AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

CLINICAL THERAPEUTICS

Effect of Genetic Variation of *NAT2* on Isoniazid and *SLCO1B1* and *CES2* on Rifampin Pharmacokinetics in Ghanaian Children with Tuberculosis

Albert Dompreh,[a] Xiaoli Tang,[b] Jianlin Zhou,[c] Hongmei Yang,[d] Ariel Topletz,[e] Eugene Adu Ahwireng,[a] Sampson Antwi,[f,g] Antony Enimil,[f,g] Taimour Langaee,[h] Charles A. Peloquin,[h] Michael H. Court,[i] Awewura Kwara[j]

[a]Department of Microbiology, Komfo Anokye Teaching Hospital, Kumasi, Ghana

[b]Department of Medicine, Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA

[c]College of Life Sciences, Hunan Normal University, Changsha, China

[d]Department of Biostatistics and Computational Biology, University of Rochester School of Medicine and Dentistry, Rochester, New York, USA

[e]Department of Medicine, The Miriam Hospital, Providence, Rhode Island, USA

[f]Department of Child Health, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

[g]Directorate of Child Health, Komfo Anokye Teaching Hospital, Kumasi, Ghana

[h]College of Pharmacy, University of Florida, Gainesville, Florida, USA

[i]College of Veterinary Medicine, Washington State University, Pullman, Washington, USA

[j]College of Medicine and Emerging Pathogens Institute, University of Florida, Gainesville, Florida, USA

ABSTRACT Isoniazid and rifampin are essential components of first-line antituberculosis (anti-TB) therapy. Understanding the relationship between genetic factors and the pharmacokinetics of these drugs could be useful in optimizing treatment outcomes, but this is understudied in children. We investigated the relationship between N-acetyltransferase type 2 (*NAT2*) genotypes and isoniazid pharmacokinetics, as well as that between the solute carrier organic anion transporter family member 1B1 (encoded by *SLCO1B1*) and carboxylesterase 2 (*CES2*) single nucleotide polymorphisms (SNPs) and rifampin pharmacokinetics in Ghanaian children. Blood samples were collected at times 0, 1, 2, 4, and 8 h postdose in children with tuberculosis on standard first-line therapy for at least 4 weeks. Isoniazid and rifampin concentrations were determined by a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, and pharmacokinetic parameters were calculated using noncompartmental analysis. Genotyping of *NAT2*, *SLCO1B1*, and *CES2* SNPs were performed using validated TaqMan genotyping assays. The Kruskal-Wallis test was used to compare pharmacokinetic parameters among the three genotypic groups and was followed by the Wilcoxon rank sum test for pairwise group comparisons. Genotype status inferred by the *NAT2* 4-SNP and 7-SNP genotyping panels identified children with a slow acetylator phenotype but not the rapid genotype. For rifampin, only the rare *SLCO1B1*1b* homozygous variant was associated with rifampin pharmacokinetics. Our findings suggest that *NAT2* and *SCLCO1B1*1b* genotyping may have minimal clinical utility in dosing decisions at the population level in Ghanaian children, but it could be useful at the individual level or in populations that have a high frequency of implicated genotypes. Further studies in other populations are warranted.

KEYWORDS *NAT2* acetylator genotypes, *SLCO1B1* gene, *CES2* gene, single nucleotide polymorphisms, rifampin pharmacokinetics, isoniazid pharmacokinetics

Received 11 October 2017 **Returned for modification** 22 November 2017 **Accepted** 13 December 2017

Accepted manuscript posted online 20 December 2017

Citation Dompreh A, Tang X, Zhou J, Yang H, Topletz A, Adu Ahwireng E, Antwi S, Enimil A, Langaee T, Peloquin CA, Court MH, Kwara A. 2018. Effect of genetic variation of *NAT2* on isoniazid and *SLCO1B1* and *CES2* on rifampin pharmacokinetics in Ghanaian children with tuberculosis. Antimicrob Agents Chemother 62:e02099-17. https://doi.org/10.1128/AAC.02099-17.

Address correspondence to Awewura Kwara, awewura.kwara@medicine.ufl.edu.

Isoniazid and rifampin are essential components of the short-course chemotherapy regimen for first-line treatment of drug-susceptible tuberculosis (TB) (1). The standard first-line regimen consists of isoniazid, rifampin, pyrazinamide, and ethambutol for 2 months, followed by isoniazid and rifampin for an additional 4 months. Resistance to or intolerance of either rifampin or isoniazid extends the treatment duration from 6 months to at least 9 months. Isoniazid alone given for 9 months and rifampin alone for 4 months are also preferred regimens for the treatment of latent TB infection in both adults and children (2). It is expected that the pharmacokinetic profiles of these drugs, associated with efficacy and safety in adults, should apply to children as well (3, 4). Although not validated in randomized or controlled trials, early bactericidal activity (EBA) of isoniazid was related to dose or pharmacokinetics (PK) in adults (5–7). Similarly, the EBAs of rifampin, rifabutin, and rifapentine in adults appear to be associated with drug dose and/or pharmacokinetics (8, 9). Thus, the variability in the pharmacokinetics of isoniazid and/or rifampin due to fixed-weight-band dosing as used in children might influence the clinical outcomes of active or latent TB treatment.

Isoniazid is metabolized primarily by the genetically polymorphic N-acetyltransferase type 2 (NAT2) enzyme (10). In adults, the trimodal distribution of isoniazid elimination and area under the concentration-time curve (AUC) is explained by a number of polymorphisms in the *NAT2* gene, which defines the acetylator phenotype (rapid, intermediate, or slow) (11, 12). In a study among South African children who were treated according to previous anti-TB drug dosing guidelines, a trimodal distribution of isoniazid AUC and 2- to 5-hour postdose concentrations was observed (13). The above-mentioned study also found that younger children eliminate isoniazid faster than older children, and as a group, children eliminate isoniazid faster than adults across all acetylator genotype groups (13). Children with rapid and intermediate acetylator genotypes were shown to be at risk of suboptimal isoniazid concentrations when given weight-based dosage similar to that of adults, prompting the investigators to recommend a higher daily dose in milligrams per kilogram of body weight for children (13, 14). In 2010, the World Health Organization (WHO) recommended a higher dosage in milligrams per kilogram of the antituberculosis drugs for all children (15), but it is not yet known if the increased dosage of isoniazid in children resolves the variability in isoniazid pharmacokinetics due to the *NAT2* acetylator genotype.

Rifampin undergoes extensive hepatic deacetylation by β-esterase to form the 25-deacetylated metabolite (16). The organic anion transporter polypeptide encoded by the solute carrier organic anion transporter family member 1B1 gene (*SLCO1B1*) mediates the hepatic uptake and elimination of a range of drugs (17). While some studies in adults found a significant relationship between *SLCO1B1* single nucleotide polymorphisms (SNPs) c.463C>A (rs11045819) and *SLCO1B1* rs4149032 (intron 2 haplotype tagging SNP; tSNP) and low rifampin plasma exposure (18, 19), others failed to replicate these associations (20, 21). The human carboxylesterase (CES) belongs to the β-esterase family, members of which are thought to be also involved in the metabolism of rifampin. Given the structural similarity between rifampin and substrates of carboxylesterase 2 (CES2), one study explored and demonstrated a relationship between *CES2* polymorphisms and rifampin metabolism (22). In particular, the *CES2* c.-2263A>G in the promoter region that is closely linked to c.269-965AG and c.1612 + 13GA was associated with rifampin metabolism through expression of the gene (22). To the best of our knowledge, the pharmacogenetic determinants of rifampin pharmacokinetics have not previously been studied in children.

