TITLE PAGE

Version: April 11, 2017

Title: Polymorphisms in the vitamin D receptor gene are associated with reduced rate of sputum culture conversion in multidrug-resistant tuberculosis patients in South Africa

Authors: Matthew J Magee (1), Yan V. Sun (2), James CM Brust (3), N. Sarita Shah (5), Yuming Ning (3), Salim Allana (2), Angela Campbell (2), Qin Hui (2), Koleka Mlisana (4), Pravi Moodley(4), Neel R Gandhi (2,6,7)

Authors' Affiliations:

1. Division of Epidemiology and Biostatistics, School of Public Health, Georgia State University, Atlanta, USA
2. Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, USA
3. Divisions of General Internal Medicine and Infectious Diseases, Albert Einstein College of Medicine, Bronx, USA
4. School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal, Durban, South Africa
5. US Centers for Disease Control and Prevention, Atlanta, USA
6. Division of Infectious Diseases, Emory School of Medicine, Emory University, Atlanta, USA
7. Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, USA

Keywords: vitamin D, vitamin D receptor gene, genetic polymorphisms, South Africa
Short title: Vitamin D receptor gene polymorphisms and MDR TB
Word count: Abstract: 298; **Manuscript:** 2664

Corresponding Author:
Matthew Magee, PhD, MPH
Email: mjmagee@gsu.edu Tel: +1 404 413 1797

Author contributions: MJM, YVS, JCMB, SS, and NRG conceived and designed the study and drafted the initial manuscript. MJM, YVS, YN, and QH performed the data analyses. All authors contributed to interpretation of the data, revised the manuscript, and approved the final version.

The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention (CDC). The use of trade names and commercial sources is for identification only and does not imply endorsement by the CDC.

Abstract

Background: Vitamin D modulates the inflammatory and immune response to tuberculosis (TB) and also mediates the induction of the antimicrobial peptide cathelicidin. Deficiency of 25-hydroxyvitamin D and single nucleotide polymorphisms (SNPs) in the vitamin D receptor (VDR) gene may increase the risk of TB disease and decrease culture conversion rates in drug susceptible TB. Whether these VDR SNPs are found in African populations or impact multidrug-resistant (MDR) TB treatment has not been established. We aimed to determine if SNPs in the VDR gene were associated with sputum culture conversion among a cohort of MDR TB patients in South Africa.

Methods: We conducted a prospective cohort study of adult MDR TB patients receiving second-line TB treatment in KwaZulu-Natal province. Subjects had monthly sputum cultures performed. In a subset of participants, whole blood samples were obtained for genomic analyses. Genomic DNA was extracted and genotyped with Affymetrix Axiom Pan-African Array. Cox proportional models were used to determine the association between VDR SNPs and rate of culture conversion.

Results: Genomic analyses were performed on 91 MDR TB subjects enrolled in the sub-study; 60% were female and median age was 35 years (interquartile range [IQR] 29-42). Smoking was reported by 21% of subjects and most subjects had HIV (80%), were smear negative (57%), and had cavitary disease (55%). Overall, 87 (96%) subjects initially converted cultures to negative, with median time to culture conversion of 57 days (IQR 17-114). Of 121 VDR SNPs examined, 10 were significantly associated ($p<0.01$) with rate of sputum conversion in multivariable analyses. Each additional risk

allele on SNP rs74085240 delayed culture conversion significantly (adjusted hazard ratio 0.30, 95% confidence interval 0.14-0.67).

Conclusions: Polymorphisms in the VDR gene were associated with rate of sputum culture conversion in MDR TB patients in this high HIV prevalence setting in South Africa.

Introduction

In 2015 there were an estimated 480,000 cases of multidrug-resistant (MDR) tuberculosis (TB) worldwide [1]. MDR TB (resistance to at least isoniazid and rifampin) treatment is less effective, more toxic and costly compared to drug susceptible TB [2]. Importantly MDR TB is associated with poor TB treatment outcomes and increased risk of death [3,4], in 2014 there were an estimated 190,000 deaths from MDR TB [5]. Despite global efforts, MDR TB remains difficult to diagnose and treat, and few new therapeutic options are available [6].

