Title: Human genetic ancestry, *Mycobacterium tuberculosis* diversity and tuberculosis disease severity in Dar es Salaam, Tanzania

Short title: Human and bacterial genomic background and TB disease severity

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Abstract

Infectious diseases have affected humanity for millennia and are among the strongest selective forces. Tuberculosis (TB) is an ancient disease, caused by the human-adapted members of the *Mycobacterium tuberculosis* complex (MTBC). The outcome of TB infection and disease is highly variable, and co-evolution between human populations and their MTBC strains may account for some of this variability. Particular human genetic ancestries have been associated with higher susceptibility to TB, but socio-demographic aspects of the disease can confound such associations. Here, we studied 1,000 TB patients from Dar es Salaam, Tanzania, together with their respective MTBC isolates, by combining human and bacterial genomics with clinical data. We found that the genetic background of the TB patient population was strongly influenced by the Bantu migrations from West Africa, which is in contrast to the corresponding MTBC genotypes that were mainly introduced from outside Africa. These findings suggest a recent evolutionary history of co-existence between the human and MTBC populations in Dar es Salaam. We detected no evidence of an effect of human genetic ancestry, or MTBC phylogenetic diversity alone, nor their interaction, on TB disease severity. Treatment-seeking, social and environmental factors are likely to be the main determinants of disease severity at the point of care in this patient population.

Author Summary

Tuberculosis (TB) is an ancient infectious disease that continues to challenge global health efforts. Here, we explored the interplay between human and bacterial genetics on TB in Dar es Salaam, Tanzania. We found that neither the genetic ancestry of the patient, nor the bacterial genotype alone, nor their interaction, influenced the severity of TB. Our finding indicate that in this patient population, social and environmental factors may be the main determinants of TB disease severity.

Introduction

Africa harbors the largest human genetic diversity worldwide (1). This continent is also inhabited by numerous ethnic and linguistic groups (2). While the long evolutionary history of modern humans in Africa and their large effective population sizes (3) explain this high genetic diversity, more recent migration events within and from outside Africa during the last 5,000 years, as well as admixture between historically separated populations, have resulted in some degree of homogenization (4). Hence, African populations nowadays are often composed of a mixture of different genetic ancestries (5, 6). One human migration that had a major influence on the population structure of present-day Africans is the so-called 'Bantu expansion', where Bantu-speaking groups migrated from central Western Africa southwards and eastwards, spreading farming technologies across sub-Saharan Africa, and admixing with local groups of hunter-gatherers and pastoralists (4, 7). Recent human genetic studies have identified moderate population structuring among Bantu-speaking populations (8, 9), yet, admixture with local populations has impacted immune responses to infectious diseases (10). Since pathogens have been one of the strongest selective forces driving human evolution (11), disease susceptibility and clinical outcomes can differ markedly between human populations. Genome-wide association studies (GWAS) have identified mutations altering the susceptibility to various infectious diseases (12-15), including tuberculosis (TB), which remains the main cause of human death due to a single infectious agent (16).

The bacteria that cause TB belong to the *Mycobacterium tuberculosis* complex (MTBC) and can be classified into ten human-adapted phylogenetic lineages: Lineage 1 (L1) to L10, plus several lineages adapted to different wild and domestic animal host species (17, 18). While TB is a global problem, the human-adapted MTBC lineages differ in their geographical distribution. L2 and L4 occur worldwide and other lineages are restricted to specific regions.

93 Specifically, L1 and L3 mainly occur around the Indian Ocean (19), L5 and L6 that are
94 limited to West Africa (20), and L7 only occurs in Ethiopia (21). This phylogeographic
95 population structure of the human-adapted MTBC led to the hypothesis that certain MTBC
96 genotypes are locally adapted to their sympatric human populations (19). This hypothesis is
97 supported by findings from cosmopolitan settings, where sympatric associations between the
98 geographical origin of TB patients and their infecting MTBC strains were observed (22-24).
99 Moreover, in immune-compromised individuals with HIV co-infection, these sympatric
100 associations were disrupted (25). The various MTBC genotypes also differ phenotypically
101 (26), regarding disease progression (27), transmission (28, 29), and disease presentation (30).

102 Human genetic diversity has also been linked to differences in TB susceptibility. While for
103 example *TYK2* has been associated with TB disease worldwide (31), several human genetic
104 loci were not consistently associated with TB in populations from different geographical
105 regions but specific to certain populations (32-36). Particular human genetic ancestries have
106 also been found to play a role in the context of TB. People with higher proportions of native
107 Peruvian genetic ancestry showed a higher risk of progressing to active TB (37), and a higher
108 proportion of San genetic ancestry was associated with an increased risk for TB among South
109 African Coloured individuals (38). In addition to the effects of human and bacterial genetic
110 diversity on TB, many social and environmental factors, as well as co-morbidities are known
111 drivers of TB. These include malnutrition (39), HIV infection, diabetes (40, 41), poverty (42),
112 smoking (43), and alcohol consumption (44).While associations between TB and individual
113 host, pathogen or environmental factors have been found (32-37, 39), studies considering all
114 these components simultaneously, remain scarce (45-47).

