

1 **TITLE PAGE**

2 Version: April 11, 2017

3

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

6

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

10 **Authors' Affiliations:**

- 11 1. Division of Epidemiology and Biostatistics, School of Public Health, Georgia
12 State University, Atlanta, USA
- 13 2. Department of Epidemiology, Rollins School of Public Health, Emory University,
14 Atlanta, USA
- 15 3. Divisions of General Internal Medicine and Infectious Diseases, Albert Einstein
16 College of Medicine, Bronx, USA
- 17 4. School of Laboratory Medicine and Medical Sciences, University of KwaZulu-
18 Natal, Durban, South Africa
- 19 5. US Centers for Disease Control and Prevention, Atlanta, USA
- 20 6. Division of Infectious Diseases, Emory School of Medicine, Emory University,
21 Atlanta, USA
- 22 7. Department of Global Health, Rollins School of Public Health, Emory University,
23 Atlanta, USA

24 **Keywords:** vitamin D, vitamin D receptor gene, genetic polymorphisms, South Africa

25 **Short title:** Vitamin D receptor gene polymorphisms and MDR TB

26 **Word count: Abstract:** 298; **Manuscript:** 2664

27

28 **Corresponding Author:**

29 Matthew Magee, PhD, MPH

30 Email: mjmagee@gsu.edu Tel: +1 404 413 1797

31

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

36

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

41 **Abstract**

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

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

56 Results: Genomic analyses were performed on 91 MDR TB subjects enrolled in the
57 sub-study; 60% were female and median age was 35 years (interquartile range [IQR]
58 29-42). Smoking was reported by 21% of subjects and most subjects had HIV (80%),
59 were smear negative (57%), and had cavitory disease (55%). Overall, 87 (96%)
60 subjects initially converted cultures to negative, with median time to culture conversion
61 of 57 days (IQR 17-114). Of 121 VDR SNPs examined, 10 were significantly associated
62 ($p < 0.01$) with rate of sputum conversion in multivariable analyses. Each additional risk

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

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

68

69

70 Introduction

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

78
79 Given the paucity of new drugs available for the treatment of MDR TB there has been
80 substantial clinical interest in adjunctive use of 25-hydroxyvitamin D (vitamin D) to
81 improve TB—including MDR TB—treatment outcomes [7]. Vitamin D has anti-
82 inflammatory and anti-bacterial properties that could theoretically improve clinical TB
83 outcomes. The active metabolite of vitamin D, calcitriol, mediates innate immune
84 responses via the induction of the antimicrobial peptide cathelicidin and reactive oxygen
85 intermediates. Vitamin D also promotes macrophage-mediated killing of *Mycobacterium*
86 *tuberculosis* and modulates both anti-inflammatory and pro-inflammatory T-helper
87 responses to TB [8,9]. Low exposure to solar ultraviolet light, inadequate intake of
88 vitamin D and its precursors, or particular genotypes of the vitamin D receptor (VDR)
89 may lead to vitamin D deficiency which, in turn, could inhibit reduction of bacillary
90 burden, inhibit culture conversion, and impair the effectiveness of TB treatment. Despite
91 the hypothesized plausibility that vitamin D supplementation may improve TB treatment
92 outcomes and the pervasiveness of vitamin D deficiency, clinical trials to date among

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

98

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

108

109 **Methods**

110 **Parent Study**

111 The SHOUT study was a prospective observational cohort study among patients
112 receiving second-line TB treatment for MDR TB from three sites in KwaZulu-Natal
113 province, South Africa between 2011 and 2015. KwaZulu-Natal is the South African
114 province that has been most severely affected by TB (incidence: 1076 cases per
115 100,000 population) and HIV (prevalence: 17%) [18,19]. Patients 18 years or older were

