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Pharmacogenetics of Between-Individual Variability in Plasma Clearance of Bedaquiline and Clofazimine in South Africa

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Background. Plasma bedaquiline clearance is reportedly more rapid with African ancestry. Our objective was to determine whether genetic polymorphisms explained between-individual variability in plasma clearance of bedaquiline, its M2 metabolite, and clofazimine in a cohort of patients treated for drug-resistant tuberculosis in South Africa.

Methods. Plasma clearance was estimated with nonlinear mixed-effects modeling. Associations between pharmacogenetic polymorphisms, genome-wide polymorphisms, and variability in clearance were examined using linear regression models.

Results. Of 195 cohort participants, 140 were evaluable for genetic associations. Among 21 polymorphisms selected based on prior genome-wide significant associations with any drug, rs776746 (*CYP3A5*3*) was associated with slower clearance of bedaquiline ($P = .0017$) but not M2 ($P = .25$). *CYP3A5*3* heterozygosity and homozygosity were associated with 15% and 30% slower bedaquiline clearance, respectively. The lowest P value for clofazimine clearance was with *VKORC1* rs9923231 ($P = .13$). In genome-wide analyses, the lowest P values for clearance of bedaquiline and clofazimine were with *RFX4* rs76345012 ($P = 6.4 \times 10^{-7}$) and *CNTN5* rs75285763 ($P = 2.9 \times 10^{-8}$), respectively.

Conclusions. Among South Africans treated for drug-resistant tuberculosis, *CYP3A5*3* was associated with slower bedaquiline clearance. Different *CYP3A5*3* frequencies among populations may help explain the more rapid bedaquiline clearance reported in Africans. Associations with *RFX4* and *CNTN5* are likely by chance alone.

Keywords. bedaquiline; clofazimine; pharmacogenomics; tuberculosis; pharmacokinetics.

Drug-resistant tuberculosis is a major public health threat, with the number of patients infected with *Mycobacterium tuberculosis* resistant to rifampicin and isoniazid (ie, multidrug-resistant [MDR] tuberculosis) increasing worldwide. Treatment of MDR tuberculosis requires 9–24 months of therapy, and outcomes are less favorable than with drug-susceptible tuberculosis. Outcomes can be improved with novel and repurposed drugs. Clinical trials are evaluating 6-month regimens to simplify management and improve outcomes [1].

Bedaquiline is an oral agent active against *M. tuberculosis* that is resistant to first- and second-line drugs [2–4]. In 2012, bedaquiline received regulatory approval for treating pulmonary MDR tuberculosis as part of combination therapy [5]. It is part of standard-of-care regimens for MDR tuberculosis [6], and it has been associated with reduced all-cause mortality rates [7]. Bedaquiline is well absorbed, its plasma exposure increases proportionally with increasing dose, and food increases its oral bioavailability [2, 3]. Bedaquiline is highly bound to plasma proteins and has a long terminal elimination half-life, likely reflecting slow release from peripheral tissues. Model-based analyses describing exposure-response relationships predict that higher bedaquiline exposure would improve mycobacterial treatment responses [8, 9].

Bedaquiline is primarily metabolized by hepatic cytochrome P450 (CYP) 3A4 into an N-monodesmethyl metabolite (M2), which is approximately 5-fold less active against *M. tuberculosis* than bedaquiline [10]. A minor N-didesmethyl metabolite (M3) lacks antimycobacterial activity. Both M2 and M3 are more toxic

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in vitro than is bedaquiline, based on cytotoxicity assays and induction of phospholipidosis [10, 11]. Bedaquiline-associated QT prolongation may also depend on M2 exposure [12]. Although CYP isoforms 1A1, 2C8 and 2C18 metabolize bedaquiline in vitro at rates ≥10% that of CYP3A4, they are unlikely to contribute substantially in vivo, given their considerably lower expression levels in liver compared with CYP3A4 [13]. In a population pharmacokinetic model, apparent clearance of bedaquiline was 52% more rapid among individuals identified as being of black race compared with others [14], suggesting that genetic polymorphisms may affect disposition. A subsequent population pharmacokinetic analysis showed that weight and albumin levels, which were inversely correlated with each other, were significantly associated with bedaquiline plasma disposition. Age and race were also significant covariates [15]. While generally well tolerated, bedaquiline has been associated with QT prolongation, arthralgias, headache, and hepatic transaminase elevation.