115 Here, we characterized the genetic ancestry of a cohort of TB patients from Dar es Salaam,
116 Tanzania, the phylogenetic lineage of their correspondent MTBC isolate, and investigated the
117 association of both with TB disease severity.

118

119 **Results**

120 **Genetic ancestry of Tanzanian TB patients**

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122 Genetic ancestries were estimated for 7,479 individuals from 249 populations
123 (Supplementary Figure S2) including 1,444 Tanzanian TB patients, using the software
124 Admixture (48) with 53,255 SNPs (Figure 1, Supplementary Figure S1).

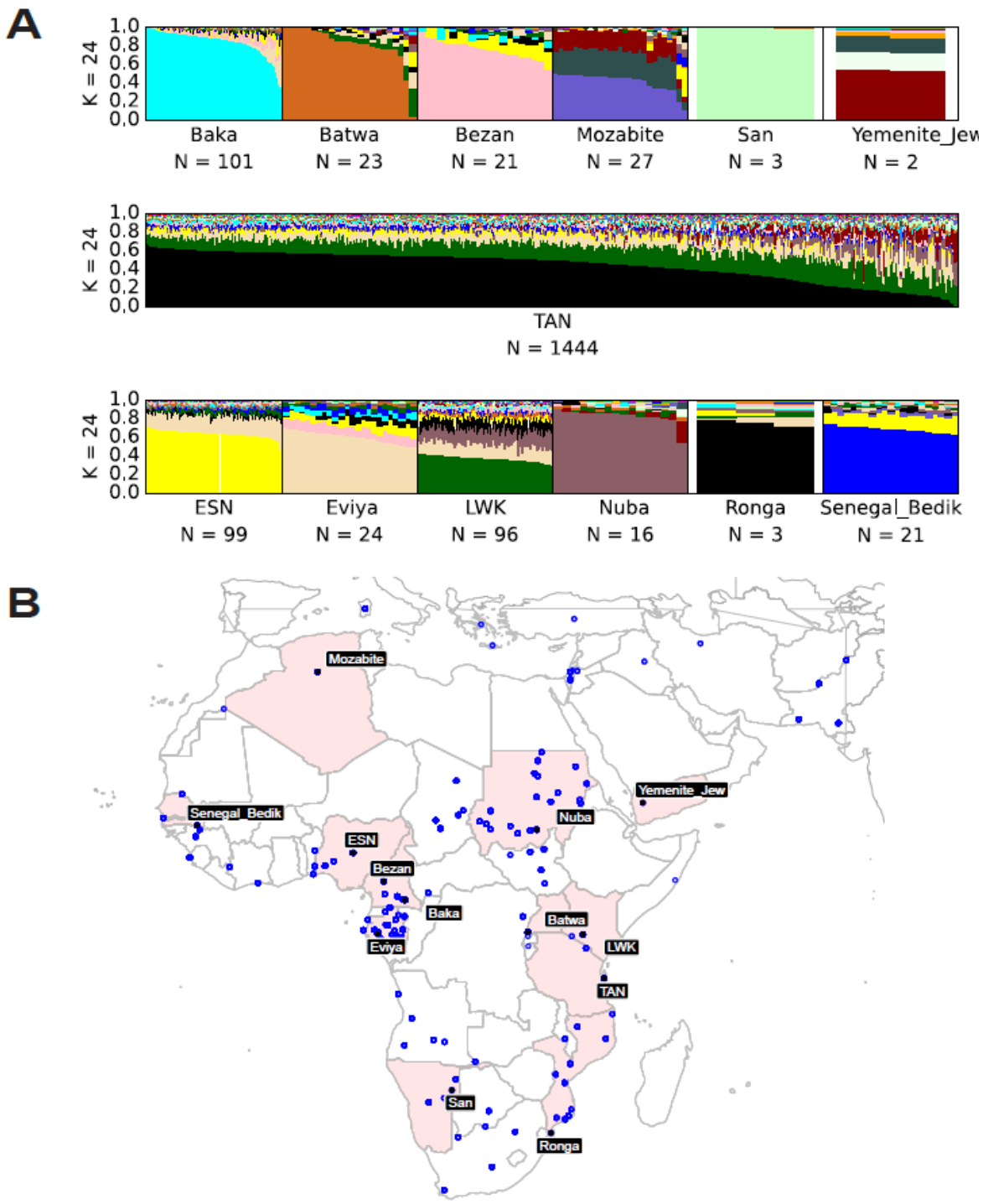

Figure 1 – Genetic ancestry analyses of Tanzanian TB patients.
A) Genetic ancestry proportions of the 1,444 Tanzanian TB patients and representative human populations who shared at least 1% of their most common genetic ancestry with the Tanzanians for K = 24. (ESN: Esan from Nigeria (1000G), LWK: Luhya from Kenya (1000G)). For all populations included in our study, see Supplementary Figure S2 for their geographic distribution and S5 for the ancestry composition of all African populations included in this study. **B**) The geographical location of the representative populations shown in A are depicted with black circles, and the corresponding country is highlighted. The remaining African populations included in the analysis are represented by blue circles.

