2C19* and *CYP2C9* are locally near in the chromosome 10q24 and there is a significant linkage disequilibrium between them, so a haplotype analysis was conducted, revealing that the haplotype (*CYP2C19* −1418 C>T_W212X CYP2C9 -1565 C>T_−1188 C > T - [h3-TATC]) was also protective for anti-TB drugs induced MPE.

GSTP1

Glutathione S-transferase (GST) genes have a major role in phase II drug metabolism, including in anti-tuberculosis drugs[27]. *GSTP1* polymorphism has been correlated with an 8-fold higher risk of liver disease[28]. Due to the hepatotoxicity aspect of anti-tuberculosis drugs, *GSTP1* polymorphisms may influence the side effects in those treatments.

Investigating this possibility, Wu and collaborators made, at first, a prospective study contemplating 287 Han Chinese TB patients, all had completed a 3-month first line

anti-TB treatment (a combination of isoniazid, rifampicin, pyrazinamide and ethambutol). Among those, 30 patients developed anti-TB drug-induced hepatotoxicity (ATDH)[27]. Genotyping of *GSTP1* was conducted considering two variants and, at first, a correlation was found for both of them: rs1695.A (OR=3.876, 95%CI: 1.258–11.91; p=0.018) and rs4147581 GG genotype (OR=2.578, 95% CI: 1.076–6.173; p=0.034) in comparison to CC or CG. But, after analyzing the linkage disequilibrium (LD) between the two variants, the authors concluded that the correlation of rs4147581 variant was likely due to the LD with rs1695. Aiming to verify those conclusions, Wu's group made an independent retrospective study of 115 cases and 116 controls, following the same inclusion and exclusion criteria for the prospective study and also analyzed *GSTP1* variants, reaching the conclusion, and further supporting their initials findings, that rs1695.A was associated with a higher risk of developing ATDH (OR=2.10, 95%CI: 1.17–3.76; p=0.012). In both prospective and retrospective studies, rs1695 AA genotype was also associated with a higher risk of ATDH (OR=3.68, 95%CI: 1.18–11.36, p=0.025) and (OR=2.00, 95%CI: 1.05–3.83; p=0.035) respectively, in comparison to GG or AG genotype. In the retrospective study, rs4147581 was not associated with a differential risk, supporting the hypothesis that the initial association was due to LD. It is important to notice that rs1695.A presents high frequency in the whole world, especially in Asians[16].

NOS2

INH is metabolized in the liver to hydrazine and acetyl isoniazid, which the latter can be further metabolized into acetyl hydrazine, accumulation of these compounds can lead to hepatotoxicity. Furthermore, this metabolism process, although necessary, creates reactive nitrogen species (RNS), that can be toxic to the cell. RNS can be produced by inducible nitric oxide synthase (iNOS), coded by the gene *NOS2*[29]. Polymorphism in *NOS2* can be correlated to different levels of RNS in hepatocytes.

Nanashima et al.[29] (2012) found an association between ATDH and *NOS2*-rs1180344 (p=0.04). However, their low number of patients may have influenced this result, because in this review, with a higher N, this significant p-value was lost. On the contrary, rs3794764 was not associated with ATDH in this previous study but increasing its N, we found an association between this SNP and ATDH (Table 2, p=0.038). Additionally, Li et al. (2018)[30] studied 157 children and found no association for *NOS2* variants. Using data from these studies, we could find some new associated loci from this gene. This demonstrates the importance of gathering data from different original studies

into a review, because we can increase the statistical power of the analyses and find new results.

Table 2: Polymorphisms with significant frequency differences between cases and controls in all genes except NAT2. Data collected from East Asian individuals.

Gene	Polymorphism	A.F. Cases	N Cases	A.F. Controls	N Controls	p-value	Reference
ABCC2	rs3740065.G	0.262	118	372	626	0.02960435	[24]
CYP2C19/CYP2C9	ht3*	0.034	59	0.187	155	0.00858617	[26]
GSTP1	rs1695.A	0.892	144	0.803	370	0.02857731	[27]
NOS2	rs3730013.T	0.476	41	0.289	116	0.03895312	[30]
	rs3794763	0.316884	677	0.265895	898	0.029658	[62]
	rs3794764.A	0.298261	520	0.218471	664	0.03814	[30], [62], [29]
	rs4795067.G	0.392	502	0.235	582	0.000000151	[30], [62]
	rs944725.T	0.341631	718	0.276814	1014	0.005271	[30], [62]