Clofazimine is an oral lipophilic riminophenazine antibiotic discovered in 1957. Although historically used to treat leprosy, it is now recommended for treating rifampicin-resistant tuberculosis [16, 17]. Its oral bioavailability is approximately 70% [18], and administration with a high-fat meal increases its area under the concentration-time curve by 60% [19]. Clofazimine is highly protein bound [20] and undergoes duration-dependent accumulation in fat, macrophages, and reticuloendothelial organs, resulting in a very long terminal elimination half-life [21]. It is metabolized by hydrolytic reactions and is excreted largely unchanged [22, 23]. Adverse reactions to clofazimine include skin discoloration and QT prolongation. The present analyses leveraged population pharmacokinetic modeling data from patients treated for drug-resistant pulmonary tuberculosis in South Africa to characterize associations between genetic polymorphisms and between-individual variability in plasma clearance of bedaquiline, its M2 metabolite, and clofazimine.

METHODS

Study Population

The Pharmacokinetics, Resistance, and Outcomes of Bedaquiline in MDR- and XDR-TB (PROBeX) study was a prospective observational cohort project conducted between 2016 and 2020 at 3 tuberculosis referral hospitals in the South African provinces of Eastern Cape, Western Cape, and KwaZulu-Natal [24]. Participants received a modified standardized regimen, which typically included bedaquiline (400 mg once daily for 2 weeks, followed by 200 mg 3 times weekly), clofazimine (100 mg once daily), linezolid (600 mg daily), levofloxacin (750–1000 mg daily), ethionamide (15–20 mg/kg; maximum 750 mg daily), terizidone (15–20 mg/kg; maximum 750 mg daily), and pyrazinamide (20–30 mg/kg; maximum 1600 mg daily). All human immunodeficiency virus (HIV)–positive participants were offered either nevirapine- or lopinavir-ritonavir-based antiretroviral therapy to avoid CYP3A4 induction by efavirenz [25].

Pharmacokinetic Parameters

In PROBeX, clinical and laboratory data were collected monthly for 6 months, and every 6 months thereafter. Sparse pharmacokinetic sampling was performed at approximately 1, 2, and 6 months after the start of treatment, at single time points after self-reported dosing. A subgroup of consecutive participants underwent intensive sampling at month 2 (before and 1, 2, 3, 4, 5, 6, 8, and 24 hours after an observed dose and standard meal). Bedaquiline and clofazimine plasma concentrations were measured at the Division of Clinical Pharmacology of the University of Cape Town, using validated liquid chromatography with tandem mass spectrometry assays with interday accuracy ranging from 101% to 105% and precision (percentage coefficient of variation [%CV]) ranging from 3.3% to 4.6% during sample analysis for clofazimine. The accuracy statistics of the low-, medium-, and high-quality control samples of both bedaquiline and M2 during sample analysis were between 95.1% and 100.1%, with precision (%CV) between 4.2%, and 7.7% [26, 27].

Population pharmacokinetics were described with nonlinear mixed-effects models, which comprise a structural component (fixed effects) and a stochastic component (random effects). The stochastic model divides unexplained variabilities into between-subject variability or within-subject variability assigned to specific parameters and the residual error.

A previous pharmacokinetic analysis of bedaquiline and M2 included data from patients with MDR tuberculosis from 2 phase IIb studies (NCT00449644 and NCT00910871). Bedaquiline and M2 disposition were well described by 3- and 1-compartment models, respectively. Weight and albumin were correlated, typically increased after starting treatment, and significantly affected bedaquiline and M2 plasma disposition. Age and race were also significant covariates [15]. Because race is related to genetics, we prevented the model from explaining variability using race by fixing the race effect to zero and weighing the clearances of the black and nonblack groups to determine the typical clearance for the full population. In addition, we incorporated the known effect of concomitant lopinavir-ritonavir treatment on bedaquiline and M2 clearances by fixing the effect sizes to previously reported values [28]. The model was then fitted to PROBeX participants with maximum a posteriori estimation.

A population pharmacokinetic model for clofazimine was reported elsewhere [26]. The population model was developed based on pooled data from a phase 2A 14-day early bactericidal activity trial of clofazimine, alone or in combination with other antituberculous drugs (NCT 01691534) [29], and from the PROBeX study [26]. Based on data from 139 participants, clofazimine pharmacokinetics were well characterized by a 3-compartment model. Body composition was found to be a key covariate affecting drug exposures and disposition.

Genetic Polymorphisms

Whole-blood samples collected from consenting participants were labeled with coded identifiers. DNA was extracted at the Centre for Proteomic and Genomic Research in Cape Town, by a method described elsewhere [30]. Genotyping was performed using the Illumina Infinium Multi-Ethnic Global BeadChip array (MEGA^{EX}), and postgenotyping quality control was performed by Vanderbilt Technologies for Advanced Genomics (VANTAGE). Quality control steps were performed using PLINK software, version 1.9 [31]. Genotyping efficiency per participant was 95% for all samples. To identify polymorphisms not directly genotyped, data were then imputed using the TOPMed program [32] after transforming to genome build 38 using liftOver software (version 3.33) [33]. Imputed polymorphisms were excluded if they had imputation scores <0.3, genotyping call rates <99%, minor allele frequency <0.05, or Hardy-Weinberg equilibrium P values $<1.0 \times 10^{-8}$.