Given the paucity of new drugs available for the treatment of MDR TB there has been substantial clinical interest in adjunctive use of 25-hydroxyvitamin D (vitamin D) to improve TB—including MDR TB—treatment outcomes [7]. Vitamin D has anti-inflammatory and anti-bacterial properties that could theoretically improve clinical TB outcomes. The active metabolite of vitamin D, calcitriol, mediates innate immune responses via the induction of the antimicrobial peptide cathelicidin and reactive oxygen intermediates. Vitamin D also promotes macrophage-mediated killing of *Mycobacterium tuberculosis* and modulates both anti-inflammatory and pro-inflammatory T-helper responses to TB [8,9]. Low exposure to solar ultraviolet light, inadequate intake of vitamin D and its precursors, or particular genotypes of the vitamin D receptor (VDR) may lead to vitamin D deficiency which, in turn, could inhibit reduction of bacillary burden, inhibit culture conversion, and impair the effectiveness of TB treatment. Despite the hypothesized plausibility that vitamin D supplementation may improve TB treatment outcomes and the pervasiveness of vitamin D deficiency, clinical trials to date among

patients with drug susceptible TB have not demonstrated efficacy in improving rate of sputum culture conversion [10-12]. However, evidence suggests that the effects of vitamin D may vary based on vitamin D receptor (VDR) genotypes, implying that supplementation may only be of clinical benefit in subpopulations with a particular VDR genotype [13].

In certain ethnic populations, single nucleotide polymorphisms (SNPs) in the vitamin D receptor (VDR) gene may increase the risk of TB disease [14]. Three previous studies have reported an association between VDR gene polymorphisms and smear or culture conversion time in patients with pulmonary TB [15-17]. However, the extent to which VDR SNPs are found in African populations or impact MDR TB treatment is limited. South Africa has a high burden of drug-resistant TB, with an estimated 13,000 MDR TB cases in 2014 and 2% MDR TB prevalence rate among all new TB cases [5]. In this context, we aimed to determine if SNPs in the VDR gene were associated with time to sputum culture conversion among a cohort of MDR TB patients in South Africa.

Methods

Parent Study

The SHOUT study was a prospective observational cohort study among patients receiving second-line TB treatment for MDR TB from three sites in KwaZulu-Natal province, South Africa between 2011 and 2015. KwaZulu-Natal is the South African province that has been most severely affected by TB (incidence: 1076 cases per 100,000 population) and HIV (prevalence: 17%) [18,19]. Patients 18 years or older were

eligible to participate if they had a sputum culture positive for *M. tuberculosis* with phenotypic resistance to both isoniazid and rifampin. Patients with unknown HIV status at the time of enrollment were offered an HIV test. Patients were excluded if they had previous MDR TB treatment, resistance to either fluoroquinolones or injectable TB medications, renal or hepatic dysfunction, or were pregnant. Subjects were treated with the standardized South African MDR TB regimen of kanamycin, moxifloxacin, ethionamide, terizidone, ethambutol, and pyrazinamide. Kanamycin was typically given for a minimum of 6 months, or 4 months after culture-conversion, and oral medications were continued without kanamycin for an additional 12-18 months after culture-conversion. All HIV co-infected participants were initiated on ART within 2 months of MDR TB treatment initiation, regardless of CD4 count, if they were not already receiving ART. Standard ART regimens consisted of efavirenz, and stavudine and lamivudine prior to 2013, or tenofovir and emtricitabine afterwards. Study participants were seen monthly for follow-up for the duration of MDR TB treatment, which is typically 21-24 months.

Study population, Measures and Definitions

The current study was conducted among a subset of patients from the parent SHOUT MDR TB study who consented to have whole blood samples collected for genomic analyses. Sub-study patients had the same inclusion/exclusion criteria as the parent study and were enrolled beginning in April 2013 until the target (n=100) was reached.

Measures and Definitions

The primary outcome of interest for the study was time to initial sputum culture conversion. Sputum cultures were performed monthly during MDR TB treatment. Mycobacterial cultures and drug-susceptibility testing (DST) were performed as previously described [4]. Time to sputum culture conversion was defined by the number of days between MDR TB treatment initiation and the first of two negative sputum cultures [20]. Patients who converted sputum cultures to negative before the start of MDR TB treatment were defined as having a 1 day conversion time. A secondary outcome of interest was poor tuberculosis treatment outcome and was defined as a patient who died, interrupted treatment, or had treatment failure.

The primary exposures of interest were VDR gene polymorphisms. Participant samples were genotyped using the Affymetrix® Axiom Pan-African Array according to the manufacturer's instructions [21]. Quality checks were performed to ensure the overall SNP call rate was $\geq$95% and that there was no sex mismatch between genotypic and phenotypic measurement. SNPs were excluded if they had an unknown chromosomal location, a call rate less than 95%, a Hardy-Weinberg Equilibrium (HWE) *p*-value less than 0.0001 or a minor allele frequency (MAF) less than 0.05. After quality control filters, 1,494,763 SNPs were available for genetic analysis. South African samples were pooled with HapMap EUR, YRI and ASW populations to identify population structure relative to European and West African ancestry [22]. Top principal components (PCs) were calculated using independent SNPs after pruning by pair-wise linkage disequilibrium R^2 larger than 0.1 within windows of 50 SNPs. Using base pair location of

human genome build 37, there were 121 SNPs annotated to the VDR gene and passed quality control filters.