The aim of this study was to examine the relationship between *NAT2* acetylator genotypes and isoniazid pharmacokinetics as well as *SLCO1B1* and *CES2* SNPs and rifampin pharmacokinetics in Ghanaian children with TB who were predominantly given the World Health Organization (WHO)-recommended revised anti-TB drug dosages for children.

TABLE 1 Pharmacokinetic parameters of isoniazid by *NAT2* acetylator genotype status in 113 Ghanaian children with TB[a]

Genotype (%)	T_{max} (h)	C_{max} (μg/ml)	AUC_{0-8h} (μg · h/ml)	CL/F (liters/h)	*V*/F (liters)
Rapid (10.6)	1.00 (0.99–1.10)	5.32 (4.14–6.83)	14.62 (10.05–18.27)	12.28 (7.26–17.02)	34.11 (17.07–53.45)
Intermediate (44.3)	1.05 (1.00–1.17)	5.27 (3.84–6.70)	16.01 (9.41–21.23)	9.79 (5.77–13.83)	21.75 (16.04–32.03)
Slow (45.1)	1.05 (1.00–1.32)	6.13 (4.77–8.40)	23.59 (19.73–32.11)	4.78 (3.50–6.75)	19.72 (14.44–24.90)
P value	0.443	0.156	<0.001	<0.001	0.121

[a]PK parameter values are medians (IQR). T_{max}, time to maximum concentration; C_{max}, maximum concentration; AUC_{0-8h}, area under the time-concentration curve from time 0 to 8 h postdose; CL/F, apparent oral clearance; *V*/F, apparent predicted volume of distribution.

RESULTS

Study population. Of the 113 study participants, 59 (52.2%) were HIV coinfected, 63 (55.8%) were male, and 24 (21.2%) were aged <2 years old as we previously reported (23). None of the HIV-infected patients were receiving antiretroviral therapy at the time of pharmacokinetic sampling. The median (interquartile range [IQR]) isoniazid dose was 11.2 (9.1 to 12.8 mg/kg of body weight) and that for rifampin was 15.8 (13.6 to 18.8 mg/kg).

The coefficient of variation of isoniazid maximum concentration in serum (C_{max}) and the area under the time-concentration curve from 0 to 8 h (AUC_{0-8h}) were 43.7% and 49.1%, respectively. There was a significant difference in median values of isoniazid AUC_{0-8h} and the predicted apparent oral clearance (CL/F) but no difference in time to C_{max} (T_{max}), C_{max}, or predicted apparent volume of distribution (*V*/F) among the three *NAT2* acetylator phenotype groups (Table 1). As shown in Fig. 1, there was a consid-

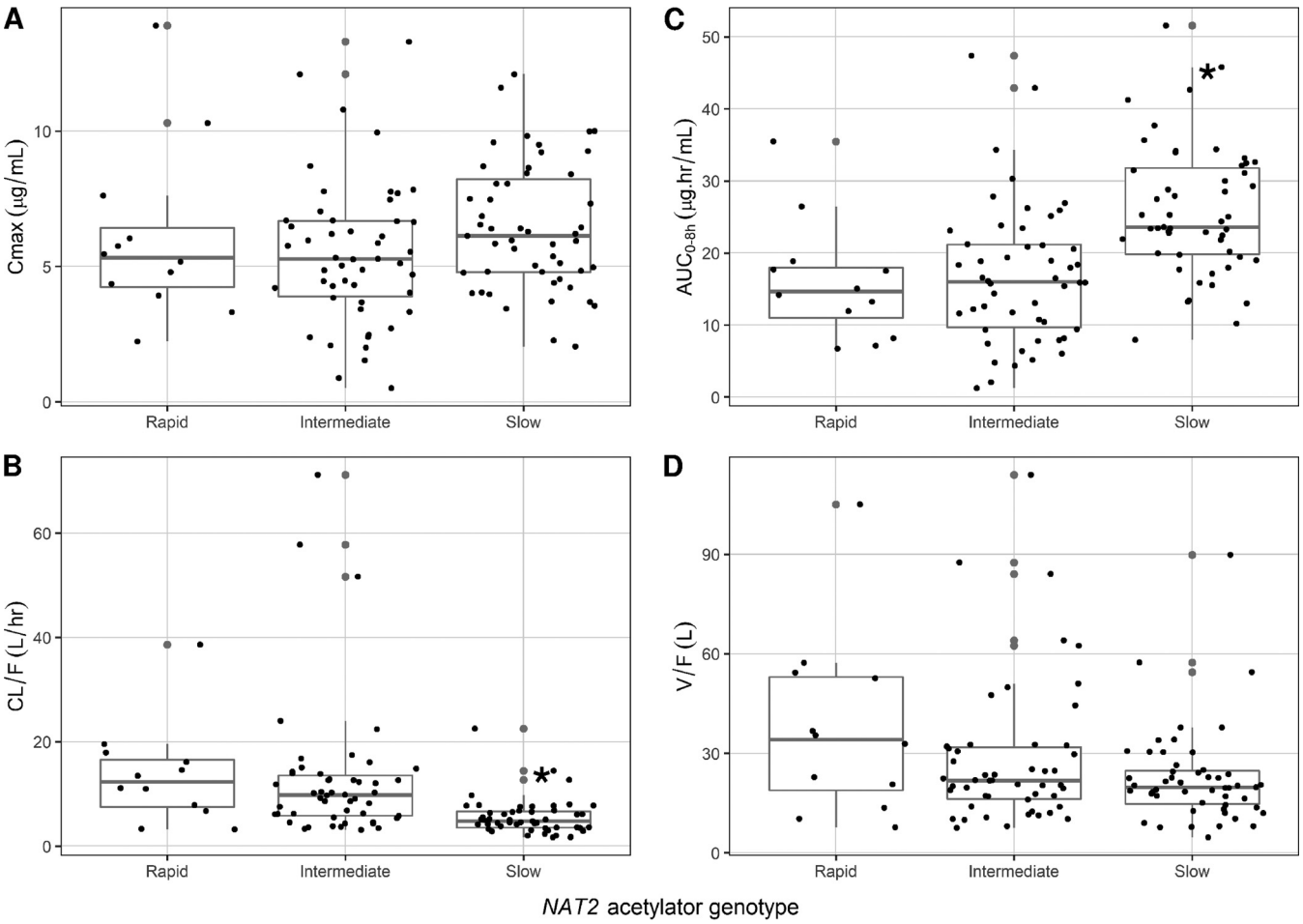

FIG 1 Relationship between isoniazid C_{max} (A), AUC_{0-8h} (B), CL/F (C), and *V*/F (D) and *NAT2* acetylator genotypes in children with tuberculosis. *, significant differences in AUC_{0-8h} and CL/F between slow and rapid or intermediate groups.