To adjust for genetic ancestry, we estimated continuous axes of ancestry incorporating the intersection of common autosomal genotypes using the EIGENSTRAT software package (version 6.0.1) [34]. We also included the 1000 Genomes Project phase 3 to provide global reference populations [35]. Principal components scree plots were inspected to ensure the components selected for analyses represented ancestral information; based on these plots, 2 principal components sufficiently adjusted for ancestry. Additional covariates were not included in association analyses because these were already evaluated and included as appropriate in population pharmacokinetic models. The bedaquiline model considered the weighted average of the typical value for nonblack and the typical value for black participants, with weighting based on the proportion of black relative to nonblack participants in the data set in which the model was developed. The bedaquiline model also accounted for difference in exposure in patients receiving lopinavir-ritonavir, which increases bedaquiline exposure [28]. The clofazimine model accounted for the effect of body composition on disposition parameters.

There was not a strong a priori rationale to focus on specific genes or polymorphisms, other than the *CYP3A* locus for bedaquiline. To decrease the burden of multiple testing, we followed an approach that stepwise prioritized sets of polymorphisms to interrogate. We reasoned that polymorphisms previously associated with ≥1 drug-related phenotype, or previously significantly associated with any trait genome wide, are more likely to be true associations than are polymorphisms not previously associated with any drug or trait. We used as references the Pharmacogenomics Knowledgebase (PharmGKB) [36] and the NHGRI-EBI (National Human Genome Research Institute–European Bioinformatics Institute) GWAS Catalog [37]. In PharmGKB, 173 polymorphisms were previously associated with ≥1 drug-related phenotype (pharmacokinetics, efficacy, or toxicity), with levels of evidence of 1 (the preponderance of evidence shows an association, replicated in multiple cohorts and preferably with strong effect size) or 2 (moderate evidence of association; replicated, but some studies may not show statistical significance or may show small effect size). In the GWAS Catalog, 89 716 polymorphisms were previously associated with any trait at $P < 5.0 \times 10^{-8}$ in ≥1 published study. A subset of 33 polymorphisms were common to both PharmGKB and the GWAS Catalog. A list of PharmGKB and GWAS Catalog polymorphisms included in our analyses are provided in the Supplementary Materials.

We considered polymorphisms common to both PharmGKB and the GWAS Catalog to have the strongest a priori evidence for true associations. We secondarily evaluated all polymorphisms from PharmGKB and from the GWAS Catalog (based on criteria described above) and all polymorphisms in our imputed genome-wide data.

Association Analyses

Outcomes of primary interest were between-individual variability in population parameter estimates for central clearance of bedaquiline and clofazimine. We also studied between-individual variability in clearance of the M2 metabolite of bedaquiline. Multivariable linear regression models were used to characterize associations with genetic polymorphisms, using 2-sided statistical tests. The first 2 genetic ancestry principal components were included as covariates. We report the regression coefficient (β) for additive associations with polymorphisms, where positive β values indicate positive associations. To correct for multiple testing, the Bonferroni method was used to determine significance thresholds, with .05 divided by the number of polymorphisms tested in prioritized analyses, and $P = 5.0 \times 10^{-8}$ for genome-wide analyses. Linkage disequilibrium (LD) estimates were determined within our data set using PLINK software. We used LocusZoom software (version 0.12.0) to visualize genetic associations and LD estimates in defined regions [38].

Ethical Approval

The PROBeX study was approved by the institutional review boards at the University of Cape Town, Albert Einstein College of Medicine, and Emory University. All participants provided written informed consent.

RESULTS

Participant Characteristics

A total of 195 individuals were enrolled in the PROBeX cohort. All were ≥18 years of age and had baseline creatinine and alanine aminotransferase values no more than 2 or 5 times the upper limit of normal, respectively. Among participants, 123 (63%) were HIV positive, the median age was 33 years, 160 (82%) were black, and 111 (57%) were female. During the study period, 190 (97%) received clofazimine, and

179 (92%) received linezolid. Of the HIV-positive participants, 113 (90%) were receiving antiretroviral therapy before enrollment, and 26 (23%) received lopinavir-ritonavir during the study.

Among cohort participants, 172 and 164 provided population pharmacokinetic data for bedaquiline and clofazimine, respectively, of whom 140 and 136, respectively, were included in genetic association analyses. The primary reason for exclusion was genotyping efficiency <95%. Individuals included in genetic analyses closely resembled the total PROBeX cohort (Table 1).