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

131

132 **Study population, Measures and Definitions**

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

137

138 **Measures and Definitions**

139

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

149

150 The primary exposures of interest were VDR gene polymorphisms. Participant samples
151 were genotyped using the Affymetrix® Axiom Pan-African Array according to the
152 manufacturer's instructions [21]. Quality checks were performed to ensure the overall
153 SNP call rate was $\geq 95\%$ and that there was no sex mismatch between genotypic and
154 phenotypic measurement. SNPs were excluded if they had an unknown chromosomal
155 location, a call rate less than 95%, a Hardy-Weinberg Equilibrium (HWE) p -value less
156 than 0.0001 or a minor allele frequency (MAF) less than 0.05. After quality control filters,
157 1,494,763 SNPs were available for genetic analysis. South African samples were
158 pooled with HapMap EUR, YRI and ASW populations to identify population structure
159 relative to European and West African ancestry [22]. Top principal components (PCs)
160 were calculated using independent SNPs after pruning by pair-wise linkage
161 disequilibrium R^2 larger than 0.1 within windows of 50 SNPs. Using base pair location of

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

164

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

167

168 **Statistical Analyses**

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

182

183 **Ethics**

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

188

189 **Results**

190 From 2011-2013, the parent cohort study screened 365 patients and enrolled 206.

191 Among patients enrolled in the parent study, 103 provided samples for the present sub-
192 study, 7 were not processed due to DNA extraction errors, and 5 patients were late
193 excluded from the parent study due to second line drug resistance. The 91 remaining
194 participants were included in the current sub-study for genomic analyses and of these,
195 55 (60%) were female and the median age was 35 years (interquartile range [IQR] 29-
196 42) (Table 1). Most patients were HIV co-infected (n=73, 80%), had undetectable viral
197 load (n=23/42, 55%) and the median baseline CD4 count of 199 cells/mm³ (IQR 143-
198 289). Thirty-nine (43%) patients were AFB smear positive, 50 (55%) had cavitary
199 disease, and 74 (81%) had previously been treated for TB. Smoking was reported by
200 21% of patients.

201

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

207 Africans and South Africans. Within South African samples in this study, no outlier
208 (3SD) was observed using top 10 PCs, indicating a genetically homogeneous
209 population.

210

211 Overall, 87 (96%) subjects converted sputum cultures to negative. Of the 87 who
212 converted sputum cultures to negative, 24% (21/87) were positive at the time of MDR
213 TB diagnosis but converted to negative before the time of MDR TB treatment start. The
214 median time to culture conversion was 57 days (IQR 17-114); among patients who were
215 not culture negative at time of treatment initiation the median time to conversion was 82
216 days (IQR 53-143). Among patients who converted, 50 (55.0%) were culture negative
217 by two months of second-line treatment (Table 1). Compared to females, males were
218 significantly more likely to be sputum culture positive at two months (36.4% [20/55] vs.
219 58.3% [21/36], $p=0.04$).

220

221 Of 121 VDR SNPs examined, 10 were significantly associated ($p<0.05$) with hazard rate
222 of sputum culture conversion in multivariable analyses (Table 2). The estimated slower
223 conversion rate (adjusted hazard ratio of sputum culture conversion <1.0) ranged from
224 0.30 (95% CI 0.14-0.67) for rs74085240 to 0.64 (95%CI 0.42-0.98) for rs11168287. For
225 example, each additional risk allele on SNP rs74085240 delayed the rate of culture
226 conversion by 70% (aHR 0.55, 95% CI 0.36-0.85). Two VDR SNPs (rs11168327 and
227 rs11574143) were associated with significantly improved rate (adjusted hazard ratio
228 >1.0) of culture conversion (Table 2).

229

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

233

234 Discussion

235 We examined 121 SNPs in the VDR gene region and found a subset to be associated
236 with rate of sputum culture conversion among patients with MDR TB in South Africa.
237 Specifically, we identified 9 VDR SNPs that were associated with an estimated 50% to
238 25% reduced (delayed conversion) rate of sputum culture conversion among patients
239 receiving second-line TB therapy. Our findings provide new data about the relationship
240 between VDR polymorphisms and sputum culture conversion among patients with MDR
241 TB in South Africa.