* ht3: CYP2C19-1418 C>T_ W212X_CYP2C9-1565 C>T-1188 C>T (TATC)
A.F.= Allele frequency

NAT2

N-acetyltransferase-2 (*NAT2)* encodes an enzyme of the same name, responsible for metabolizing lipophilic compounds making them more water soluble, easing its elimination. For example, isoniazid can be acetylated and further metabolized into diacetyl hydrazine, which is nontoxic, by NAT2. But in its absence or lower functionality, isoniazid can suffer hydrolysis and form various toxic compounds, such as isoniazid hydrazine, N-hydroxy Acetyl-hydrazine and acetyl diazine[31].

We included 51 articles investigating the association between *NAT2* polymorphisms and variability in the response to tuberculosis treatments. A total of 45 genetic polymorphisms were evaluated across 8 ethnicities. Based on our statistical analyses, we identified potentially protective and risk alleles regarding treatment response for different ethnic groups, considering the significant frequency differences ($p<0.05$) between cases and controls. We observed 12 potential protective alleles and 3 potential risk alleles for Southeast Asians, and respectively, 1 and 3 for East Asians, 2 and 1 for South Asians, 1 and 1 for the not informed ethnicity group, and 1 protective allele for Africans. Alleles and frequencies of cases and controls, sorted by ethnicity, are available in Table 3. No significant differences were observed for Europeans, Middle Easterns, and Admixed.

Individuals homozygous for the *4 allele are classified as fast acetylators, while heterozygotes with one slow allele are considered intermediate acetylators. Previous studies show that allele *4 carriers are less frequent in the group of patients who developed hepatotoxicity[32,33]. Significant differences of *4 in frequencies of cases and controls were observed in four out of the eight analyzed ethnicities, with higher frequencies found in controls: East Asian, Southeast Asian, South Asian, and African ($p=0.000001$, 0.00001, 0.00001 and 0.0348, respectively). This was the only significant finding for the African population.

Table 3: Polymorphisms with significant frequency differences between cases and controls in NAT2 gene, separated by ethnic group. Bold values indicate variants with higher frequency in control group (possibly protective).

Ethnic group	Polymorphism	A.F. Cases	N Cases	A.F. Controls	N Controls	p-value	Reference
Not Informed	Allele *6	0.253	99	0.1327	421	0.0081	[59]; [60]; [61]
	Allele *12	**0.142**	**99**	**0.331**	**421**	**<0.0001**	[59]; [60]; [61]
African	**Allele *4**	**0.09025**	**81**	**0.307**	**327**	**0.0347**	[35]; [58]
South Asian	Allele *7	0.3373	361	0.24	1103	0.000006	[54];[55]; [34]; [56]; [57]
	Allele *4	**0.3024**	**361**	**0.442**	**1103**	**0.000018**	[54];[55]; [34]; [56]; [57]
	rs1495741.G	**0.168**	**24**	**0.555**	**79**	**0.0017**	[39]
Southeast Asian	Allele *6A	0.4407	410	0.338	825	0.0004	[51]; [52]; [53]; [38]; [50]
	rs1799931.A	0.222	113	0.1765	338	0.00076	[51]; [52], [50]
	Allele *4	**0.3026**	**410**	**0.382**	**825**	**0.00001**	[51]; [52]; [53]; [38]; [50]
	rs10109286.G	**0.21**	**79**	**0.39**	**239**	**0.0073**	[38]
	rs4921911.G	**0.36**	**79**	**0.55**	**239**	**0.0078**	[38]
	rs12674710.A	**0.16**	**79**	**0.38**	**239**	**0.0006**	[38]
	rs7816847.A	**0.16**	**79**	**0.4**	**239**	**0.0002**	[38]
	rs10088333.A	**0.16**	**79**	**0.38**	**239**	**0.0006**	[38]
	rs10103029.G	**0.16**	**79**	**0.38**	**239**	**0.0006**	[38]
	rs4921913.G	**0.16**	**79**	**0.41**	**239**	**0.0001**	[38]
	rs4921914.G	**0.16**	**79**	**0.41**	**239**	**0.0001**	[38]
	rs1044183.C	**0.32**	**60**	**0.53**	**62**	**0.0261**	[50]
	rs4646267.G	**0.16**	**79**	**0.38**	**239**	**0.0006**	[38]
	rs4646246.G	**0.16**	**79**	**0.38**	**239**	**0.0006**	[38]
	rs1495741.A	**0.16**	**79**	**0.41**	**239**	**0.0001**	[38]
East Asian	Allele *7B	0.1907	409	0.1177	1367	0.0024	[42]; [43]; [32]; [44]; [45]; [46]; [47]
	Allele *6A	0.3073	409	0.2325	1367	0.00006	[42]; [43]; [32]; [44]; [45]; [46]; [47]
	rs1495741.A	0.4884	417	0.4210	664	0.0308	[48]; [49]
	Allele *4	**0.5106**	**409**	**0.6394**	**1367**	**<0.0001**	[42]; [43];[32]; [44]; [45]; [46]; [47]