Smoking status and alcohol use were self-reported by study patients. Both smoking status and alcohol use were categorized dichotomously as current use (yes/no).

Statistical Analyses

We examined 121 VDR SNPs to assess their association with rate of sputum culture conversion among patients with MDR TB. Each SNP was coded using the additive genetic effect. Cox proportional models were used to determine the association between VDR SNPs (additive effect) and the hazard rate of initial sputum culture conversion. Patients who had a positive culture at time of MDR TB diagnosis but converted sputum cultures to negative before the start of MDR TB treatment were censored at day 1. Patients who died or failed treatment before an initial sputum culture conversion were censored. All models were adjusted for age, sex, smoking status, alcohol, AFB smear status, HIV status, and cavitary disease. For the secondary outcome of TB treatment result, we used logistic regression to estimate the odds of poor TB treatment outcome among a subset of SNPs associated with reduced rate of culture conversion. Patients who withdrew from the study before treatment completion were excluded from the secondary outcome analysis.

Ethics

The study protocol was approved by the institutional review boards at the University of KwaZulu-Natal, Albert Einstein College of Medicine, and Emory University, and by the KwaZulu-Natal Department of Health and CDC's National Center for HIV, Hepatitis, STDs and Tuberculosis. All participants signed written informed consent.

Results

From 2011-2013, the parent cohort study screened 365 patients and enrolled 206. Among patients enrolled in the parent study, 103 provided samples for the present sub-study, 7 were not processed due to DNA extraction errors, and 5 patients were late excluded from the parent study due to second line drug resistance. The 91 remaining participants were included in the current sub-study for genomic analyses and of these, 55 (60%) were female and the median age was 35 years (interquartile range [IQR] 29-42) (Table 1). Most patients were HIV co-infected (n=73, 80%), had undetectable viral load (n=23/42, 55%) and the median baseline CD4 count of 199 cells/mm^3 (IQR 143-289). Thirty-nine (43%) patients were AFB smear positive, 50 (55%) had cavitary disease, and 74 (81%) had previously been treated for TB. Smoking was reported by 21% of patients.

All samples passed standard genome-wide association study (GWAS) quality control filters with the lowest individual level SNP call rate of 98.2%. Using top two PCs from GWAS data (Figure 1), we observed the separation of South Africans from populations with known African ancestry (YRI and ASW), and European ancestry (EUR). PC1 clustered European versus African ancestry, while PC2 further distinguished West

Africans and South Africans. Within South African samples in this study, no outlier (3SD) was observed using top 10 PCs, indicating a genetically homogeneous population.

Overall, 87 (96%) subjects converted sputum cultures to negative. Of the 87 who converted sputum cultures to negative, 24% (21/87) were positive at the time of MDR TB diagnosis but converted to negative before the time of MDR TB treatment start. The median time to culture conversion was 57 days (IQR 17-114); among patients who were not culture negative at time of treatment initiation the median time to conversion was 82 days (IQR 53-143). Among patients who converted, 50 (55.0%) were culture negative by two months of second-line treatment (Table 1). Compared to females, males were significantly more likely to be sputum culture positive at two months (36.4% [20/55] vs. 58.3% [21/36], p=0.04).

Of 121 VDR SNPs examined, 10 were significantly associated ($p<0.05$) with hazard rate of sputum culture conversion in multivariable analyses (Table 2). The estimated slower conversion rate (adjusted hazard ratio of sputum culture conversion <1.0) ranged from 0.30 (95% CI 0.14-0.67) for rs74085240 to 0.64 (95%CI 0.42-0.98) for rs11168287. For example, each additional risk allele on SNP rs74085240 delayed the rate of culture conversion by 70% (aHR 0.55, 95% CI 0.36-0.85). Two VDR SNPs (rs11168327 and rs11574143) were associated with significantly improved rate (adjusted hazard ratio >1.0) of culture conversion (Table 2).

Overall, 19% (17/88) of patients had a poor TB treatment outcome and 3 additional patients withdrew treatment. We did not detect any genotypes associated with a significant increased odds of poor TB treatment outcome (Table 3).

Discussion

We examined 121 SNPs in the VDR gene region and found a subset to be associated with rate of sputum culture conversion among patients with MDR TB in South Africa. Specifically, we identified 9 VDR SNPs that were associated with an estimated 50% to 25% reduced (delayed conversion) rate of sputum culture conversion among patients receiving second-line TB therapy. Our findings provide new data about the relationship between VDR polymorphisms and sputum culture conversion among patients with MDR TB in South Africa.