TABLE 2 Multivariate analysis showing coefficient estimates factors that were jointly associated with isoniazid and rifampin pharmacokinetic parameters[a]

Drug	PK parameter	Predictor	Estimate	SE	Standardized estimate	*P* value
Isoniazid	C_{max}	Dose	0.312	0.078	0.362	<0.001
		Male versus female	−0.750	0.476	−0.144	0.118
		Slow versus nonslow *NAT2* genotype	1.058	0.463	0.203	0.024
	AUC_{0-8h}	Age	0.393	0.226	0.147	0.086
		Dose	1.353	0.287	0.400	<0.001
		Slow versus nonslow *NAT2* genotype	9.642	1.602	0.472	<0.001
	CL/F	Age	0.593	0.234	0.222	0.013
		Male versus female	3.118	1.800	0.152	0.086
		Slow versus nonslow *NAT2* genotype	−7.668	1.770	−0.375	<0.001
	V/F	Age	2.313	0.477	0.447	<0.001
		Dose	1.369	0.624	0.209	0.030
		BMI	1.325	0.787	0.148	0.095
		Slow versus nonslow *NAT2* genotype	−6.977	3.377	−0.176	0.041
Rifampin	C_{max}	Age	0.255	0.079	0.298	0.002
		Dose	0.329	0.081	0.379	<0.001
		Male versus female	−0.994	0.585	−0.151	0.092
		TB/HIV coinfection versus TB	−1.132	0.566	−0.173	0.048
	AUC_{0-8h}	Age	1.201	0.330	0.326	<0.001
		Dose	1.495	0.338	0.401	<0.001
		Male versus female	−5.649	2.433	−0.200	0.022
		TB/HIV coinfection	−6.016	2.351	−0.214	0.012
	CL/F	BMI	0.639	0.393	0.151	0.106
		SLCO1B1 c.388GG versus AG/AA	−3.430	1.850	−0.172	0.066
	V/F	Age	1.771	0.756	0.214	0.021
		SLCO1B1 c.388GG versus AG/AA	−12.746	6.178	−0.189	0.041

[a]C_{max}, maximum concentration; AUC_{0-8h}, area under the time concentration curve from time 0 to 8 h postdose; CL/F, apparent oral clearance; *V*/F, apparent predicted volume of distribution.

erable overlap of isoniazid pharmacokinetic parameters among the three groups, and the differences in median AUC_{0-8h} and CL/F values were significant between slow and rapid as well as slow and intermediate acetylator phenotype groups. There was no significant difference in isoniazid pharmacokinetics in children with a rapid genotype compared to those with an intermediate genotype. Of the 113 study participants, 12 (11%) had an isoniazid C_{max} of <3 μg/ml (low C_{max}) and 49 (43%) had a C_{max} of >6 μg/ml (high) compared to the typical 2-h postdose sample concentration range. Of the 12 participants with low C_{max}, only 1 (0.8%) had a rapid and 2 (16.7%) had a slow *NAT2* genotype. Of the 49 with high isoniazid, only 26 (53%) had a slow *NAT2* genotype. Multivariate analysis suggested that isoniazid dose and slow *NAT2* genotype were jointly associated with isoniazid C_{max} and AUC_{0-8h}, age and slow *NAT2* genotype were associated with CL/F, while isoniazid dose, age, and slow *NAT2* genotype were jointly associated with *V*/F (Table 2).

The coefficient of variation of rifampin C_{max} and AUC_{0-8h} were 48.0% and 48.9%, respectively. None of the evaluated *SLCO1B1* and *CES2* SNPs were significantly associated with rifampin pharmacokinetics in the primary analysis (Table 3). The genetic variation in *SLCO1B1* c.388A>G (**1b*) SNP showed a trend toward a significant relationship with rifampin PK parameters (Table 3). The two patients with the **1b* homozygous variant (AA genotype) had significantly lower rifampin C_{max} and AUC_{0-8h} and higher CL/F and *V*/F than did those with the wild type (GG genotype) in pairwise analysis (Fig. 2). Also, the one participant with *SLCO1B1* c.463AA had much lower rifampin C_{max} and AUC and higher CL/F and *V*/F than did participants with *SLCO1B1* c.463 CC or CA (Table 3), but pairwise comparison was not done because there was just one participant with the homozygous variant (Fig. 3). Of the 113 participants, 73 (65%) had rifampin C_{max} of <8 μg/ml (low C_{max}), but we found no association between any of the studied SNPs and risk of low C_{max}. In a multivariate model, none of the studied SNPs were associated with rifampin C_{max}, AUC_{0-8h}, and CL/F (Table 2). The *SLCO1B1*

TABLE 3 Rifampin pharmacokinetic parameter by *SLCO1B1* and *CES2* single nucleotide polymorphism status in 113 Ghanaian children with TB[a]

Genetic factor (%)	C_{max} (μg/ml)	AUC_{0-8h} (μg · h/ml)	CL/F (liters/h)	*V*/F (liters)
*SLCO1B1*c.388A>G (**1b*)				
AA (1.8)	1.81 (0.81–2.80)	9.33 (2.35–16.31)	44.54 (15.38–73.69)	109.23 (54.86–163.59)
AG (30.1)	5.86 (4.71–8.72)	26.84 (20.90–34.71)	7.38 (5.22–10.37)	20.73 (15.64–32.76)
GG (68.1)	7.11 (5.08–8.79)	29.50 (20.79–38.71)	7.43 (4.86–10.06)	21.98 (14.20–30.12)
P value	0.052	0.085	0.093	0.067
*SLCO1B1*c.388A>G (**1b*)				
AA/AG (31.9)	5.84 (4.08–8.34)	26.35 (16.67–34.27)	7.58 (5.26–12.73)	22.07 (15.95–40.54)
GG (68.1)	7.11 (5.08–8.79)	29.50 (20.79–38.71)	7.43 (4.86–10.06)	21.98 (14.20–30.12)
P value	0.147	0.181	0.583	0.265
SLCO1B1 rs4149032 (intron SNP)				
CC (57.5)	7.00 (5.10–8.77)	29.50 (21.23–36.58)	7.34 (4.99–9.79)	21.20 (13.46–31.00)
CT (31.9)	6.55 (4.59–8.81)	26.35 (16.74–37.82)	7.58 (4.62–11.56)	21.80 (16.64–30.48)
TT (10.6)	5.43 (3.25–6.90)	24.07 (13.58–29.58)	8.37 (5.88–14.74)	23.59 (13.02–49.79)
P value	0.228	0.262	0.462	0.657
SLCO1B1 c.463C>A (**4*)				
CC (84.1)	6.50 (4.94–8.79)	27.25 (20.37–37.46)	7.52 (4.63–10.67)	22.13 (14.72–32.10)
CA (15.0)	5.70 (4.28–7.96)	27.67 (23.05–30.06)	7.18 (5.78–9.10)	17.72 (13.16–28.79)
AA (0.09)	1.72 (1.72, 1.72)	9.35 (9.35, 9.35)	14.81 (14.81, 14.81)	63.01 (63.01, 63.01)
P value	0.262	0.304	0.422	0.248
SLCO1B1 c.463C>A (**4*)				
CC (84.1)	6.50 (4.94–8.79)	27.25 (20.37–37.46)	7.52 (4.63–10.67)	22.13 (14.72–32.10)
CA/AA (15.9)	5.68 (4.02–7.96)	27.30 (21.11–30.06)	7.26 (5.78–9.79)	18.46 (13.16–28.95)
P value	0.417	0.621	0.919	0.701
SLCO1B1 c.521T>C (**5*)				
TT (97.3)	6.32 (4.92–8.79)	27.08 (20.62–36.58)	7.49 (4.86–10.37)	21.59 (14.72–31.00)
TC (2.7)	8.05 (3.23–8.68)	29.83 (16.44–35.30)	5.10 (4.99–12.50)	49.09 (7.82–58.79)
P value	0.986	0.915	0.761	0.464
CES2 c.–2263A>G				
AA (49.6)	7.17 (4.94–8.81)	29.00 (20.95–38.31)	7.41 (5.04–9.69)	19.89 (14.02–30.56)
AG (41.6)	5.85 (3.69–8.17)	24.48 (16.31–34.71)	7.71 (5.57–12.50)	23.36 (15.64–36.76)
GG (8.8)	6.90 (5.90–9.24)	30.62 (25.12–43.45)	4.48 (2.25–9.80)	21.80 (10.17–27.42)
P value	0.134	0.179	0.207	0.487