Associations With Between-Individual Variability in Bedaquiline and M2 Clearance

We primarily characterized associations with between-individual variability in plasma bedaquiline clearance. Of 33 polymorphisms common to PharmGKB and the GWAS Catalog (described in Methods), we were able to test for associations with 21 (64%). The other 12 polymorphisms failed imputation score, minor allele frequency, or Hardy-Weinberg equilibrium cutoffs. For bedaquiline clearance, the lowest *P* value among these 21 polymorphisms was for *CYP3A5* rs776746 ($P = .0017$; $\beta = -.18$), with the C allele associated with slower clearance. This withstood correction for multiple testing (cutoff, 0.0024). This T→C polymorphism defines the *CYP3A5*3* allele which results in nonfunctional CYP3A5 protein [39, 40].

Given the association of *CYP3A5*3* with bedaquiline clearance, we more thoroughly interrogated the *CYP3A* gene locus, including 150 kB on either side of *CYP3A4* and *CYP3A5*. Among 1214 polymorphisms in this region, the lowest *P* values for association with bedaquiline clearance were seen with 4 polymorphisms, approximately 73 kB 3' of rs776746 (rs1011024, rs10254729, rs12333760, and rs34777615) (each $P = 1.0 \times 10^{-4}$; $\beta = -.25$), which were in strong LD with *CYP3A5* rs776746 ($r^2 = 0.70$). Two additional polymorphisms were in complete LD with rs776746, rs6465750, and rs4646457 ($r^2 = 1.0$). A LocusZoom plot for the *CYP3A* locus ±150 kB is presented in Figure 1.

Secondarily considering PharmGKB polymorphisms that were not in the GWAS Catalog, we were able to test for associations with 36 of 136 (26%). The other 100 failed imputation score, minor allele frequency, or Hardy-Weinberg equilibrium cutoffs. For bedaquiline clearance, the lowest *P* value for association among these 36 polymorphisms was *ERCC1* rs11615 ($P = .081$; $\beta = .12$). Considering polymorphisms previously associated with any GWAS Catalog trait, we were able to test for associations with 63 429 of 89 716 (71%). The lowest *P* value for association with bedaquiline clearance was *RIC8B* rs7977247 ($P = 1.1 \times 10^{-6}$; $\beta = -.18$). Considering genome-wide associations regardless of the GWAS Catalog, the lowest *P* value for association with bedaquiline clearance was for *RFX4* rs763450 ($P = 6.4 \times 10^{-7}$; $\beta = .20$). A genome-wide Manhattan plot for bedaquiline clearance is presented in Figure 2. The 5 lowest *P* value polymorphisms for association with bedaquiline clearance in each of the above stepwise-prioritized analyses are provided in Table 2. Only *CYP3A5* rs776746 withstood correction for multiple testing within any analysis. There were not strong genetic associations with between-individual variability in M2 clearance. For *CYP3A5* rs776746 and M2 clearance, $P = .25$.

We next assessed whether including *CYP3A5* rs776746 in the population pharmacokinetic model improved model fit. Based on log-likelihood profiling, rs776746 heterozygosity was associated with 15% slower (95% confidence interval, 3.5%–25.5%) and homozygosity with 30% slower (7.0%–51%) bedaquiline clearance. The model-predicted effects of rs776746 genotype on bedaquiline and M2 exposure are shown in the Supplementary Materials.

Genetic Associations With Between-Individual Variability in Clofazimine Clearance

As we did for bedaquiline, we characterized associations with between-individual variability in plasma clofazimine clearance. We were able to test for associations with 21 of 33 polymorphisms (64%) common to PharmGKB and the GWAS Catalog. For clofazimine clearance, the lowest *P* value

Table 1. Baseline Characteristics of Study Participants

Characteristic	Total PROBeX Cohort (n = 195)	Bedaquiline Genetic Analysis Group (n = 140)	Clofazimine Genetic Analysis Group (n = 136)
Age, median (IQR), y	33 (28–42)	33 (27–41)	33 (27–42)
Female sex, no. (%)	111 (57)	71 (51)	71 (51)
Race, no. (%)			
Black	160 (82)	112 (80)	106 (78)
Mixed race	33 (17)	26 (19)	28 (21)
White	2 (1)	2 (1)	2 (1)
BMI, median (IQR)[a]	20 (18–23)	20 (18–23)	20 (18–23)
HIV positive, no. (%)	123 (63)	82 (59)	80 (59)

Abbreviations: BMI, body mass index; HIV, human immunodeficiency virus; IQR, interquartile range; PROBeX, Pharmacokinetics, Resistance, and Outcomes of Bedaquiline in MDR- and XDR-TB.

[a]BMI calculated as weight in kilograms divided by height in meters squared.