242

243 Our findings in patients with MDR TB are consistent with three previous studies that
244 reported significantly lower rates of sputum culture conversion among patients with drug
245 susceptible pulmonary TB who had specific VDR polymorphisms [10,15,17]. First, an
246 observational longitudinal study among 78 Peruvian patients with confirmed pulmonary
247 TB reported that patients with the *TT TaqI* genotype (previous nomenclature now
248 typically replaced by endonuclease digestion pattern) had significantly longer time to
249 sputum culture conversion (median 46 days for *TT* genotype vs. 16 days for *Tt*
250 genotype) [15]. Second, in 2011 Martineau et al conducted a randomized control trial in
251 London, testing the efficacy of 2.5mg vitamin D₃ supplementation to reduce culture
252 conversion time in smear positive TB patients [10]. The trial reported no overall effect of

253 supplementation on culture conversion rates, but the authors did report an interaction
254 between vitamin D₃ supplementation and culture conversion with *TaqI* genotype.
255 Specifically, supplementation was beneficial among patients with *tt* genotype (HR 8.09,
256 95%CI 1.36-48.01). Unlike the studies from Peru and London, we did not observe an
257 association between rs731236 (*TaqI* genotype defined by endonuclease digestion
258 pattern) and rate of sputum culture conversion. Third, an observational longitudinal
259 study of HIV-negative patients with pulmonary TB from the Western Cape of South
260 Africa reported that a significantly lower proportion of patients with *Apal aa* genotype
261 had converted cultures by month 2 of TB treatment compared to patients with *Apal Aa*
262 genotype (26% vs. 51%, p=0.03) [17]. All three studies are consistent with our findings
263 that VDR polymorphisms are associated with rate of culture conversion. Unlike the
264 previous studies, our study identified SNPs significantly associated with rate of culture
265 conversion in patients with MDR TB.

266
267 To our knowledge, only one previous study examined the association between VDR
268 genotypes and time to culture conversion in patients with MDR TB. In 2012 Rathored et
269 al. followed 236 HIV-negative patients with MDR TB during DOTS-Plus treatment in
270 India and reported no significant differences in bivariate analyses between the three
271 VDR genotypes examined (*BsmI*, *TaqI*, *FokI*) and time to culture conversion [16].
272 However, in our study we examined 121 specific SNPs on the VDR gene region and
273 adjusted for important confounding factors (i.e., smoking status); Rathored et al. did not
274 adjust for confounding which may partially explain why we reported significant
275 differences in rates of culture conversion and the previous study did not. Similar to the

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

278

279 Several hypothesized biologic mechanisms may explain why polymorphisms in the VDR
280 gene are associated with rate of sputum culture conversion in patients with MDR TB.

281 Although vitamin D supplementation has not been demonstrated to be efficacious in
282 improving culture conversion time in drug-susceptible TB treatment [10-12], vitamin D
283 likely has a role in TB treatment-induced modulation of circulating immunologic signals.

284 Circulating immune signals are affected by TB treatment alone, for example interleukin
285 (IL)-10, cathelicidin LL-37, and neutrophil gelatinase-associated lipocalin (NGAL) are
286 suppressed by medications administered during the intensive phase TB treatment.

287 Moreover, a randomized trial from London demonstrated that vitamin D

288 supplementation enhanced the immune effects of TB treatment. In the trial of 126

289 smear-positive pulmonary TB patients, Coussens et al. reported that patients receiving
290 vitamin D supplementation during first-line treatment had TB treatment-induced

291 increases in lymphocytes and reduced concentrations of inflammatory markers [23].

292 Therefore, it is plausible that effects of second-line TB treatment on the immune

293 responses may be modified differently by vitamin D compared to first-line TB treatment
294 immune modulation by vitamin D.

295

296 Our study was subject to limitations. First, we did not measure plasma levels of vitamin

297 D or calcitriol. Therefore, we were unable to verify if vitamin D levels were affected by

298 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 metalloproteinase-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*, *Apal*, 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

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

324

325 **Acknowledgements**

326 We are grateful to the study team at the University of KwaZulu-Natal for their tireless
327 efforts in data collection, record abstraction, participant recruitment and interviews.