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126 The optimal number of source populations to describe our dataset was 24, based on the
127 lowest cross-validation error (49) (Error: Reference source not found). For the purpose of this
128 study, we named the genetic ancestries according to the geographical distribution and/or
129 ethnicity of the reference populations that they are most prevalent in. The genetic ancestry
130 with the highest contribution among Tanzanians with a mean of 44% (maximum 68%,
131 minimum 0%) was also the most abundant in Bantu-speaking people from Southern and
132 southeastern Africa (e.g. the Ronga population in Figure 1), and hence hereafter, we will refer
133 to this ancestry as "Southeastern Bantu" (Figure 1). The second most common genetic
134 ancestry with a mean of 22% (maximum 42%, minimum 6%) in the Tanzanian TB patients
135 was most common among Kenyans (e.g. the Luhya population in Figure 1, 1000G and
136 HGDP, see methods), and will be referred to as the "Eastern Bantu" genetic ancestry.
137 Additionally, the Tanzanian TB patients had a mean of 9% (maximum 53%, minimum 0%)
138 of a genetic ancestry that was most common among Bantu-speaking populations from
139 western Central Africa, (e.g. the Eviya population in Figure 1); we will refer to it as the
140 "Western Bantu" genetic ancestry. Furthermore, the Tanzanian TB patients contained on
141 average 4% of a genetic ancestry most abundant among Nigerians represented by the Esan
142 population ("Nigerian" genetic ancestry in Figure 1), and 4% of a genetic ancestry most
143 abundant in people from Chad and Sudan (represented by the Nuba population in Figure 1).
144 In addition, the genetic ancestry of the Tanzanian TB patients was composed of 3% of a
145 genetic ancestry most prevalent in people from Western Africa (Gambia and Senegal
146 represented by the Senegal Bedik population, in Figure 1), as well as 3% of a genetic ancestry
147 most prevalent in individuals from western Africa rainforest hunter-gatherer populations
148 (Bezan population in Figure 1). A mean of 2% belonged to a genetic ancestry most common
149 among Bedouin individuals (represented by the Yemenite Jew population in Figure 1). The

150 proportions of the remaining genetic ancestries were all smaller than 2% (Error: Reference
151 source not found, admixture plots for all African populations included can be found in Error:
152 Reference source not found). Finally, most Tanzanian TB patients had little non-African
153 genetic ancestry (mean 5%, maximum 65%), with only 14 patients (~1%) showing more than
154 30% non-African genetic ancestry. In summary, the ancestry of Tanzanian TB patients was,
155 for the most part, a mixture of three different Bantu components. Thus, for the remaining
156 sections, we will focus on the ancestries termed Eastern Bantu, Southeastern Bantu, and
157 Western Bantu.