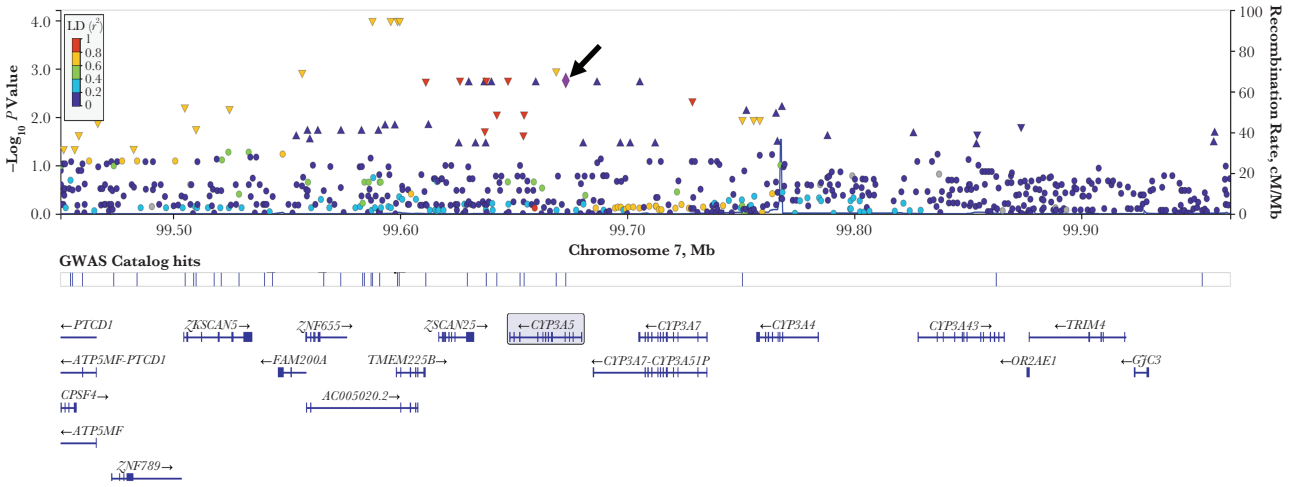

Figure 1. LocusZoom plot of associations with between-individual variability in bedaquiline clearance, across the *CYP3A* locus on chromosome 7. Represented are $-\log_{10}$ *P* values among 140 individuals for 1214 polymorphisms in the region, ranging from –150 kB from the 3′ end of *CYP3A5* to +150 kB from the 5′ end of *CYP3A4*. Purple diamond and arrow identify *CYP3A5* rs776746 (*CYP3A5*3* [*arrow*]), which is used as the reference for the linkage disequilibrium (LD) values in the graph. Each other marker is color coded to indicate r^2 value categories for LD with rs776746, as shown in the legend. These LD values are based on the "ALL" LocusZoom setting for the reference population

among these 21 polymorphisms was for *VKORC1* rs9923231 ($P = .13$; $\beta = .07$). Considering polymorphisms in PharmGKB but not the GWAS Catalog, we were able to test for associations with 36 of 140 (26%). The lowest *P* value among these 36 polymorphisms was for *IFNL3* rs11881222 ($P = .047$; $\beta = .34$). Considering polymorphisms previously associated with any trait in the GWAS Catalog, we were able to test for associations with 63 502 of 89 716 (71%). The lowest *P* value was for rs3827592, an intergenic chromosome 4 polymorphism ($P = 1.7 \times 10^{-5}$; $\beta = .29$). Considering genome-wide associations regardless of the GWAS Catalog, the lowest *P* value was for *CNTN5* rs75285763 ($P = 2.9 \times 10^{-8}$; $\beta = .46$). A genome-wide Manhattan plot for clofazimine clearance is presented in Figure 3. The lowest *P* value polymorphisms for association with clofazimine clearance stratified by each of the above stepwise-prioritized analyses are presented in Table 3. Only *CNTN5* rs75285763 was significant after correction for multiple testing within each analysis.

DISCUSSION

Bedaquiline and clofazimine are important for treating MDR tuberculosis. At the time of this writing, this is the first pharmacogenetic study of either bedaquiline or clofazimine. In our analyses that leveraged modeled pharmacokinetic data from the PROBeX cohort study of adults treated for MDR tuberculosis in South Africa, *CYP3A5* rs776746 was associated with slower plasma bedaquiline clearance ($P = .0017$). When included as a covariate in the population pharmacokinetic model, rs776746 was significant, with heterozygosity associated with 15% slower