Our findings in patients with MDR TB are consistent with three previous studies that reported significantly lower rates of sputum culture conversion among patients with drug susceptible pulmonary TB who had specific VDR polymorphisms [10,15,17]. First, an observational longitudinal study among 78 Peruvian patients with confirmed pulmonary TB reported that patients with the *TT TaqI* genotype (previous nomenclature now typically replaced by endonuclease digestion pattern) had significantly longer time to sputum culture conversion (median 46 days for *TT* genotype vs. 16 days for *Tt* genotype) [15]. Second, in 2011 Martineau et al conducted a randomized control trial in London, testing the efficacy of 2.5mg vitamin D_3 supplementation to reduce culture conversion time in smear positive TB patients [10]. The trial reported no overall effect of

supplementation on culture conversion rates, but the authors did report an interaction between vitamin D_3 supplementation and culture conversion with *TaqI* genotype. Specifically, supplementation was beneficial among patients with *tt* genotype (HR 8.09, 95%CI 1.36-48.01). Unlike the studies from Peru and London, we did not observe an association between rs731236 (*TaqI* genotype defined by endonuclease digestion pattern) and rate of sputum culture conversion. Third, an observational longitudinal study of HIV-negative patients with pulmonary TB from the Western Cape of South Africa reported that a significantly lower proportion of patients with *ApaI aa* genotype had converted cultures by month 2 of TB treatment compared to patients with *ApaI Aa* genotype (26% vs. 51%, p=0.03) [17]. All three studies are consistent with our findings that VDR polymorphisms are associated with rate of culture conversion. Unlike the previous studies, our study identified SNPs significantly associated with rate of culture conversion in patients with MDR TB.

To our knowledge, only one previous study examined the association between VDR genotypes and time to culture conversion in patients with MDR TB. In 2012 Rathored et al. followed 236 HIV-negative patients with MDR TB during DOTS-Plus treatment in India and reported no significant differences in bivariate analyses between the three VDR genotypes examined (*BsmI, TaqI, FokI*) and time to culture conversion [16]. However, in our study we examined 121 specific SNPs on the VDR gene region and adjusted for important confounding factors (i.e., smoking status); Rathored et al. did not adjust for confounding which may partially explain why we reported significant differences in rates of culture conversion and the previous study did not. Similar to the

study in India, we did not observe a significant association between *BsmI* (rs1544410) or *TaqI* (rs731236) genotypes and time to culture conversion.

Several hypothesized biologic mechanisms may explain why polymorphisms in the VDR gene are associated with rate of sputum culture conversion in patients with MDR TB. Although vitamin D supplementation has not been demonstrated to be efficacious in improving culture conversion time in drug-susceptible TB treatment [10-12], vitamin D likely has a role in TB treatment-induced modulation of circulating immunologic signals. Circulating immune signals are affected by TB treatment alone, for example interleukin (IL)-10, cathelicidin LL-37, and neutrophil gelatinase-associated lipocalin (NGAL) are suppressed by medications administered during the intensive phase TB treatment. Moreover, a randomized trial from London demonstrated that vitamin D supplementation enhanced the immune effects of TB treatment. In the trial of 126 smear-positive pulmonary TB patients, Coussens et al. reported that patients receiving vitamin D supplementation during first-line treatment had TB treatment-induced increases in lymphocytes and reduced concentrations of inflammatory markers [23]. Therefore, it is plausible that effects of second-line TB treatment on the immune responses may be modified differently by vitamin D compared to first-line TB treatment immune modulation by vitamin D.

Our study was subject to limitations. First, we did not measure plasma levels of vitamin D or calcitriol. Therefore, we were unable to verify if vitamin D levels were affected by polymorphisms in the VDR SNPs or if the polymorphisms affected culture conversion

299 directly. Second, we did not measure any immune modulating signals. Vitamin D is
300 hypothesized to affect sputum culture conversion through immune modulation of
301 cytokines (interferon-gamma, IL-2, IL-12) chemokines (chemokine ligand (CXCL)-9,
302 CXCL-10, matrix metallopeptidase-9) and antigen stimulated responses (Th1) [23].
303 Consequently, we were unable to determine if VDR SNPs influenced the expression of
304 immune modulating signals that may affect rate of culture conversion. Third, our sample
305 had relatively low power and we were therefore unable to adjust statistical tests for
306 multiple comparisons. We did not have power to adjust for covariates in the logistic
307 regression models that were used to estimate the odds of poor MDR TB treatment
308 outcome by VDR SNPs. Fourth, we did not examine sputum culture reversions to
309 positive. The analysis only analyzed the association between VDR SNPs and the
310 patients' first sputum culture conversion and therefore does not assess the association
311 with sustained conversion.
312
313 Despite limitations, our study had several strengths. Foremost, we examined 121
314 specific SNPs in the VDR gene region among patients with MDR TB from a genetically
315 distinct population. Previous similar studies have focused on VDR polymorphisms at the
316 level of *FokI*, *ApaI*, and *TaqI* genotypes but did not measure specific SNPs and only one
317 previous study enrolled patients with TB from South Africa (which included only drug-
318 susceptible patients). Second, our study only included patients with culture- and DST-
319 confirmed MDR TB and followed patients monthly to obtain cultures during second-line
320 TB treatment. Previous studies examining the association between VDR polymorphisms
321 and response to TB treatment were primarily among drug susceptible patients, included

patients without culture confirmed TB, largely examined sputum smear conversion at one time point, and few adjusted for key confounders [24,25].