[a]PK parameter values are medians (IQR). T_{max}, time to maximum concentration; C_{max}, maximum concentration; AUC_{0-8h}, area under the time-concentration curve from time 0 to 8 h postdose; CL/F, apparent oral clearance; *V*/F, apparent predicted volume of distribution.

c.388GG compared to AA/AG and age were jointly associated with rifampin *V*/F (Table 2).

Overall, 99 (87.6%) subjects completed therapy, 12 (10.6%) were lost to follow-up, and 2 (1.8%) died. No study participants discontinued therapy or required modification of therapy because of medication side effects. Of 74 children who had liver enzyme test results available at baseline and at week 4 of therapy, there were no significant differences in the median changes in aspartate transferase (AST) or alanine transferase (ALT) levels at week 4 from baseline by *NAT2* acetylator genotype status ($P > 0.05$).

DISCUSSION

In this study, the 4-SNP or 7-SNP *NAT2* acetylator genotype identified slow compared to rapid or intermediate metabolizers of isoniazid among children treated according to the revised WHO dosing guidelines. However, we found no difference in isoniazid pharmacokinetics in children with a rapid genotype compared to those with an intermediate genotype. For rifampin, we found no significant relationship between pharmacokinetic parameters and *SLCO1B1* and *CES2* SNPs, except that the rare *SLCO1B1*1b* homozygous AA variant (found in only 2% of participants) was associated with low rifampin concentrations. Overall, the modest effect of *NAT2* genotypes to fully discriminate between children with low, intermediate, and high isoniazid plasma

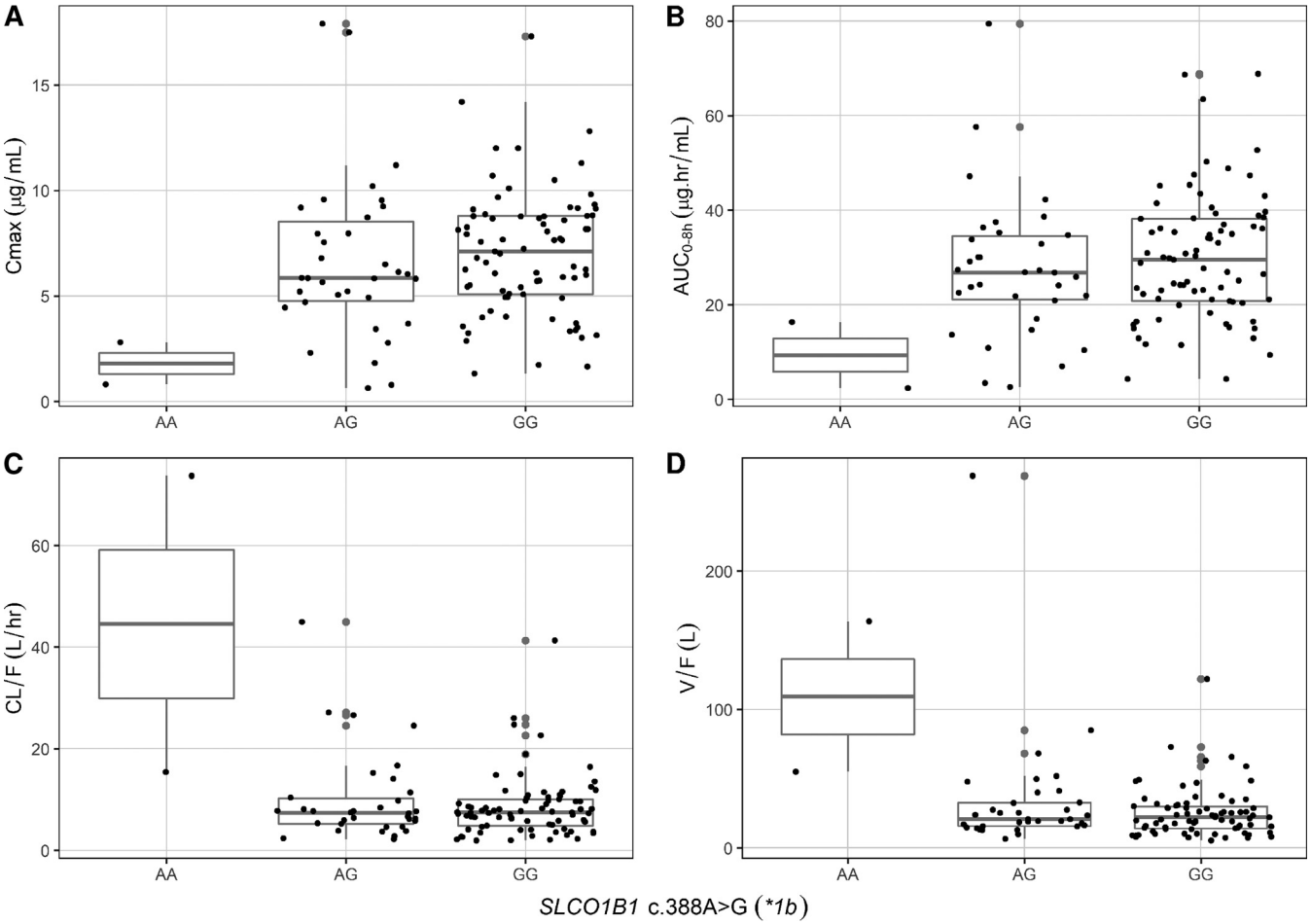

FIG 2 Relationship between rifampin C_{max} (A), AUC_{0-8h} (B), CL/F (C), and *V*/F (D) and *SLCO1B1* c.388A>G genotypes in children with tuberculosis. The differences in median values of all pharmacokinetic parameters between genotypes AA and GG were significant in *post hoc* analysis.

exposure without significant overlap and the rare occurrence of the *SLCO1B1* c.388AA genotype suggest that genotyping for the studied SNPs in Ghanaian children may have minimal clinical utility in making isoniazid and rifampin dosing decisions at the population level.