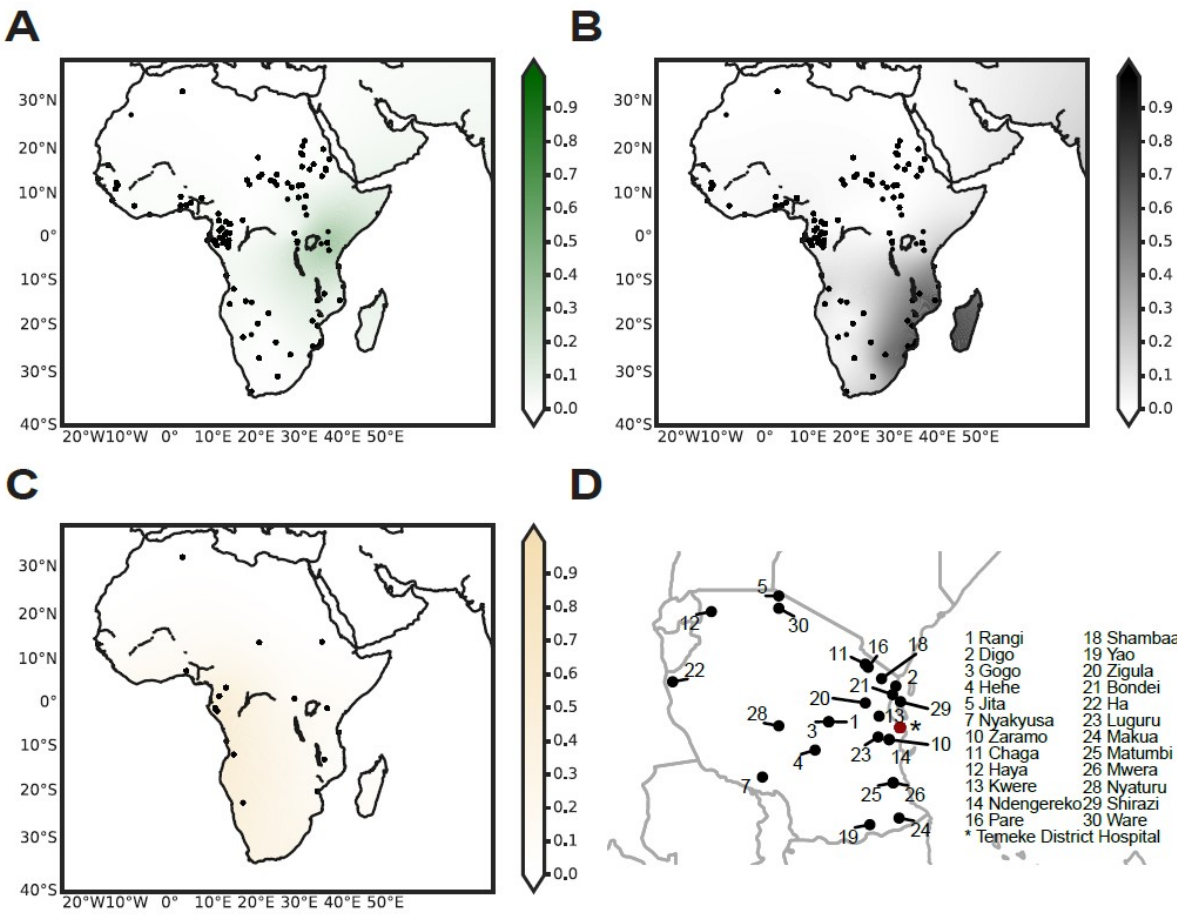
158

159 **Insights into the Bantu genetic ancestries in Africa and Tanzania**

160 Compiling several datasets, including many different Bantu populations, allowed for a closer
161 look at the distribution of Bantu ancestries across African populations. Like Tanzanians, the
162 populations from the neighboring Kenya and Mozambique showed strong contributions of
163 Bantu ancestries resulting from different admixture events (Figure 1). While the Eastern
164 Bantu genetic ancestry was highest in Kenya and Tanzania, decreasing from there to the
165 south and to the west of the continent (Figure 2A), the Southeastern Bantu genetic ancestry
166 generally increased towards the south and decreased towards the west as observed by others
167 (Figure 2B) (8). The Western Bantu genetic ancestry was mainly seen in Bantu-speaking
168 populations from Gabon and Cameroon, as well as in South African populations (Figure 2C).
169 At a continental-wide level, the geographical distribution and the genetic distances of the
170 human populations analyzed were significantly correlated (Mantel test: veridical correlation =
171 0.18, p-value < 0.001, Supplementary Figure S6).

172 Even though all our TB patients were recruited in Dar es Salaam, we found them to belong to
173 a variety of self-defined ethnic groups linked to different geographical regions within

Tanzania (Figure 2D). Even at this smaller scale, we found a significant correlation between the geographic distance of the self-defined ethnic groups of our TB patients and their genetic distances (Mantel test: veridical correlation = 0.12, p-value < 0.001). The geographical structuring of human genetic diversity within Tanzania was further supported by our finding that the Southeastern Bantu genetic ancestry increased from West to East, and from North to South. The Eastern Bantu genetic ancestry increased from South to North and decreased from West to East. The Western Bantu genetic ancestry increased to the North and decreased to the East (Error: Reference source not foundA).