Acknowledgements

We are grateful to the study team at the University of KwaZulu-Natal for their tireless efforts in data collection, record abstraction, participant recruitment and interviews. Finally, we thank the participants and their families who consented to participate in this study.

References

1. WHO (2016) Global tuberculosis report 2016.
2. Centers for Disease C, Prevention (2013) Provisional CDC guidelines for the use and safety monitoring of bedaquiline fumarate (Sirturo) for the treatment of multidrug-resistant tuberculosis. MMWR Recomm Rep 62: 1-12.
3. Gandhi NR, Nunn P, Dheda K, Schaaf HS, Zignol M, et al. (2010) Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. Lancet 375: 1830-1843.
4. Gandhi NR, Moll A, Sturm AW, Pawinski R, Govender T, et al. (2006) Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet 368: 1575-1580.
5. WHO (2015) Global tuberculosis report 2015. Geneva: World Health Organization.
6. WHO (2016) WHO Treatment guidelines for drug-resistant tuberculosis – 2016 update.
7. Cegielski P, Vernon A (2015) Tuberculosis and vitamin D: what's the rest of the story? Lancet Infect Dis 15: 489-490.
8. Bruns H, Stenger S (2014) New insights into the interaction of Mycobacterium tuberculosis and human macrophages. Future Microbiol 9: 327-341.
9. Coussens AK, Martineau AR, Wilkinson RJ (2014) Anti-Inflammatory and Antimicrobial Actions of Vitamin D in Combating TB/HIV. Scientifica (Cairo) 2014: 903680.
10. Martineau AR, Timms PM, Bothamley GH, Hanifa Y, Islam K, et al. (2011) High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. Lancet 377: 242-250.
11. Tukvadze N, Sanikidze E, Kipiani M, Hebbar G, Easley KA, et al. (2015) High-dose vitamin D3 in adults with pulmonary tuberculosis: a double-blind randomized controlled trial. Am J Clin Nutr 102: 1059-1069.
12. Daley P, Jagannathan V, John KR, Sarojini J, Latha A, et al. (2015) Adjunctive vitamin D for treatment of active tuberculosis in India: a randomised, double-blind, placebo-controlled trial. Lancet Infect Dis 15: 528-534.
13. Ralph AP, Lucas RM, Norval M (2013) Vitamin D and solar ultraviolet radiation in the risk and treatment of tuberculosis. Lancet Infect Dis 13: 77-88.
14. Gao L, Tao Y, Zhang L, Jin Q (2010) Vitamin D receptor genetic polymorphisms and tuberculosis: updated systematic review and meta-analysis. Int J Tuberc Lung Dis 14: 15-23.
15. Roth DE, Soto G, Arenas F, Bautista CT, Ortiz J, et al. (2004) Association between vitamin D receptor gene polymorphisms and response to treatment of pulmonary tuberculosis. J Infect Dis 190: 920-927.
16. Rathored J, Sharma SK, Singh B, Banavaliker JN, Sreenivas V, et al. (2012) Risk and outcome of multidrug-resistant tuberculosis: vitamin D receptor polymorphisms and serum 25(OH)D. Int J Tuberc Lung Dis 16: 1522-1528.
17. Babb C, van der Merwe L, Beyers N, Pheiffer C, Walzl G, et al. (2007) Vitamin D receptor gene polymorphisms and sputum conversion time in pulmonary tuberculosis patients. Tuberculosis (Edinb) 87: 295-302.