328 Finally, we thank the participants and their families who consented to participate in this
329 study.

330

331

332 References

- 333
- 334 1. WHO (2016) Global tuberculosis report 2016.
- 335 2. Centers for Disease C, Prevention (2013) Provisional CDC guidelines for the use and
- 336 safety monitoring of bedaquiline fumarate (Sirturo) for the treatment of multidrug-
- 337 resistant tuberculosis. *MMWR Recomm Rep* 62: 1-12.
- 338 3. Gandhi NR, Nunn P, Dheda K, Schaaf HS, Zignol M, et al. (2010) Multidrug-resistant
- 339 and extensively drug-resistant tuberculosis: a threat to global control of
- 340 tuberculosis. *Lancet* 375: 1830-1843.
- 341 4. Gandhi NR, Moll A, Sturm AW, Pawinski R, Govender T, et al. (2006) Extensively
- 342 drug-resistant tuberculosis as a cause of death in patients co-infected with
- 343 tuberculosis and HIV in a rural area of South Africa. *Lancet* 368: 1575-1580.
- 344 5. WHO (2015) Global tuberculosis report 2015. Geneva: World Health Organization.
- 345 6. WHO (2016) WHO Treatment guidelines for drug-resistant tuberculosis – 2016
- 346 update.
- 347 7. Cegielski P, Vernon A (2015) Tuberculosis and vitamin D: what's the rest of the
- 348 story? *Lancet Infect Dis* 15: 489-490.
- 349 8. Bruns H, Stenger S (2014) New insights into the interaction of Mycobacterium
- 350 tuberculosis and human macrophages. *Future Microbiol* 9: 327-341.
- 351 9. Coussens AK, Martineau AR, Wilkinson RJ (2014) Anti-Inflammatory and
- 352 Antimicrobial Actions of Vitamin D in Combating TB/HIV. *Scientifica (Cairo)* 2014:
- 353 903680.
- 354 10. Martineau AR, Timms PM, Bothamley GH, Hanifa Y, Islam K, et al. (2011) High-
- 355 dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary
- 356 tuberculosis: a double-blind randomised controlled trial. *Lancet* 377: 242-250.
- 357 11. Tukvadze N, Sanikidze E, Kipiani M, Hebbar G, Easley KA, et al. (2015) High-dose
- 358 vitamin D3 in adults with pulmonary tuberculosis: a double-blind randomized
- 359 controlled trial. *Am J Clin Nutr* 102: 1059-1069.
- 360 12. Daley P, Jagannathan V, John KR, Sarojini J, Latha A, et al. (2015) Adjunctive
- 361 vitamin D for treatment of active tuberculosis in India: a randomised, double-
- 362 blind, placebo-controlled trial. *Lancet Infect Dis* 15: 528-534.
- 363 13. Ralph AP, Lucas RM, Norval M (2013) Vitamin D and solar ultraviolet radiation in
- 364 the risk and treatment of tuberculosis. *Lancet Infect Dis* 13: 77-88.
- 365 14. Gao L, Tao Y, Zhang L, Jin Q (2010) Vitamin D receptor genetic polymorphisms and
- 366 tuberculosis: updated systematic review and meta-analysis. *Int J Tuberc Lung*
- 367 *Dis* 14: 15-23.
- 368 15. Roth DE, Soto G, Arenas F, Bautista CT, Ortiz J, et al. (2004) Association between
- 369 vitamin D receptor gene polymorphisms and response to treatment of pulmonary
- 370 tuberculosis. *J Infect Dis* 190: 920-927.
- 371 16. Rathored J, Sharma SK, Singh B, Banavaliker JN, Sreenivas V, et al. (2012) Risk
- 372 and outcome of multidrug-resistant tuberculosis: vitamin D receptor
- 373 polymorphisms and serum 25(OH)D. *Int J Tuberc Lung Dis* 16: 1522-1528.
- 374 17. Babb C, van der Merwe L, Beyers N, Pheiffer C, Walzl G, et al. (2007) Vitamin D
- 375 receptor gene polymorphisms and sputum conversion time in pulmonary
- 376 tuberculosis patients. *Tuberculosis (Edinb)* 87: 295-302.