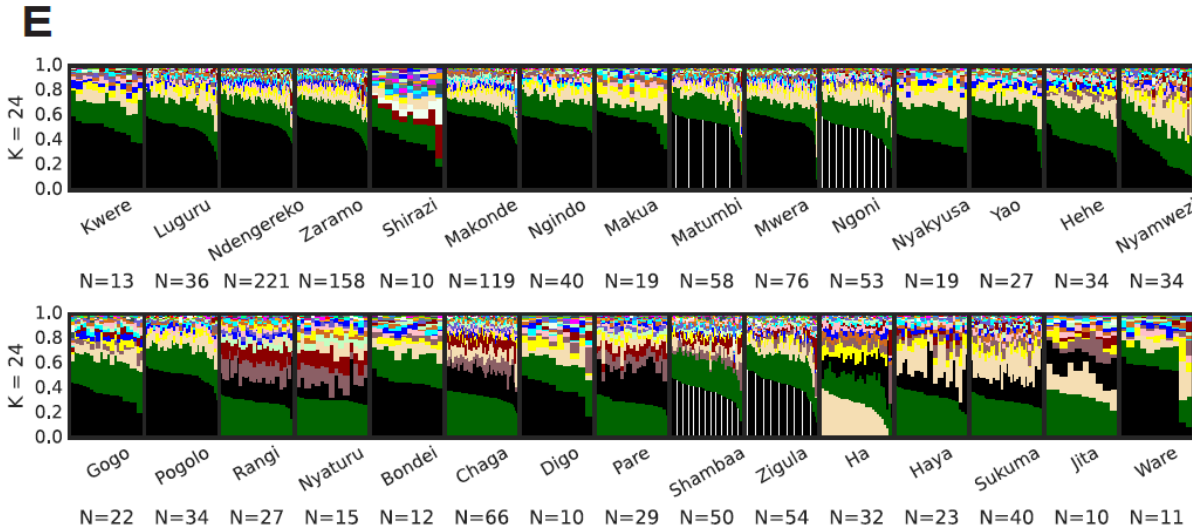


Figure 2 - Spatial visualizations of the Bantu genetic ancestries and the genetic ancestries of the different self-identified ethnic groups among the TB patients in Tanzania. The genetic ancestry was inferred by admixture with K = 24, and the interpolation of the ancestries was performed by using the pykrige module in Python (see methods). **A**) Eastern Bantu genetic ancestry, **B**) Southeastern Bantu genetic ancestry, and **C**) Western Bantu genetic ancestry. The populations included for spatial interpolations are marked with a black dot on the maps. The maps were created using the basemap module in Python. **D**) Geographical origin of the ethnic groups among our TB patient cohort. The Temeke Distric hospital in Dar es Salaam where the patients were recruited is marked with a red point. Note that for some ethnic groups, no geographical origin could be identified (Supplementary Table 1). **E**) Ancestry plots for the different ethnic groups with at least 10 patients from our TB patient cohort.

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183 **The MTBC genotypes circulating in Tanzania and their association with TB disease**
184 **severity**

185 In previous work (50), we investigated the MTBC genotypes causing TB in the patient cohort
186 analyzed here. We found a high diversity of MTBC genotypes, with approximately half of the
187 TB cases caused by four main MTBC genotypes estimated to have been introduced into
188 Tanzania starting 320 years ago. After adding the genomic information of an additional 389
189 MTBC isolates to our dataset (total N=1,471), the prevalence of the four dominant MTBC
190 genotypes was very similar to our previous findings (50). The most successful genotype
191 within L3.1.1 (referred to as "Introduction 10") contributed 39% of the current TB cases,
192 followed by a genotype within L1.1.2 ("Introduction 9") with 9%, a genotype within L4.3.4
193 ("Introduction 5") with 6%, and a genotype within L2.2.1 ("Introduction 1") with 5%. The
194 remaining TB cases were caused by a variety of other genotypes within L1-L4, but occurred
195 at frequencies of 1% or less (50). Despite a 36% increase in sample size compared to our
196 previous analysis (50), we still find no evidence of an association between the different
197 MTBC genotypes circulating in Dar es Salaam and TB disease severity using as proxies; X-
198 ray scores (mild or severe), bacterial load (inferred from GeneXpert cycle threshold) and TB-
199 score (mild or severe) (Table 1, Table 3) (see Methods).

Table 1- Human and bacterial genotypes by the severity measures

		TB-score				Lung damage (X-ray score)					Bacterial load (Ct-value)		
	levels	Total N (%)	Missing N (%)	Severe N (%)	Mild N (%)	Total N	Missing N	severe	mild	not available	Total N	Missing N	Mean (SD)
Total N (%)				624 (33)	1280 (67)			177 (9)	849 (45)	878 (46)			
MTBC Genotype	other	1471 (77.3)	433	207 (42)	406 (41)	764 (74.5)	262	51 (39)	269 (43)	293 (41)	863 (78.3)	239	19.4 (4.9)
	L2.2.1 - Intro 1			22 (4)	49 (5)			7 (5)	28 (4)	36 (5)			20.5 (5.1)
	L3.1.1 - Intro 10			184 (38)	388 (40)			59 (45)	239 (38)	274 (39)			19.1 (4.7)
	L4.3.4 - Intro 5			26 (5)	60 (6)			4 (3)	38 (6)	44 (6)			19.5 (4.1)
	L1.1.2 - Intro 9			51 (10)	78 (8)			11 (8)	58 (9)	60 (8)			19.1 (4.7)
Southeastern Bantu	Mean (SD)	1442 (75.7)	462	0.44 (0.2)	0.44 (0.2)	840 (81.9)	186	0.45 (0.1)	0.43 (0.2)	0.45 (0.2)	810 (73.5)	292	19.9 (5.2)
Eastern Bantu	Mean (SD)	1442 (75.7)	462	0.23 (0.1)	0.22 (0.1)	840 (81.9)	186	0.22 (0.1)	0.23 (0.1)	0.22 (0.1)	810 (73.5)	292	19.9 (5.2)
Western Bantu	Mean (SD)	1442 (75.7)	462	0.08 (0.1)	0.09 (0.1)	840 (81.9)	186	0.08 (0.1)	0.09 (0.1)	0.08 (0.1)	810 (73.5)	292	19.9 (5.2)
Other ancestry	Mean (SD)	1442 (75.7)	462	0.25 (0.1)	0.25 (0.1)	840 (81.9)	186	0.25 (0.1)	0.25 (0.1)	0.25 (0.1)	810 (73.5)	292	19.9 (5.2)

204 **Relationship between the human and MTBC population structures**

205 We previously found that the four dominant MTBC genotypes in Dar es Salaam differed in
206 their transmission rate and in the duration of the infectious period (50). Here, we assessed
207 whether there might be an additional host genetic contribution to these differences. We first
208 compared the genetic ancestry proportions between patients infected with the four dominant
209 genotypes, and then tested whether patients who were genetically more closely related were
210 infected with MTBC genotypes that were also more closely related as would be expected
211 from a co-evolutionary process (51). However, the human genetic ancestry proportions
212 differed only marginally between the TB patients infected by the four main MTBC genotypes
213 (Table 2). Moreover, there was no correlation between the human and bacterial genetic
214 distances (Mantel test: veridical correlation = -0.02, p-value = 0.85). Taken together, we
215 found no evidence for any statistically significant relationship between the human and
216 bacterial genetic population structure in Dar es Salaam. These results also suggest that the
217 genetic composition of this human population is unlikely to have a measurable effect on the
218 differences in bacterial transmission rate and duration of the infectious period reported
219 previously (50).