18. Shisana O, Rehle T, Simbayi L, Zuma K, Jooste S, et al. (2014) South African National HIV Prevalence, Incidence and Behaviour Survey, 2012. Cape Town: HSRC Press.
19. Ndjecka N (2014) Multi-drug resistant tuberculosis: strategic overview on MDR-TB care in South Africa. Pretoria: Department of Health, Republic of South Africa.
20. Brust JC, Lygizos M, Chaiyachati K, Scott M, van der Merwe TL, et al. (2011) Culture conversion among HIV co-infected multidrug-resistant tuberculosis patients in Tugela Ferry, South Africa. PLoS One 6: e15841.
21. Scientific ATF Axiom® Genome-Wide Population-Optimized Human Arrays Technical Documentation
22. International HapMap C, Frazer KA, Ballinger DG, Cox DR, Hinds DA, et al. (2007) A second generation human haplotype map of over 3.1 million SNPs. Nature 449: 851-861.
23. Coussens AK, Wilkinson RJ, Hanifa Y, Nikolayevskyy V, Elkington PT, et al. (2012) Vitamin D accelerates resolution of inflammatory responses during tuberculosis treatment. Proc Natl Acad Sci U S A 109: 15449-15454.
24. Salahuddin N, Ali F, Hasan Z, Rao N, Aqeel M, et al. (2013) Vitamin D accelerates clinical recovery from tuberculosis: results of the SUCCINCT Study [Supplementary Cholecalciferol in recovery from tuberculosis]. A randomized, placebo-controlled, clinical trial of vitamin D supplementation in patients with pulmonary tuberculosis'. BMC Infect Dis 13: 22.
25. Sutaria N, Liu CT, Chen TC (2014) Vitamin D Status, Receptor Gene Polymorphisms, and Supplementation on Tuberculosis: A Systematic Review of Case-Control Studies and Randomized Controlled Trials. J Clin Transl Endocrinol 1: 151-160.

403 Table 1. Baseline participant characteristics and 2-month sputum culture status

Characteristic	Total N=91 N (%)	Sputum culture negative at 2-months N=50 (55.0) N (%)	Sputum culture positive at 2-months N=41 (45.1) N (%)	*P* value[A]
Male	36 (39.6)	15 (30.0)	21 (51.2)	0.04
Median age, years (IQR)	35 (29-42)	35 (27-41)	38 (32-42)	0.12
Current smoker[B]	19 (20.9)	7 (14.0)	12 (29.3)	0.07
Alcohol[B]	28 (30.7)	14 (28.0)	14 (34.2)	0.53
Baseline AFB positive	39 (42.9)	18 (36.0)	21 (51.2)	0.14
Baseline cavity	50 (55.0)	26 (52.0)	24 (58.5)	0.53
Median baseline BMI (IQR) N=82	21.6 (18.5-24.6)	21.8 (19.4-24.4)	21.3 (16.8-24.9)	0.42
Previous TB treatment	74 (81.3)	40 (80.0)	34 (82.9)	0.72
HIV seropositive	73 (80.2)	40 (80.0)	33 (80.5)	0.95
Median baseline CD4 (IQR) N=46[C]	199 (143-289)	185 (125-266)	221 (143-309)	0.61
On ARV at baseline[C] N=57	44 (75.7)	24 (75.0)	20 (76.9)	0.66
Median baseline viral load (IQR) N=42[C]	83 (39-13000)	65 (39-75278)	100 (39-3200)	0.94
Undetectable viral load N=42[C]	23 (54.8)	13 (56.5)	10 (52.6)	0.80

404

405 Table 1 abbreviations: IQR-interquartile range; AFB-acid fast bacilli; ARV-antiretroviral

406 A. 2-side chi-square p-value, except for age (2-sided Wilcoxon rank sum)
407 B. Self-reported
408 C. Among HIV positive only

409

Table 2. Hazard of sputum culture conversion by vitamin D receptor gene single nucleotide polymorphism

SNP	Model N	Days[A] (IQR)	HR	95% CI	*P* value[B]	Adjusted N	aHR[C]	95% CI	*P* value[B]
rs74085240	88	57 (17-113)	0.54	0.28-1.07	0.077	88	0.30	0.14-0.67	0.003
rs1015390	88	55 (19-90)	0.67	0.46-0.99	0.045	88	0.54	0.35-0.82	0.004
rs4073729	91	56 (27-114)	0.72	0.50-1.05	0.085	91	0.56	0.37-0.85	0.006
rs11168268	91	55 (1-111)	0.63	0.42-0.94	0.024	91	0.55	0.36-0.85	0.008
rs2525044	91	55 (1-111)	0.57	0.33-0.97	0.038	91	0.46	0.24-0.86	0.015
rs11168287	91	57 (19-122)	0.76	0.52-1.09	0.139	91	0.64	0.42-0.98	0.040
rs2238139	91	55 (1-108)	0.66	0.43-1.01	0.054	91	0.60	0.37-0.98	0.042
rs11574138	91	57 (1-113)	0.78	0.39-1.57	0.486	91	0.43	0.19-1	0.049
rs11168327	91	84 (41-131)	1.30	0.90-1.88	0.155	91	1.81	1.16-2.84	0.009
rs11574143	91	72 (26-156)	1.73	1.02-2.93	0.041	91	2.05	1.16-3.63	0.014

Table 2A abbreviations: IQR-interquartile range; SNP-single nucleotide polymorphism; CI-confidence interval; rs-reference SNP

A. Median days to sputum culture conversion among SNPs carrying 0 effect alleles
B. Wald test p-value, SNPs are listed in ascending order based on p-value
C. Hazard ratios estimated from Cox Proportional regression models adjusted for age, sex, smoking status, alcohol, AFB smear status, HIV status, and cavitary disease; SNPs modelled additively, hazard ratio indicates per additional risk allele on each SNP.