- 377 18. Shisana O, Rehle T, Simbayi L, Zuma K, Jooste S, et al. (2014) South African
378 National HIV Prevalence, Incidence and Behaviour Survey, 2012. Cape Town:
379 HSRC Press.
- 380 19. Ndjecka N (2014) Multi-drug resistant tuberculosis: strategic overview on MDR-TB
381 care in South Africa. Pretoria: Department of Health, Republic of South Africa.
- 382 20. Brust JC, Lygizos M, Chaiyachati K, Scott M, van der Merwe TL, et al. (2011)
383 Culture conversion among HIV co-infected multidrug-resistant tuberculosis
384 patients in Tugela Ferry, South Africa. PLoS One 6: e15841.
- 385 21. Scientific ATF Axiom® Genome-Wide Population-Optimized Human Arrays
386 Technical Documentation
- 387 22. International HapMap C, Frazer KA, Ballinger DG, Cox DR, Hinds DA, et al. (2007)
388 A second generation human haplotype map of over 3.1 million SNPs. Nature
389 449: 851-861.
- 390 23. Coussens AK, Wilkinson RJ, Hanifa Y, Nikolayevskyy V, Elkington PT, et al. (2012)
391 Vitamin D accelerates resolution of inflammatory responses during tuberculosis
392 treatment. Proc Natl Acad Sci U S A 109: 15449-15454.
- 393 24. Salahuddin N, Ali F, Hasan Z, Rao N, Aqeel M, et al. (2013) Vitamin D accelerates
394 clinical recovery from tuberculosis: results of the SUCCINCT Study
395 [Supplementary Cholecalciferol in recovery from tuberculosis]. A randomized,
396 placebo-controlled, clinical trial of vitamin D supplementation in patients with
397 pulmonary tuberculosis'. BMC Infect Dis 13: 22.
- 398 25. Sutaria N, Liu CT, Chen TC (2014) Vitamin D Status, Receptor Gene
399 Polymorphisms, and Supplementation on Tuberculosis: A Systematic Review of
400 Case-Control Studies and Randomized Controlled Trials. J Clin Transl
401 Endocrinol 1: 151-160.

402

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 (%)	<i>P</i> 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

410

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

SNP	Model N	Days ^A (IQR)	HR	95% CI	<i>P</i> value ^B	Adjusted N	aHR ^C	95% CI	<i>P</i> 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

412

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

414

A. Median days to sputum culture conversion among SNPs carrying 0 effect alleles

415

B. Wald test p-value, SNPs are listed in ascending order based on p-value

416

C. Hazard ratios estimated from Cox Proportional regression models adjusted for age, sex, smoking status, alcohol, AFB smear status, HIV status, and cavitory disease; SNPs modelled additively, hazard ratio indicates per additional risk allele on each SNP.