Table 2- Summary of ancestry proportions, sociodemographic and clinical data stratified by MTBC genotype.

	Total N (%)	Missing N	levels	Other genotype	L2.2.1 - Intro 1	L3.1.1 - Intro 10	L4.3.4 - Intro 5	L1.1.2 - Intro 9	Missing
Total N (%)				613 (32)	71 (4)	572 (30)	86 (5)	129 (7)	433 (23)
Sex	1471 (100.0)	0	male	424 (69)	52 (73)	425 (74)	55 (64)	87 (67)	302 (70)
			female	189 (31)	19 (27)	147 (26)	31 (36)	42 (33)	131 (30)
Age	1471 (100.0)	0	Mean (SD)	34.64 (10.4)	32.03 (8.4)	34.59 (10.5)	33.85 (11.0)	36.01 (12.4)	35.94 (11.4)
HIV Status	1452 (98.7)	19	negative	515 (85)	60 (86)	462 (82)	67 (78)	95 (75)	330 (77)
			infected	90 (15)	10 (14)	103 (18)	19 (22)	31 (25)	97 (23)
Smoker	1443 (98.1)	28	no	477 (79)	52 (74)	408 (73)	73 (87)	102 (80)	334 (77)
			yes	127 (21)	18 (26)	149 (27)	11 (13)	26 (20)	97 (23)
Cough duration (weeks)	1454 (98.8)	17	Mean (SD)	3.69 (1.9)	3.74 (2.0)	3.76 (1.8)	3.77 (2.0)	3.68 (1.7)	3.61 (1.9)
Southeastern Bantu	1009 (68.6)	462	Mean (SD)	0.43 (0.2)	0.45 (0.1)	0.45 (0.2)	0.46 (0.2)	0.46 (0.1)	0.44 (0.2)
Eastern Bantu	1009 (68.6)	462	Mean (SD)	0.23 (0.1)	0.22 (0.1)	0.22 (0.1)	0.23 (0.1)	0.22 (0.1)	0.22 (0.1)
Western Bantu	1009 (68.6)	462	Mean (SD)	0.09 (0.1)	0.09 (0.1)	0.08 (0.1)	0.08 (0.1)	0.08 (0.1)	0.09 (0.1)
Other ancestry	1009 (68.6)	462	Mean (SD)	0.26 (0.1)	0.24 (0.1)	0.24 (0.1)	0.23 (0.1)	0.23 (0.1)	0.25 (0.1)

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225 **Association of human genetic ancestry with TB disease severity**

226 We next investigated whether human genetic ancestry could have contributed to the
227 differences in disease severity observed between our TB patients. We fitted logistic
228 regression models using the three proxies of TB disease severity mentioned previously as
229 outcome variables. We included the three human genetic ancestries with the highest
230 proportions among the Tanzanian TB patients (Southeastern Bantu, Eastern Bantu, Western
231 Bantu) as covariates, together with age, sex, HIV status, age, smoking and cough duration to
232 control for potential confounding. We found no evidence of an association between human
233 genetic ancestry and any of these three proxies of TB disease severity (Table 1).

234 **The combined effect of human and MTBC genetic diversity on TB disease severity**

235 For a subset of 1,000 TB patients, we had both an MTBC genome and a human genome or
236 genotype available. Genetic interactions between the host and the pathogen have been show
237 to affect TB severity in other settings (37, 52). To test for potential interactions between
238 human and bacterial diversity on TB severity, we added to the logistic regression models
239 described in the previous section the most common MTBC genotype as an additional
240 explanatory variable (L3.1.1-Introduction 10), as well as the interaction between human
241 ancestry and MTBC genotype. We only tested L3.1.1-Introduction 10, since the numbers of
242 the other genotypes were too few for meaningful testing. However, there we found no
243 evidence for any interaction between the MTBC genotype and human ancestry influencing
244 TB disease severity in this patient population (able 3).

Table 3 - Associations between the different proxies for disease severity, human genetic ancestry, bacterial genotype and the interaction between human genetic ancestry and bacterial genotype. Binomial logistic regressions were performed on the data of HIV-negative patients, adjusting for age, sex, smoking and cough duration.