Inferring the genotype status by the 4-SNP *NAT2* genotyping panel is considered more economical than using the recommended 7-SNP panel. The experimentally determined *NAT2* 4-SNP acetylator genotype inferred *NAT2* acetylator phenotype status with 98.4% accuracy (24). The 4-SNP *NAT2* genotyping panel also had a 100% agreement with acetylator phenotypes inferred by the recommended 7-SNP panel in a diverse population of non-European ancestry (25). In the current study, we found 100% agreement between acetylator status inferred by the 4-SNP and 7-SNP genotyping panels. However, *NAT2* genotypes failed to clearly discriminate between isoniazid phenotype groups, as we observed a considerable overlap of isoniazid pharmacokinetic parameters across the three groups (Fig. 1). Unlike the trimodal distribution of isoniazid pharmacokinetics reported in some studies in adults (11, 12), the bimodal distribution observed in our study is consistent with other studies in children, in which the pharmacokinetic parameters in those rapid and intermediate genotypes were similar (13, 26, 27). The bimodal distribution of NAT2 acetylator phenotype in children may be explained by differences in enzyme maturation with age. NAT2 enzyme maturation based on weight-normalized CL/F was demonstrated to increase with age in children up to 2 years old for rapid and intermediate acetylators, but no significant change was observed in slow acetylators (28).

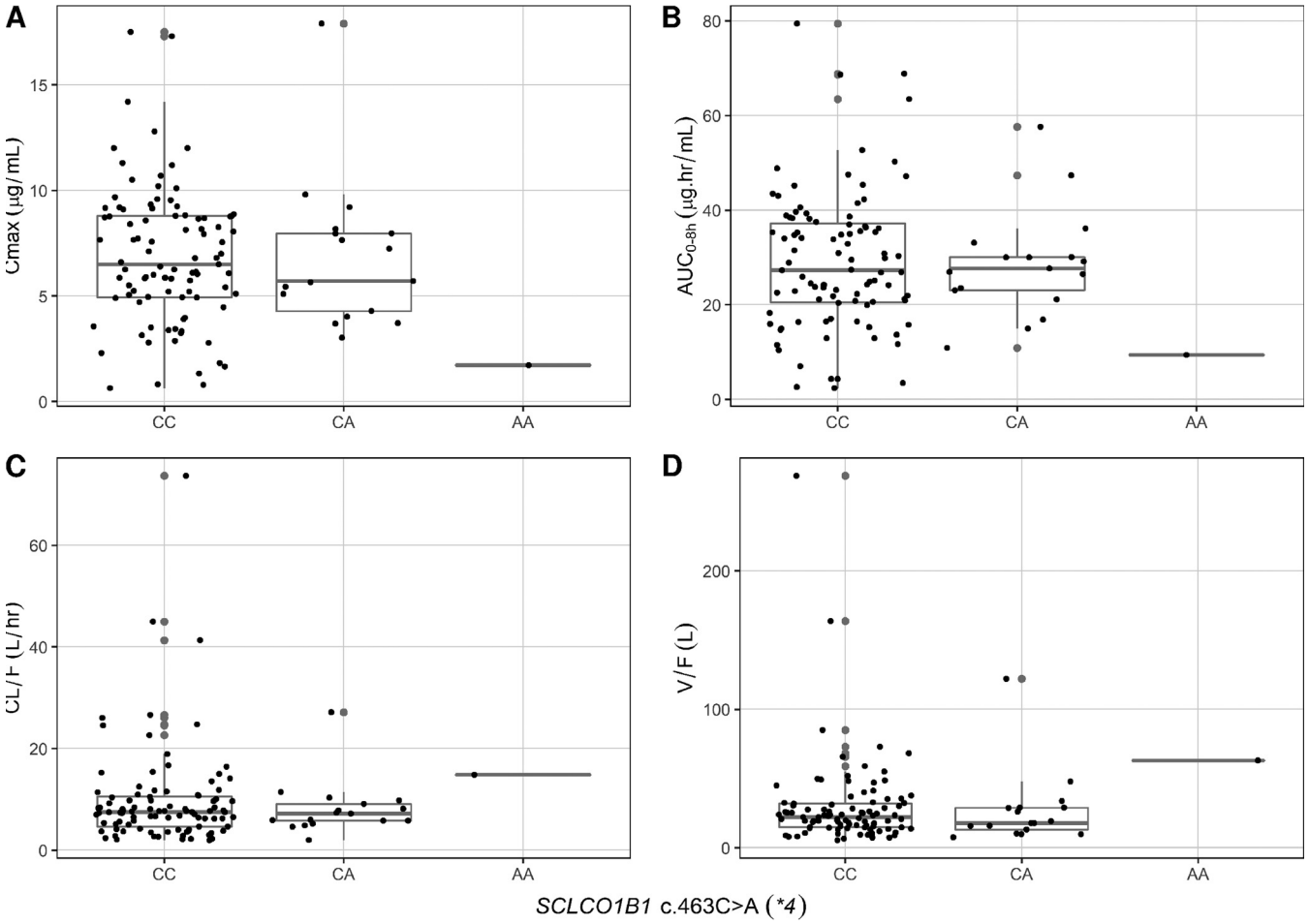

FIG 3 Relationship between rifampin C_{max} (A), AUC_{0-8h} (B), CL/F (C), and *V*/F (D) and *SLCO1B1* c.463C>A genotypes in children with tuberculosis.

In our multivariate analysis, isoniazid dose and *NAT2* acetylator genotype status were joint predictors of isoniazid C_{max} and AUC_{0-8h}, while dose, age, and acetylator *NAT2* genotype jointly predicted CL/F. Thus, the dose of isoniazid likely influences the relationship between *NAT2* acetylator genotypes and isoniazid pharmacokinetics in children. In a study that used the previous recommended isoniazid dose of 5 mg/kg for children in South Africa, 35% of children with homozygous rapid genotype had a low 2-hour concentration with a faster elimination of isoniazid in younger than older children, prompting the investigators to recommend an isoniazid dose of at least 10 mg/kg for children younger than 5 years old (13). In our study, in which the median isoniazid dose was 11.2 mg/kg, a low isoniazid C_{max} was uncommon and only one child with rapid acetylator genotype had a low C_{max}.