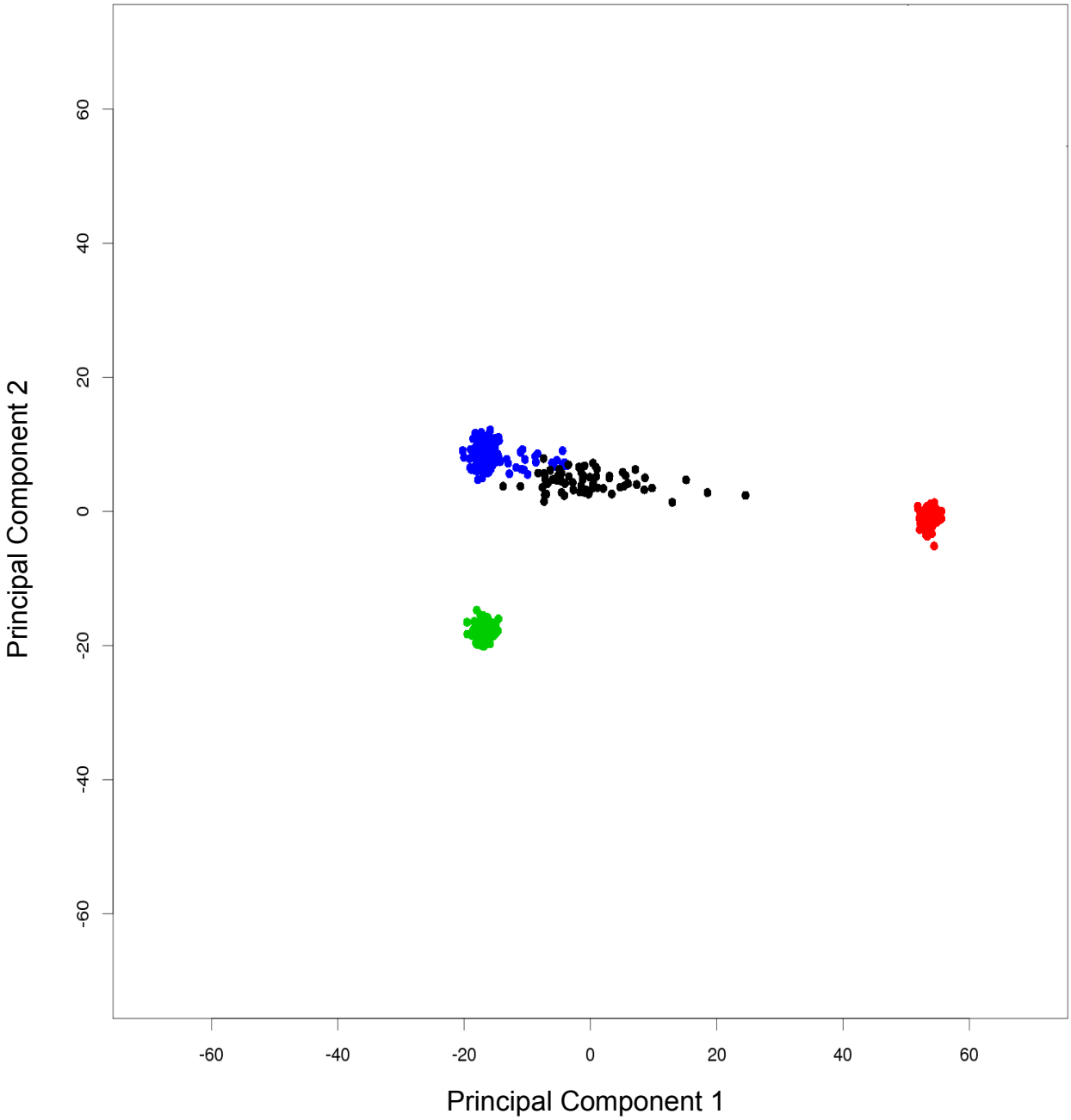
Table 3. Poor tuberculosis treatment outcome by vitamin D receptor gene single nucleotide polymorphism, N=88