417

418

419

420

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

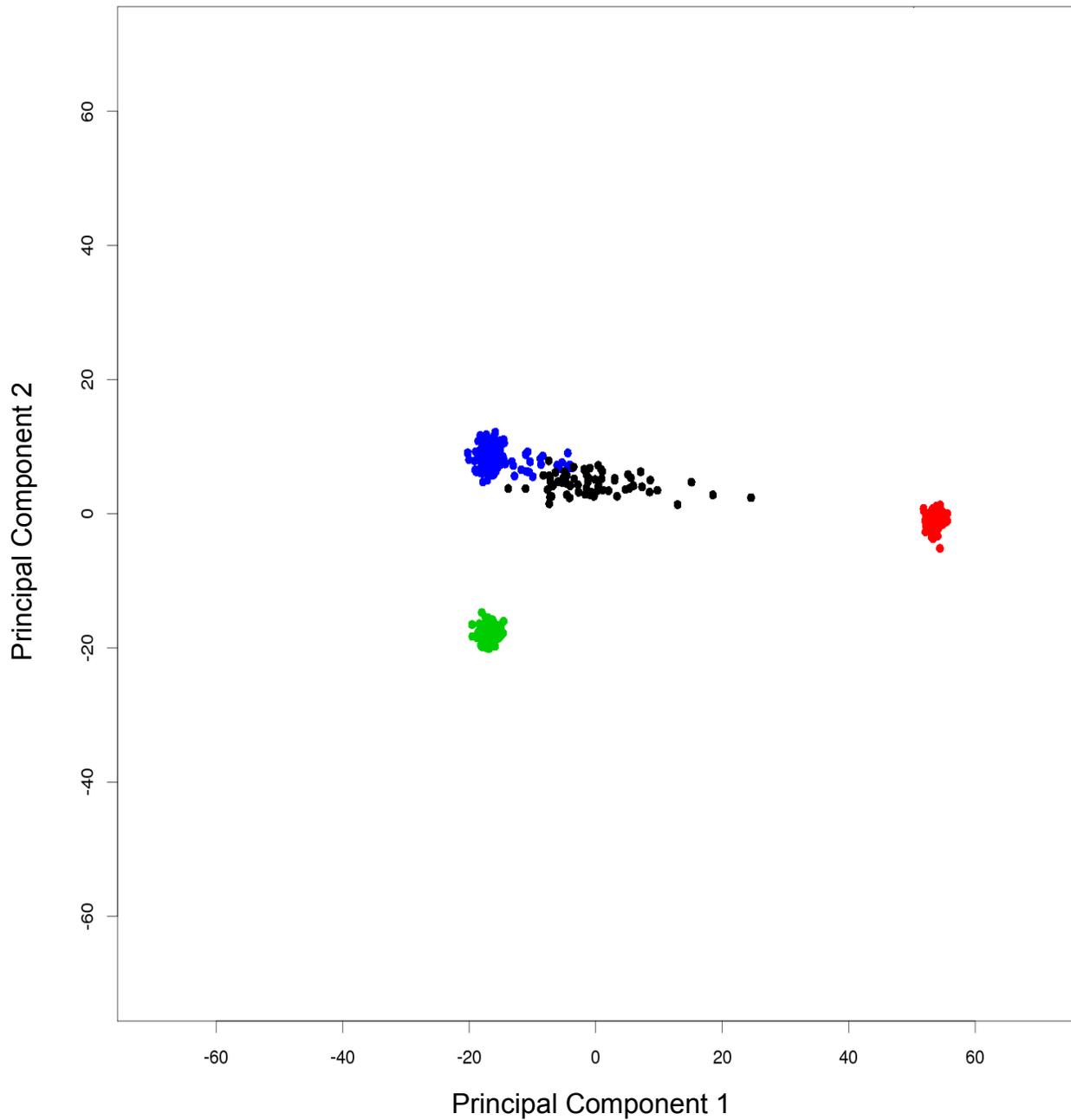
SNP	Total N=88	Poor Outcome ^A N=17 (19.3) N (%)	Cured/Completed N=71 (80.7) N (%)	Odds ratio (95% CI)
rs74085240*				
CC	2	1 (50.0)	1 (50.0)	7.50 (0.24-68.83)
CA	8	1 (12.5)	7 (87.5)	0.58 (0.07-50.09)
AA	76	15 (19.7)	61 (80.3)	1.00
NA	2	0	2 (100)	
rs11574138*				
CC	1	0	1 (100)	NA
CT	8	3 (37.5)	5 (62.5)	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)	24 (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

423

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

426 **A.** Poor outcome defined as death or failure.

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



429 ■ Participants with MDR TB, KwaZulu-Natal, South Africa

430
431 HapMap Ethnicity Groups:

432 ■ Yoruba, Nigeria

433 ■ African ancestry in Southwest, USA

434 ■ European ancestry, Utah, USA

435
436

437 **Supplemental Table 1.** Vitamin D receptor gene single nucleotide polymorphisms not
 438 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

439
 440 A. Wald test p-value from Cox Proportional regression models adjusted for age, sex, smoking
 441 status, alcohol, AFB smear status, HIV status, and cavitory disease; SNPs modelled additively.
 442

443

444