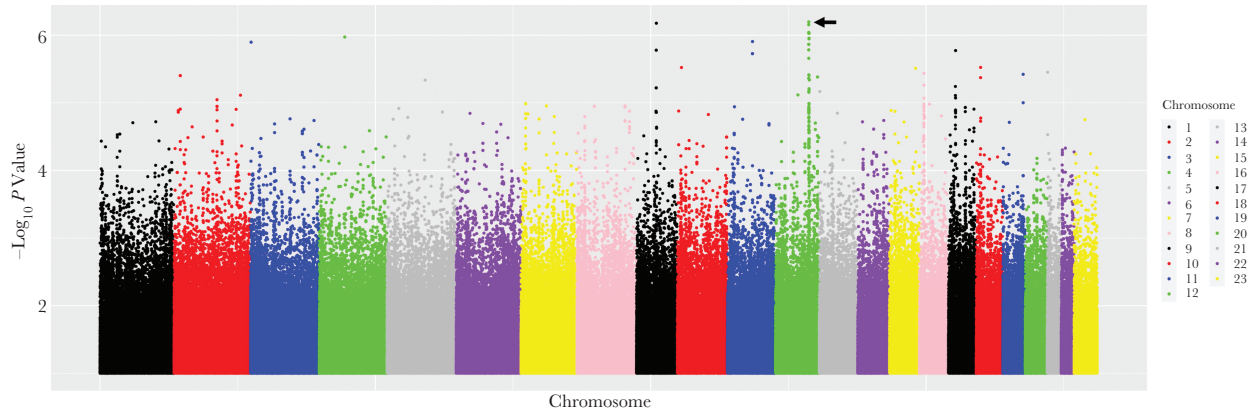

Figure 2. Manhattan plot of associations with between-individual variability in bedaquiline clearance. Shown are associations among 140 individuals who received bedaquiline during participation in PROBeX and were evaluable for genetic associations. The lowest *P* value is for *RFX4* rs763450 on chromosome 12 (*arrow*) ($P = 6.4 \times 10^{-7}$; $\beta = .20$).

Table 2. Lowest *P* Values for Genetic Association With Between-Subject Variability in Plasma Bedaquiline Clearance in 140 PROBeX Participants

Polymorphism	Gene	Chromosome	Reference Allele	Variant Allele	MAF	β Value	*P* Value
PharmGKB and GWAS Catalog (n = 21)[a]							
rs776746	*CYP3A5*	7	T	C	0.17	−.18	.0017
rs8050894	*VKORC1*	16	C	G	0.23	.11	.033
rs1800629	*TNF*	6	G	A	0.14	.11	.068
rs17782313	*MC4R*	18	T	C	0.24	−.09	.073
rs12979860	*IFNL4*	19	T	C	0.47	.04	.335
PharmGKB, not GWAS Catalog (n = 36)[a]							
rs11615	*ERCC1*	19	G	A	0.13	.12	.08
rs2359612	*VKORC1*	16	G	A	0.26	−.07	.14
rs7997012	*HTR2A*	13	G	A	0.05	− .11	.23
rs1045642	*ABCB1*	7	G	A	0.16	−.07	.24
rs2298383	*ADORA2A*	22	C	T	0.38	−.05	.27
GWAS Catalog (n = 63 502)[a]							
rs7977247	*RIC8B*	12	C	T	0.43	−.18	1.1×10^{-6}
rs10778495	*RFX4*	12	G	A	0.47	−.19	1.7×10^{-6}
rs10161520	*RFX4*	12	T	C	0.49	.17	1.2×10^{-5}
rs11113071	*RFX4*	12	T	C	0.45	.17	1.2×10^{-5}
rs10861637	*RFX4*	12	A	G	0.28	.19	1.3×10^{-5}
Genome-wide genotype data[b] (n = 9 074 402)[a]							
rs763450	*RFX4*	12	A	G	0.49	.20	6.4×10^{-7}
rs78277930	Intergenic	9	A	T	0.05	−.46	6.6×10^{-7}
rs80098193	Intergenic	9	A	G	0.05	−.46	6.6×10^{-7}
rs114384536	Intergenic	9	C	A	0.05	−.46	6.6×10^{-7}
rs12310706	*RFX4*	12	G	A	0.45	−.19	7.0×10^{-7}

Abbreviations: MAF, minor allele frequency; PROBeX, Pharmacokinetics, Resistance, and Outcomes of Bedaquiline in MDR- and XDR-TB.

[a]Parenthetical numbers are numbers of polymorphisms.

[b]All polymorphisms with *P* values $<1.0 \times 10^{-6}$ are shown.

and homozygosity with 30% slower clearance of bedaquiline. We also found a genome-wide significant association between *CNTN5* rs75285763 and slower plasma clofazimine clearance ($P = 2.9 \times 10^{-8}$). Because both bedaquiline and clofazimine accumulate slowly in the body with repeated dosing, it was important that population pharmacokinetic models were used to evaluate their disposition.