SNP	Total N=88	Poor Outcome[A] N=17 (19.3) N (%)	Cured/Completed N=71 (80.7) N (%)	Odds ratio (95% CI)
rs74085240*				
CC	2	1 (50.0)	1 (50.0)	7.50 (0.24-68.83)
CA	8	1 (12.5)	7 (87.5)	0.58 (0.07-50.09)
AA	76	15 (19.7)	61 (80.3)	1.00
NA	2	0	2 (100)	
rs11574138*				
CC	1	0	1 (100)	NA
CT	8	3 (37.5)	5 (62.5)	2.79 (0.60-13.04)
TT	79	14 (17.7)	65 (82.3)	1.00
rs11168268*				
GG	9	3 (33.3)	6 (66.7)	2.50 (0.50-12.46)
GA	37	7 (18.9)	30 (81.2)	1.17 (0.37-3.71)
AA	42	7 (16.7)	35 (83.3)	1.00
rs2525044*				
AA	2	1 (50.0)	1 (50.0)	5.09 (0.30-87.68)
AG	19	5 (26.3)	14 (73.7)	1.82 (0.54-6.09)
GG	67	11 (16.4)	56 (83.6)	1.00
rs1015390*				
TT	9	2 (22.2)	7 (77.8)	0.86 (0.15-1.53)
TC	44	6 (13.6)	38 (86.4)	0.47 (0.15-5.00)
CC	32	8 (25.0)	245 (75.0)	1.00
NA	3	1 (33.3)	2 (66.7)	
rs2238139				
GG	5	2 (40.0)	3 (60.0)	3.33 (0.49-22.60)
GA	23	5 (21.7)	18 (78.3)	1.39 (0.42-4.62)
AA	60	10 (16.7)	50 (83.3)	1.00

Table 3 abbreviations: SNP-single nucleotide polymorphism; rs-reference SNP; CI-confidence interval; NA-snp information not available

A. Poor outcome defined as death or failure.

Figure 1. Principal component analysis of study participants compared to HapMap ethnic groups



Participants with MDR TB, KwaZulu-Natal, South Africa

HapMap Ethnicity Groups:

- Yoruba, Nigeria
- African ancestry in Southwest, USA
- European ancestry, Utah, USA

Supplemental Table 1. Vitamin D receptor gene single nucleotide polymorphisms not significantly associated with initial time to sputum culture conversion

SNP	P-value[A]	SNP	P-value[A]	SNP	P-value[A]
rs10875705	0.052	rs7963776	0.337	rs2525049	0.634
rs987849	0.062	rs11568820	0.341	rs11574110	0.668
rs2239185	0.063	rs2239179	0.354	rs7302235	0.671
rs35609792	0.070	rs11168309	0.359	rs60556433	0.676
rs10875700	0.079	rs11168328	0.366	rs58187695	0.683
rs74088704	0.087	rs11574070	0.387	rs58426141	0.718
rs7309452	0.092	rs61919101	0.399	rs4237855	0.725
rs11168319	0.101	rs6580642	0.401	rs10467099	0.732
rs74086592	0.104	rs7976091	0.416	rs58436504	0.735
rs7974905	0.110	rs739837	0.419	rs3819545	0.737
rs2246001	0.118	rs12313208	0.421	rs12308082	0.740
rs1544410	0.120	rs12717991	0.425	rs10747526	0.772
rs4334089	0.143	rs12721416	0.428	rs11168307	0.773
rs58379944	0.144	rs2228572	0.431	rs2107301	0.778
rs7965281	0.158	rs58789572	0.438	rs11168263	0.780
rs11168280	0.177	rs7965943	0.444	rs11574100	0.787
rs4442605	0.180	rs11168306	0.449	rs7974708	0.796
rs2525043	0.183	rs11574050	0.455	rs10747524	0.798
rs757555	0.183	rs7305032	0.465	rs7311030	0.799
rs1859281	0.186	rs12314197	0.471	rs2544037	0.802
rs4237856	0.216	rs7965274	0.475	rs2239182	0.819
rs4393380	0.221	rs11574053	0.483	rs2544039	0.834
rs11574044	0.222	rs73109883	0.506	rs10783221	0.839
rs2525045	0.225	rs10459227	0.515	rs12721397	0.847
rs886441	0.241	rs7970376	0.517	rs12303561	0.848
rs2238136	0.243	rs11168325	0.538	rs10459217	0.850
rs4254129	0.246	rs7967673	0.542	rs4760648	0.856
rs11574041	0.252	rs11574081	0.548	rs12299534	0.863
rs7975128	0.255	rs731236	0.549	rs11168261	0.877
rs12721370	0.270	rs61553170	0.555	rs61558228	0.882
rs11574005	0.273	rs12321826	0.558	rs74085273	0.898
rs2238140	0.278	rs11168314	0.563	rs11168264	0.910
rs2853560	0.291	rs4341603	0.565	rs2239186	0.925
rs4307774	0.292	rs2238138	0.577	rs2189480	0.934
rs11168311	0.308	rs2408876	0.580	rs11168277	0.953
rs4760674	0.331	rs2544038	0.580	rs3890734	0.954
rs4328263	0.334	rs2853561	0.619	rs4760658	0.954

A. Wald test p-value from Cox Proportional regression models adjusted for age, sex, smoking status, alcohol, AFB smear status, HIV status, and cavitary disease; SNPs modelled additively.