		Disease severity measures					
		Lung damage		**TB-score**		**Bacterial load (Ct-value)**	
		OR adjusted	p-value adjusted	OR adjusted	p-value adjusted	OR adjusted	p-value adjusted
MTBC genotype[1]	L3.1.1-Introduction 10	1.59 (0.95-2.66)	0.08	1.05 (0.77-1.44)	0.76	0.99 (0.97-1.01)	0.24
	other genotypes	1		1		1.00	
Human ancestry[2]	Southeastern Bantu category 3	2.26 (1.06-5.21)	0.17	1.09 (0.71-1.68)	0.29	1.00 (0.97-1.02)	0.22
	Southeastern Bantu category 2	2.27 (1.10-5.10)		0.69 (0.46-1.02)		1.01 (0.99-1.05)	
	Southeastern Bantu category 1	1		1		1.00	
	Eastern Bantu category 3	1.36 (0.55-3.90)		0.93 (0.56-1.52)		1.02 (0.99-1.05)	
	Eastern Bantu category 2	1.55 (0.64-4.37)		1.09 (0.67-1.76)		1.00 (0.97-1.03)	
	Eastern Bantu category 1	1		1		1.00	
	Western Bantu category 4	0.54 (0.22-1.42)		1.16 (0.64-2.06)		1.01 (0.98-1.05)	
	Western Bantu category 3	0.51 (0.20-1.39)		1.30 (0.72-2.32)		1.03 (0.99-1.06)	

	Western Bantu category 2	0.70 (0.25-2.05)		1.13 (0.58-2.17)		1.03 (0.99-1.06)	
	Western Bantu category 1	1		1		1.00	
Interaction between MTBC genotype and human ancestry	Southeastern Bantu category 3 & L3.1.1-Intro 10	1.10 (0.21-5.65)	0.06	1.03 (0.42-2.53)	0.90	0.99 (0.94-1.04)	0.91
	Southeastern Bantu category 2 & L3.1.1-Intro 10	0.39 (0.08-1.91)		1.28 (0.55-2.98)		0.98 (0.93-1.03)	
	Southeastern Bantu category 1 & L3.1.1-Intro 10	1		1		1.00	
	Eastern Bantu category 3 & L3.1.1-Intro 10	7.79 (0.87-177.55)		1.69 (0.59-4.76)		1.01 (0.94-1.07)	
	Eastern Bantu category 2 & L3.1.1-Intro 10	6.55 (0.74-147.98)		1.38 (0.50-3.83)		1.02 (0.96-1.09)	
	Eastern Bantu category 1 & L3.1.1-Intro 10	1		1		1.00	
	Western Bantu category 4 & L3.1.1-Intro 10	0.21 (0.03-1.46)		1.44 (0.44-4.75)		0.97 (0.90-1.05)	
	Western Bantu category 3 & L3.1.1-Intro 10	0.45 (0.05-3.45)		1.06 (0.32-3.55)		0.99 (0.92-1.07)	
	Western Bantu category 2 & L3.1.1-Intro 10	1.19 (0.12-11.04)		1.62 (0.41-6.54)		0.98 (0.91-1.07)	
	Western Bantu category 1 & L3.1.1-Intro 10	1		1		1.00	

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[1] The odds ratio for genotype represents the odds of severe disease for Introduction 10 compared to the odds for other genotypes.

[2] The odds ratios represent the estimated multiple in the odds of severe disease for a one-unit increase in the additive log-transformed ancestry variable.

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Discussion

In this study, we analyzed the genetic ancestry of TB patients, the MTBC diversity underlying their TB infection, and estimated the associations of both with disease severity in Dar es Salaam, Tanzania. We found a strong component of Bantu genetic ancestries among the Tanzanian TB patients, similar to those of neighboring populations from Mozambique and Kenya, and little non-African genetic ancestry. Genetic ancestry proportions did not differ between patients infected with different MTBC genotypes. There was no evidence that the patient genetic ancestry or the MTBC genotype on their own, nor their interaction, had any effect on TB disease severity.

Despite the fact that Tanzania is one of the few countries in sub-Saharan Africa where all four main African linguistic groups co-exist (53), and that its largest city and economic capital, Dar es Salaam, is strongly influenced by different human populations from within and outside Africa, our cohort of TB patients mostly comprised Bantu-speaking ethnicities. Comparing this TB patient cohort to a large number of modern human populations revealed major components of Eastern and Southeastern Bantu genetic ancestries. This genetic population structure probably resulted from several admixture events estimated to have happened between 1500 to 150 years ago, between local populations and Bantu-speaking populations who migrated from West Africa to the East and South of the continent (4). The TB patients investigated here were recruited in one district hospital of Dar es Salaam. Yet, we found the genetic distances between the patients to be correlated with the original geographic range of their self-identified ethnicities, suggesting that the corresponding human populations are not fully admixed. The population of Dar es Salaam has increased by several millions in the last 40 years, mainly as a result of immigration from rural parts of Tanzania (54). Thus,

275 our findings suggest that our TB patient population mostly represents recent migrants to Dar
276 es Salaam from other regions of Tanzania. Moreover, we found little evidence of Eurasian
277 genomic influence in the TB patient population (on average 5% genetic ancestry). This is in
278 strong contrast to coastal Swahili populations, as recent findings comparing modern and
279 medieval Swahili people revealed large components of genetic ancestry derived from
280 exchanges between local East African Bantu populations and people from India, Persia, and
281 Arabia, starting as early as 1000 AD (55). We conclude that our TB patient population does
282 not represent the full spectrum of human genetic diversity in Tanzania.