Several considerations suggest that the association between *CYP3A5* rs776746 and bedaquiline clearance is not by chance alone. First, bedaquiline is known to be metabolized by CYP3A4, and substrate specificity often overlaps between CYP3A4 and CYP3A5. The *CYP3A5* rs776746 polymorphism (where T = *CYP3A5*1* and C = *CYP3A5*3*) causes an alternatively spliced isoform that results in a premature stop codon

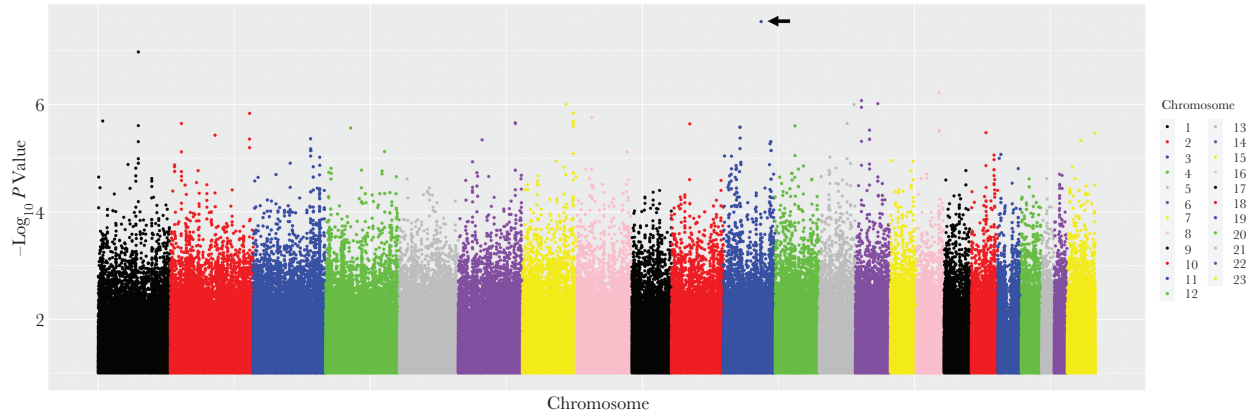

Figure 3. Manhattan plot of associations with unexplained variability in clofazimine clearance. Shown are associations among 136 individuals who received clofazimine during participation in PROBeX and were evaluable for pharmacogenomics associations. The lowest *P* value is for *CNTN5* rs75285763 on chromosome 11 (*arrow*) ($P = 2.9 \times 10^{-8}$; β = .46).

Table 3. Lowest *P* Values for Genetic Association With Unexplained Variability in Plasma Clofazimine Clearance in 136 PROBeX Participants

Polymorphism	Gene	Chromosome	Reference Allele	Variant Allele	MAF	β Value	*P* Value
PharmGKB and GWAS Catalog (n = 21)[a]							
rs9923231	*VKORC1*	16	C	T	0.07	−.11	.13
rs887829	*UGT1A10*	2	C	T	0.37	−.05	.17
rs8099917	Intergenic	19	T	G	0.07	.09	.21
rs489693	Intergenic	18	A	C	0.47	−.04	.23
rs1800629	*TNF*	6	G	A	0.14	.06	.24
PharmGKB, not GWAS Catalog (n = 36)[a]							
rs11881222	*IFNL3*	19	A	G	0.34	.07	.047
rs2359612	*VKORC1*	16	G	A	0.25	−.07	.058
rs1042713	*ADRB2*	5	G	A	0.42	.06	.083
rs2740574	*LOC110366354*	7	C	T	0.29	−.06	.115
rs9934438	*VKORC1*	16	G	A	0.07	−.11	.126
GWAS Catalog (n = 63 502)[a]							
rs3827592	Intergenic	4	G	A	0.29	−.17	1.73×10^{-5}
rs7689452	Intergenic	4	A	G	0.29	−.17	1.96×10^{-5}
rs79709502	LINC01800	2	C	G	0.20	.19	2.84×10^{-5}
rs5749446	FBXO7	22	T	C	0.37	−.16	3.21×10^{-5}
rs3827335	FBXO7	22	A	G	0.38	−.16	3.64×10^{-5}
Genome-wide genotype data[b] (n = 9 074 402)[a]							
rs75285763	*CNTN5*	11	A	G	0.04	.46	2.87×10^{-8}
rs35274012	Intergenic	1	G	A	0.16	.25	1.05×10^{-7}
rs562673502[c]	Intergenic	16	C	A	0.05	.39	6.05×10^{-7}
rs147293114	*NPAS3*	14	TTTAG	T	0.05	.40	8.47×10^{-7}
rs140444407	Intergenic	14	C	T	0.06	−.35	9.62×10^{-7}
rs11769507	Intergenic	7	C	T	0.15	.23	9.910×10^{-7}

Abbreviations: MAF, minor allele frequency; PROBeX, Pharmacokinetics, Resistance, and Outcomes of Bedaquiline in MDR- and XDR-TB.

[a]Parenthetical numbers are numbers of polymorphisms.

[b]All polymorphisms with $P < 1.0 \times 10^{-6}$ are shown.