Adult studies that examined the relationship between rifampin pharmacokinetics and *SLCO1B1* SNPs reported 36% lower rifampin AUC_{0-24} in participants with the *SLCO1B1* c.463CA genotype than in those with the c.463CC genotype (19) and 18% and 28% lower AUC in patients heterozygous and homozygous, respectively, for the rs4149032 allele (18). However, two recent studies in Indian and Malawian adult patients failed to replicate or confirm the above-mentioned associations (20, 21). We found no significant association between the studied *SCLCO1B1* and *CES2* SNPs in the primary analysis. In *post hoc* analysis, the rare *SLCO1B1* c.388AA genotype (found in 2 children) was associated with low rifampin concentrations compared to those with c.388GG. Also, one patient with the *SLCO1B1* c.463AA genotype appeared to have low rifampin C_{max} and AUC, but this could be due to chance. The relationship between

TABLE 4 Distribution of studied *NAT2*, *SLCO1B1*, and *CES2* single nucleotide polymorphisms in Ghanaian children with TB[a]

Single nucleotide polymorphism	Mutation	dbSNP ID	MAF	Genotype	No. of times observed	Frequency (%)	HWE *P* value
NAT2 191G>A	Arg64Gln	rs1801279	0.12	GG	87	77	0.38
				GA	26	23	
				AA	0	0	
NAT2 282C>T	Tyr94Tyr	rs1041983	0.42	CC	45	39.8	0.044
				CT	42	37.2	
				TT	26	23	
NAT2 341C>T	Ile114Thr	rs1801280	0.30	CC	10	8.5	0.99
				CT	48	42.5	
				TT	55	49	
NAT2 481C>T	Leu161Leu	rs1799929	0.25	CC	61	54	0.55
				CT	47	42	
				TT	5	4	
NAT2 590G>A	Arg197Gln	rs1799930	0.26	GG	64	57	0.74
				GA	40	35	
				AA	9	8	
NAT2 803A>G	Arg268Lys	rs1208	0.41	AA	42	37	0.68
				AG	50	44	
				GG	21	19	
NAT2 191G>A	Gly286Glu	rs1799931	0.01	GG	111	98.2	0.99
				GA	2	1.8	
				AA	0	0.0	
NAT2 genotype[b]							
				Rapid	12	10.6	
				Intermediate	50	44.3	
				Slow	51	45.1	
*SLCO1B1*c.388A>G (**1b*)	Asn130Asp	rs2306283	0.17	AA	2	1.8	0.72
				AG	34	30.1	
				GG	77	68.1	
SLCO1B1 (tSNP) C>T		rs4149032	0.27	CC	65	57.5	0.15
				CT	36	31.9	
				TT	12	10.6	
SLCO1B1 c.463C>A (**4*)	Pro155Thr	rs11045819	0.08	CC	95	84.1	0.97
				CA	17	15.0	
				AA	1	0.9	
SLCO1B1 c.521T>C (**5*)	Val174Ala	rs4149056	0.01	TT	110	97.3	0.99
				TC	3	2.7	
				CC	0	0.0	
CES2 c.-2263A>G	2263A>G		0.30	AA	56	49.6	0.99
				AG	47	41.6	
				GG	10	10.8	

[a]HWE, Hardy Weinberg equilibrium; MAF, minor allele frequency; *SLCO1B1* tSNP, *SLCO1B1* intron 2 haplotype tagging SNP.
[b]*NAT2* genotypes defined by the 4-SNP panel (191G>A, 341T>C, 590G>A, and 857G>A) and the 7-SNP panel (191G>A, 341T>C, 590G>A, and 857G>A plus 282C>T, 481C>T, and 803A>G) were similar.

SLCO1B1 c.388A>G SNP and rifampin pharmacokinetics requires further evaluation, especially in populations where the homozygous variant is frequent, as our study was highly limited by sample size and the rare occurrence of the variant. For the *CES2* genetic variation and rifampin pharmacokinetics, our study was limited since we examined only one SNP with the strongest effect *in vitro* (22). It is possible that other *CES2* SNPs that we did not include in our study may influence rifampin metabolism.

In summary, our results suggest that genotyping for the *NAT2* acetylator genotype status or the selected *SCLCO1B1* and *CES2* SNPs may have only minimal clinical utility in isoniazid and rifampin dosing decisions at the population level in Ghanaian children given the modest effect of the *NAT2* acetylator genotype and the rarity of the implicated *SLCO1B1* SNP. However, at the individual level or in other populations with different allele frequencies of the implicated SNPs, host genetics may have a role in individualizing therapy. Further study with a larger sample size or in other populations in which the distribution of the studied SNPs may be different from that of our population is warranted.

MATERIALS AND METHODS

Study population and design. Children aged 3 months to 14 years old with clinical diagnosis of TB were enrolled in a study at Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana. Briefly, enrolled children were treated with a regimen consisting of 7 to 15 mg/kg isoniazid, 10 to 20 mg/kg rifampin, 30 to 40 mg/kg pyrazinamide, and 15 to 25 mg/kg ethambutol daily for 2 months and then 7 to 15 mg/kg isoniazid and 10 to 20 mg/kg rifampin daily for 4 months. Pharmacokinetic sampling was performed after 4 weeks of anti-TB treatment as previously described (23, 29). The details of the study population and study procedures were previously reported (23). The Institutional Review Board (IRB) of KATH, Ghana, and Lifespan Hospitals, Providence, RI, USA, reviewed and approved the study. All parents or guardians of study participants provided signed informed consent.

Blood samples were collected at times 0 (predose), 1, 2, 4, and 8 hours postdose. This sampling scheme, when conducted at steady state, is considered sufficient to estimate key pharmacokinetic parameters such as C_{max} and area under the concentration-time curve (AUC) (30). The blood samples collected in EDTA-coated tubes were placed immediately on ice and centrifuged within 30 min at 3,000 × *g* for 10 min. Plasma was stored at −80°C until shipment on dry ice to University of Cape Town, Cape Town, South Africa, for drug concentration assays. Drug concentrations were determined using validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods as we previously described (29). The observed C_{max} and time to C_{max} (T_{max}) were determined by inspection of the concentration of drug in serum-time graphs for each drug. The calculation of AUC from time 0 to 8 h (AUC_{0-8h}), predicted apparent oral clearance (CL/F), and predicted apparent volume of distribution (*V*/F) was performed using noncompartmental analysis (Phoenix Software; Pharsight Corporation, Mountain View, CA).

Genotyping. The isolated genomic DNA samples from blood leukocytes were genotyped by the TaqMan genotyping method on ViiA 7 real-time PCR system according to the manufacturer's recommendations (Applied Biosystems, Thermo Fisher Scientific, Foster City, CA). For *NAT2*, four SNPs were genotyped, rs1801279 (191G>A), rs1801280 (341T>C), rs1799930 (590G>A), and rs1799931 (857G>A). Those homozygous wild-type samples for all SNPs were classified as rapid acetylator phenotype, those heterozygous for any one of the SNPs were classified as intermediate acetylator phenotype, and those homozygous variants for one or more SNPs or heterozygous for two or more SNPs were classified as slow acetylator phenotype according to the established criteria for the 4-SNP genotype panel (24). We further genotyped the samples for *NAT2* gene SNPs, rs1041983 (282C>T), rs1799929 (481C>T), and rs1208 (803A>G) to infer 7-SNP genotypes. In our study participants, the 4-SNP- and 7-SNP panel-inferred acetylator genotypes were similar. Thus, in this paper, genotype-phenotype associations described for 4-SNP are the same as for 7-SNP. For rifampin pharmacogenetics, we genotyped for *SLCO1B1*c.388A>G (**1b*, rs2306283), c.463C>A (**4*, rs11045819), c.521T>C (**5*, rs4149056), and rs4149032 (intron 2 haplotype tagging SNP; tSNP) and *CES2* c.-2263A>G. These SNPs were selected based on reported effects on rifampin pharmacokinetics in previous studies in adults (18, 19, 22). The departure from Hardy Weinberg equilibrium (HWE) was tested for all the SNPs from *NAT2*, *CES2*, and *SLCO1B1* using the chi-square test with 1 degree of freedom. The genotype distribution of all SNPs (except *NAT2*) 282C>T (rs1041983) in the 7-SNP *NAT2* panel (Table 4) were in HWE by χ^2 analysis (31).