A.F. = Allele frequency

The *6 (rs1799930) and *7 (rs1799931) alleles, when present in homozygosity, are associated with slow acetylation phenotypes; they were previously found at a higher frequency among individuals who developed hepatotoxicity in response to tuberculosis treatment[34]. Furthermore, heterozygous individuals carrying at least one of these alleles are classified as intermediate acetylators and may exhibit elevated levels of liver enzymes during treatment[35]. Significant differences in their frequencies were found between cases and controls in all 3 Asian populations we analyzed. In Brazilian populations, a study observed that patients diagnosed with HIV/AIDS and tuberculosis who carried the *6B

allele had a higher risk of developing hepatotoxicity in response to treatment[36]. These are SNPs that have been highlighted as important tagSNPs in pharmacogenetic testing for tuberculosis treatments in the literature[37,38].

It is worth noting that the analyzed SNPs were not only shown to be associated with the occurrence of adverse effects but were also sometimes related to the severity of these events. For instance, rs1495741 exhibited an increasing frequency in different degrees of severity of drug-induced liver injury. This association indicates that this SNP may play a role in the severity of adverse effects in patients undergoing tuberculosis treatment[39].

GWAS (genome-wide association studies) are valuable tools in PGx, allowing to identify genetic biomarkers that are relevant to some complex phenotypes, such as drug response, especially in neglected populations. However, it is important to notice that these studies are high-cost due to the need of high scale genotyping of the involved subjects[40]. According to the WHO[41], it is estimated that 130,000 new cases of tuberculosis were reported in Thailand in 2021. A GWAS in Thai individuals have shown some promising results concerning *NAT2*[38]. The authors have identified 9 *NAT2* SNPs associated with anti-TB drug liver injury (rs1495741, rs4646246, rs4646267, rs4921914, rs4921913, rs10103029, rs10088333, rs7816847, rs12674710; $p=5.0 \times 10^{-8}$). These results highlight the importance of *NAT2* polymorphisms and the risk of anti-TB drug liver injury.

Several limitations were identified in the conduct of this study. In some instances, there was a discrepancy in the number of cases and controls, which could potentially impact statistical analyses. Additionally, a few papers failed to differentiate between cases and controls when reporting polymorphism frequencies or reported combined frequencies of heterozygotes and homozygotes. Such cases were excluded from the statistical analyses. The use of multiple terms to refer to a single polymorphism or gene was also considered a limitation. Furthermore, it was observed that in certain studies, the ethnicity of the individuals was not disclosed; when ethnicity was provided, there were instances where polymorphism frequencies were not reported separately for each ethnic group, and thus were classified as "Not informed".

Moreover, a pattern of not infering star alleles from rs ID was noticeable. Some authors have also performed the inference using only one SNP, whereas others used two or more SNPs. For this reason, we have collected data comprising rs ID and star alleles, as reported in the article, so in this case an overlapping of individuals and genetic variants could have occurred.

CONCLUSIONS

Through the analyses performed here, we found significant differences in the frequencies of cases and controls regarding 5 genes: *NAT2*, mainly, *GSTP1*, *CYP2C19*/*CYP2C9*, *ABCC2*, and *NOS2*. As a result, we were able to pinpoint potential protective and risk variants that are specific to each ethnicity, which is of utmost importance if personalized treatments are to become accessible to all populations in the future. Furthermore, it is important to highlight the importance of systematic reviews in this context: in an effort to bring together the currently available pharmacogenetic data in reference to tuberculosis one can re-analyze such data, this time with a larger number of studied individuals and therefore greater statistical power, so as to obtain more statistically reliable results. Here we suggest further directions to be taken by future initiatives aiming to maximize the efficacy and minimize adverse effects of anti-tuberculosis treatments.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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