[c]Polymorphism rs562673502 was in complete linkage with rs534375032, rs147681927, rs569881175, rs148413871, rs141244461, and rs141114616 in our imputed genotype data, so it has identical association results.

and nonfunctional CYP3A5 protein [40]. Among individuals homozygous for *CYP3A5*3*, CYP3A5 comprises only 5% of hepatic CYP3A expression, compared with as much as 50% among individuals carrying ≥1 copy of *CYP3A5*1* [39]. The *CYP3A5*3* allele also predicts plasma exposure of the immunosuppressant drug, tacrolimus [41]. Second, among 21 polymorphisms common to PharmGKB and the GWAS Catalog, and for which we characterized associations, *CYP3A5* rs776746 had the lowest *P* value, was in the expected direction (ie, the C allele with slower bedaquiline clearance), and withstood correction for multiple testing. Finally, considering 1214 polymorphisms within the *CYP3A* locus, the polymorphisms with the lowest *P* value for association with bedaquiline clearance were in strong LD with *CYP3A5* rs776746. Despite these considerations, the association between *CYP3A5* rs776746 and bedaquiline clearance should be replicated in independent cohorts.

We found no association between *CYP3A5* rs776746 and clearance of M2. As speculation, this could be explained by CYP3A isoforms being involved in both generating M2 from bedaquiline, and in metabolizing M2 to M3, which may make it harder to detect an effect on M2 clearance.

The *CYP3A5* rs776746 loss-of-function C allele varies markedly in frequency depending on ancestry, ranging from approximately 30% among Africans to 70% among East Asians and 93% among Europeans [42]. Thus, Africans overall express substantially more CYP3A5 than do other populations. This may help to explain the 52% greater apparent clearance of bedaquiline among individuals identified as black in a previous population pharmacokinetic model [14]. In the present study the minor allele frequency of *CYP3A5* rs776746 was 17%. Given that rs776746 is relatively frequent and that higher bedaquiline exposure may improve mycobacterial treatment responses [8, 9], genetic testing to guide bedaquiline dosing has the potential to improve treatment outcomes.

Regarding clofazimine clearance, we found a genome-wide significant association with *CNTN5* rs75285763. We suspect that this is by chance alone. The gene *CNTN5* encodes contactin 4, a member of the immunoglobulin superfamily [43]. Contactin 4 is a neuronal cell membrane adhesion molecule that helps to form axon connections in the developing nervous system. This protein seems unlikely to affect clofazimine exposure. In addition, this polymorphism is infrequent, with a

minor allele frequency of only 0.05 in our study. Furthermore, no pharmacogenes reside near *CNTN5* on chromosome 11, and the *P* value for rs75285763 ($P = 2.9 \times 10^{-8}$) was barely significant genome wide.

Other than the *CYP3A* locus with bedaquiline, there was no strong a priori evidence for an association of any specific polymorphisms or genes with bedaquiline or clofazimine pharmackinetics. For this reason, and to decrease the burden of multiple testing, we used an approach that leveraged the imputed genome-wide data generated with the PROBeX cohort against the vast knowledge generated by prior genetic association studies represented in PharmGKB [36] and the GWAS Catalog [37]. We reasoned that polymorphisms associated with ≥1 drug-related phenotype in PharmGKB with levels of evidence of 1 or 2 (as described in Methods), and also associated with any trait in the GWAS Catalog at $P < 5.0 \times 10^{-8}$ in ≥1 published study, would most likely be true-positives in the present study. This approach appeared to work well for *CYP3A5* rs776746, which was common to PharmGKB and the GWAS Catalog. For secondarily prioritized analyses, we did not identify compelling associations (ie, in PharmGKB but not the GWAS Catalog, in the GWAS Catalog regardless of PharmGKB, and genome wide regardless of the GWAS Catalog),

The present study had limitations. The sample size was relatively modest. However, unlike genome-wide studies of some complex traits such as diabetes, large effect sizes with pharmacogenes and off-target genes often reveal significant associations with small sample sizes. The modest sample size limited our ability to detect associations with infrequent polymorphisms, those with small effect sizes, or those not previously associated with a drug or trait in PharmGKB or the GWAS Catalog. In addition to bedaquiline and clofazimine, cohort participants were receiving multiple medications to treat tuberculosis and HIV-1. Although not anticipated, it is conceivable that interactions between these drugs and bedaquiline or clofazimine may have obscured genetic associations. Finally, the present analyses focused on a cohort of African ancestry. Results may be different in other populations.

In summary, among patients treated for MDR tuberculosis in a prospective, observational cohort study in South Africa, the *CYP3A5*3* loss-of-function allele was associated with slower plasma clearance of bedaquiline. The variable frequency of this allele between populations suggests that *CYP3A5*3* may help to explain the more rapid clearance of bedaquiline reported among Africans. This association should be considered tentative until replicated in independent cohorts. A genome-wide significant association between a *CNTN5* polymorphism and plasma clearance of clofazimine is likely by chance alone.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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