Statistical analysis. Multiple-group comparisons of pharmacokinetic parameters were examined by both one-way analysis of variance (ANOVA) and the Kruskal-Wallis test, followed by pairwise group comparisons by two-sample *t* test and its nonparametric version of the Wilcoxon rank sum test. Multivariate analysis with best subset variable selection based on corrected Akaike's information criterion (AICC) was used to find joint predictors of PK parameters. In addition to genotypes, HIV coinfection status (positive versus negative), sex (male versus female), age, body mass index (BMI), and drug dose were included in the multivariate model fitting. Statistical analyses were performed using software SAS 9.4 (SAS Institute Inc., Cary, NC). For all analyses, a *P* value of <0.05 was considered significant.

ACKNOWLEDGMENTS

We thank the study participants, the supportive staff of the TB and HIV clinics, and the malnutrition ward at KATH who helped with patient enrollment.

The work was supported primarily by the Eunice Kennedy Shriver National Institute of Child Health and Human Development at the National Institutes of Health (grant number HD071779). X. Tang was supported in part by Lifespan/Tufts/Brown Center for AIDS Research [P30 AI042853] and A. Topletz by T32 grant award DA013911. The pharmacokinetic laboratory at University of Cape Town is supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health Awards UM1 AI068634, UM1 AI068636, and UM1 AI106701, under the auspices of the Adult Clinical Trial Group. H. Yang utilized core services and support from the University of Rochester Center for AIDS Research (CFAR), an NIH-funded program (P30 AI078498). M. H. Court was supported by the National Institute of General Medical Sciences at the National Institutes of Health (grant number R01 GM102130). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

REFERENCES

1. Nahid P, Dorman SE, Alipanah N, Barry PM, Brozek JL, Cattamanchi A, Chaisson LH, Chaisson RE, Daley CL, Grzemska M, Higashi JM, Ho CS, Hopewell PC, Keshavjee SA, Lienhardt C, Menzies R, Merrifield C, Narita M, O'Brien R, Peloquin CA, Raftery A, Saukkonen J, Schaaf HS, Sotgiu G, Starke JR, Migliori GB, Vernon A. 2016. Official American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America clinical practice guidelines: treatment of drug-susceptible tuberculosis. Clin Infect Dis 63:e147–e195. https://doi.org/10.1093/cid/ciw376.
2. Getahun H, Matteelli A, Abubakar I, Aziz MA, Baddeley A, Barreira D, Den Boon S, Borroto Gutierrez SM, Bruchfeld J, Burhan E, Cavalcante S, Cedillos R, Chaisson R, Chee CB, Chesire L, Corbett E, Dara M, Denholm J, de Vries G, Falzon D, Ford N, Gale-Rowe M, Gilpin C, Girardi E, Go UY, Govindasamy D, D Grant A, Grzemska M, Harris R, Horsburgh CR, Jr, Ismayilov A, Jaramillo E, Kik S, Kranzer K, Lienhardt C, LoBue P, Lonnroth K, Marks G, Menzies D, Migliori GB, Mosca D, Mukadi YD, Mwinga A, Nelson L, Nishikiori N, Oordt-Speets A, Rangaka MX, Reis A, Rotz L, Sandgren A, Sañé Schepisi M, Schünemann HJ, Sharma SK, Sotgiu G, Stagg HR, Sterling TR, Tayeb T, Uplekar M, van der Werf MJ, Vandevelde W, van Kessel F, van't Hoog A, Varma JK, Vezhnina N, Voniatis C, Vonk Noordegraaf-Schouten M, Weil D, Weyer K, Wilkinson RJ, Yoshiyama T, Zellweger JP, Raviglione M. 2015. Management of latent Mycobacterium tuberculosis infection: WHO guidelines for low tuberculosis burden countries. Eur Respir J 46:1563–1576. https://doi.org/10.1183/13993003.01245-2015.
3. Donald PR, Ahmed A, Burman WJ, Cotton MF, Graham SM, Mendel C, McIlleron H, Mac Kenzie WR, Nachman S, Schaaf HS, Starke JR, Wingfield C, Hesseling AC. 2013. Requirements for the clinical evaluation of new anti-tuberculosis agents in children. Int J Tuberc Lung Dis 17:794–799. https://doi.org/10.5588/ijtld.12.0567.
4. Schaaf HS, Garcia-Prats AJ, Donald PR. 2015. Antituberculosis drugs in children. Clin Pharmacol Ther 98:252–265. https://doi.org/10.1002/cpt.164.
5. Donald PR, Parkin DP, Seifart HI, Schaaf HS, van Helden PD, Werely CJ, Sirgel FA, Venter A, Maritz JS. 2007. The influence of dose and N-acetyltransferase-2 (NAT2) genotype and phenotype on the pharmacokinetics and pharmacodynamics of isoniazid. Eur J Clin Pharmacol 63:633–639. https://doi.org/10.1007/s00228-007-0305-5.
6. Donald PR, Sirgel FA, Botha FJ, Seifart HI, Parkin DP, Vandenplas ML, Van de Wal BW, Maritz JS, Mitchison DA. 1997. The early bactericidal activity of isoniazid related to its dose size in pulmonary tuberculosis. Am J Respir Crit Care Med 156:895–900. https://doi.org/10.1164/ajrccm.156.3.9609132.
7. Jindani A, Aber VR, Edwards EA, Mitchison DA. 1980. The early bactericidal activity of drugs in patients with pulmonary tuberculosis. Am Rev Respir Dis 121:939–949.
8. Sirgel FA, Botha FJ, Parkin DP, Van De Wal BW, Donald PR, Clark PK, Mitchison DA. 1993. The early bactericidal activity of rifabutin in patients with pulmonary tuberculosis measured by sputum viable counts: a new method of drug assessment. J Antimicrob Chemother 32:867–875. https://doi.org/10.1093/jac/32.6.867.
9. Sirgel FA, Fourie PB, Donald PR, Padayatchi N, Rustomjee R, Levin J, Roscigno G, Norman J, McIlleron H, Mitchison DA. 2005. The early bactericidal activities of rifampin and rifapentine in pulmonary tuberculosis. Am J Respir Crit Care Med 172:128–135. https://doi.org/10.1164/rccm.200411-1557OC.
10. Preziosi P. 2007. Isoniazid: metabolic aspects and toxicological correlates. Curr Drug Metab 8:839–851. https://doi.org/10.2174/138920007782798216.
11. Kinzig-Schippers M, Tomalik-Scharte D, Jetter A, Scheidel B, Jakob V, Rodamer M, Cascorbi I, Doroshyenko O, Sorgel F, Fuhr U. 2005. Should we use N-acetyltransferase type 2 genotyping to personalize isoniazid doses? Antimicrob Agents Chemother 49:1733–1738. https://doi.org/10.1128/AAC.49.5.1733-1738.2005.
12. Parkin DP, Vandenplas S, Botha FJ, Vandenplas ML, Seifart HI, van Helden PD, van der Walt BJ, Donald PR, van Jaarsveld PP. 1997. Trimodality of isoniazid elimination: phenotype and genotype in patients with tuberculosis. Am J Respir Crit Care Med 155:1717–1722. https://doi.org/10.1164/ajrccm.155.5.9154882.
13. Schaaf HS, Parkin DP, Seifart HI, Werely CJ, Hesseling PB, van Helden PD, Maritz JS, Donald PR. 2005. Isoniazid pharmacokinetics in children treated for respiratory tuberculosis. Arch Dis Child 90:614–618. https://doi.org/10.1136/adc.2004.052175.
14. McIlleron H, Willemse M, Werely CJ, Hussey GD, Schaaf HS, Smith PJ, Donald PR. 2009. Isoniazid plasma concentrations in a cohort of South African children with tuberculosis: implications for international pediatric dosing guidelines. Clin Infect Dis 48:1547–1553. https://doi.org/10.1086/598192.
15. World Health Organization. 2014. Guidance for national tuberculosis programmes on the management of tuberculosis in children, 2nd ed. WHO/HTM/TB/2014.03.1-146. World Health Organization, Geneva, Switzerland.
16. Jamis-Dow CA, Katki AG, Collins JM, Klecker RW. 1997. Rifampin and rifabutin and their metabolism by human liver esterases. Xenobiotica 27:1015–1024. https://doi.org/10.1080/004982597239994.
17. Giacomini KM, Huang SM, Tweedie DJ, Benet LZ, Brouwer KL, Chu X, Dahlin A, Evers R, Fischer V, Hillgren KM, Hoffmaster KA, Ishikawa T, Keppler D, Kim RB, Lee CA, Niemi M, Polli JW, Sugiyama Y, Swaan PW, Ware JA, Wright SH, Yee SW, Zamek-Gliszczynski MJ, Zhang L. 2010. Membrane transporters in drug development. Nat Rev Drug Discov 9:215–236. https://doi.org/10.1038/nrd3028.
18. Chigutsa E, Visser ME, Swart EC, Denti P, Pushpakom S, Egan D, Holford NH, Smith PJ, Maartens G, Owen A, McIlleron H. 2011. The SLCO1B1 rs4149032 polymorphism is highly prevalent in South Africans and is associated with reduced rifampin concentrations: dosing implications. Antimicrob Agents Chemother 55:4122–4127. https://doi.org/10.1128/AAC.01833-10.
19. Weiner M, Peloquin C, Burman W, Luo CC, Engle M, Prihoda TJ, Mac Kenzie WR, Bliven-Sizemore E, Johnson JL, Vernon A. 2010. Effects of tuberculosis, race, and human gene SLCO1B1 polymorphisms on rifampin concentrations. Antimicrob Agents Chemother 54:4192–4200. https://doi.org/10.1128/AAC.00353-10.
20. Ramesh K, Hemanth Kumar AK, Kannan T, Vijayalakshmi R, Sudha V, Manohar Nesakumar S, Bharathiraja T, Lavanya J, Swaminathan S, Ramachandran G. 2016. SLCO1B1 gene polymorphisms do not influence plasma rifampicin concentrations in a South Indian population. Int J Tuberc Lung Dis 20:1231–1235. https://doi.org/10.5588/ijtld.15.1007.
21. Sloan DJ, McCallum AD, Schipani A, Egan D, Mwandumba HC, Ward SA, Waterhouse D, Banda G, Allain TJ, Owen A, Khoo SH, Davies GR. 2017. Genetic determinants of the pharmacokinetic variability of rifampin in Malawian adults with pulmonary tuberculosis. Antimicrob Agents Chemother 61:e00210-17. https://doi.org/10.1128/AAC.00210-17.
22. Song SH, Chang HE, Jun SH, Park KU, Lee JH, Lee EM, Song YH, Song J. 2013. Relationship between CES2 genetic variations and rifampicin metabolism. J Antimicrob Chemother 68:1281–1284. https://doi.org/10.1093/jac/dkt036.
23. Antwi S, Yang H, Enimil A, Sarfo AM, Gillani FS, Ansong D, Dompreh A, Orstin A, Opoku T, Bosomtwe D, Wiesner L, Norman J, Peloquin CA, Kwara A. 2017. Pharmacokinetics of the first-line antituberculosis drugs in Ghanaian children with tuberculosis with or without HIV coinfection. Antimicrob Agents Chemother 61:e01701-16. https://doi.org/10.1128/AAC.01701-16.
24. Hein DW, Doll MA. 2012. Accuracy of various human NAT2 SNP genotyping panels to infer rapid, intermediate and slow acetylator phenotypes. Pharmacogenomics 13:31–41. https://doi.org/10.2217/pgs.11.122.
25. Suarez-Kurtz G, Vargens DD, Sortica VA, Hutz MH. 2012. Accuracy of NAT2 SNP genotyping panels to infer acetylator phenotypes in African, Asian, Amerindian and admixed populations. Pharmacogenomics 13:851–854, 855. https://doi.org/10.2217/pgs.12.48.
26. Cranswick N, Mulholland K. 2005. Isoniazid treatment of children: can genetics help guide treatment? Arch Dis Child 90:551–553. https://doi.org/10.1136/adc.2004.063610.
27. Verhagen LM, Coenen MJ, Lopez D, Garcia JF, de Waard JH, Schijvenaars MM, Hermans PW, Aarnoutse RE. 2014. Full-gene sequencing analysis of NAT2 and its relationship with isoniazid pharmacokinetics in Venezuelan children with tuberculosis. Pharmacogenomics 15:285–296. https://doi.org/10.2217/pgs.13.230.
28. Zhu R, Kiser JJ, Seifart HI, Werely CJ, Mitchell CD, D'Argenio DZ, Fletcher CV. 2012. The pharmacogenetics of NAT2 enzyme maturation in perinatally HIV exposed infants receiving isoniazid. J Clin Pharmacol 52:511–519. https://doi.org/10.1177/0091270011402826.
29. Kwara A, Enimil A, Gillani FS, Yang H, Sarfo AM, Dompreh A, Ortsin A, Osei-Tutu L, Kwarteng Owusu S, Wiesner L, Norman J, Kurpewski J,

Peloquin CA, Ansong D, Antwi S. 2016. Pharmacokinetics of first-line antituberculosis drugs using WHO revised dosage in children with tuberculosis with and without HIV coinfection. J Pediatr Infect Dis Soc 5:356–365. https://doi.org/10.1093/jpids/piv035.

30. Thee S, Seddon JA, Donald PR, Seifar HI, Werely CJ, Hesseling AC, Rosenkranz B, Roll S, Magdorf K, Schaaf HS. 2011. Pharmacokinetics of isoniazid, rifampin, and pyrazinamide in children younger than two years of age with tuberculosis; evidence for implementation of revised World Health Organization recommendations. Antimicrob Agents Chemother 55:5560–5567. https://doi.org/10.1128/AAC.05429-11.
31. Schaid DJ, Batzler AJ, Jenkins GD, Hildebrandt MA. 2006. Exact tests of Hardy-Weinberg equilibrium and homogeneity of disequilibrium across strata. Am J Hum Genet 79:1071–1080. https://doi.org/10.1086/510257.