283 In contrast to the genetic ancestry of the TB patients, we found that the MTBC genotypes
284 infecting these patients descend from multiple historical introductions, which mainly resulted
285 from the human exchanges that took place across the Indian Ocean during the last few
286 centuries (50). Some of these recently introduced MTBC genotypes became dominant, in
287 particular the MTBC genotype L3.1.1-Introduction 10, which caused TB in approximately
288 40% of our patients. These strains descended from an introduction that occurred
289 approximately 300 years ago from South or Central Asia to East-Africa (50). Hence, while
290 our TB patient population reflects little historical gene flow from non-African populations,
291 the underlying MTBC diversity indicates that the MTBC genotypes introduced from outside
292 successfully spread in this newly encountered host population, eventually outcompeting
293 native MTBC genotypes (56).

294

295 We previously reported that in this TB patient cohort, some of the dominant MTBC
296 genotypes had a higher transmission rate than others, while some other MTBC genotypes
297 induced patients to remain infectious for longer (50). Based on the similar proportions of
298 MTBC genotypes among self-reported ethnic groups we observed at the time, we had already

299 hypothesized that human genetic heterogeneity of the host population is unlikely to be
300 responsible for those differences (50). Here we formally addressed this hypothesis, and found
301 that there was no evidence that the human genetic ancestry proportions differed between
302 patients infected with different MTBC genotypes in our cohort. This finding further supports
303 the notion that the differences in epidemiological parameters we reported previously are
304 mainly determined by the pathogen genotype.

305

306 Disease severity is one aspect of the clinical presentation of TB with a direct impact on
307 patient mortality and morbidity, as well as on pathogen transmission, as it influences patient
308 infectiousness. It is thus likely that both host and pathogen genetic characteristics can
309 modulate TB disease severity (51). Experimental infections in various animal models suggest
310 that different MTBC strains vary in virulence (57-59). However, *in clinico* evidence for
311 differences in disease severity caused by different MTBC genotypes are inconsistent (60, 61).
312 We found no evidence of differences in disease severity at the point of care caused by the
313 different MTBC genotypes in our study. Moreover, we did not observe any association
314 between human genetic ancestry and disease severity, which is in contrast to a recent study
315 from Peru, where human genetic ancestry was found to influence progression to active TB
316 (37).

317 Our previous work found evidence of such genetic interactions when considering the
318 complete genomes of the TB patients and their infecting MTBC strain (46), and identified
319 associations between human and pathogen variants. Such associations reflect host-pathogen
320 genetic interactions that determine susceptibility to symptomatic TB or intra-host selection
321 during mycobacterial replication. Here, in the context of co-evolutionary history between
322 humans and MTBC, we specifically tested whether an interaction between the main human

323 ancestry components and being infected with the most dominant MTBC strain in Dar es
324 Salaam could explain the variability in TB disease severity. However, we did not find
325 evidence of such an effect. Others have reported an association between TB disease severity,
326 a particular bacterial genotype and a particular human SNP in Uganda, but did not explicitly
327 link this human SNP to a particular human genetic ancestry (52, 62). Several factors could
328 account for the lack of effect we observed. First, our patient population was relatively
329 genetically homogeneous, given that the different Bantu components represent populations
330 with only moderate levels of genetic differentiation (63). Second, there is likely to be
331 selection bias in our cohort since only patients presenting at the clinic were recruited. The
332 disease severity measures included in this study mainly reflect disease stages, at which
333 patients felt ill enough to go to the hospital; i.e. we did not consider intermediate, more
334 contrasting disease states that are known to occur between infection and the development of
335 symptoms (64). To at least partially account for that, we included the number of weeks a
336 patient was coughing as a covariate in our analyses. Third, the lack of a measurable
337 interaction between host genetic ancestry and MTBC genotype could reflect the relatively
338 recent presence of these MTBC genotypes in Tanzania, and the distinct (i.e. allopatric)
339 geographical origins of the host and pathogen populations. This indicates that none of the
340 ancestral human populations that compose modern Tanzanians has lived in sympatry with the
341 ancestors of the modern MTBC genotypes that circulate in Dar es Salaam today, thus
342 rendering human susceptibility alleles linked to genetic ancestry unlikely. With the exception
343 of West Africa, where the geographically restricted West-African MTBC lineages L5 and L6
344 remain an important cause of human TB (65), the situation in Tanzania might be
345 representative of the TB epidemics in many African countries, as evidence suggests that
346 many MTBC genotypes dominating the continent today have been introduced from outside
347 Africa during recent centuries (56, 66-68).

348

349 In conclusion, our study shows that the TB patients from Dar es Salaam were mainly of
350 Bantu genetic ancestry reflecting limited Eurasian genetic influx. Neither the human genetic
351 ancestry or the MTBC genotype alone, nor their interaction, were associated with TB disease
352 severity. Our results highlight the dominant role of social and environmental factors in human
353 TB in Tanzania.

354

355 **Methods**

356 **Study population**

357 This study is based on a previously described dataset (46, 50). Briefly, adult active TB
358 patients (sputum smear-positive and GeneXpert-positive) were recruited between November
359 2013 and June 2022 at the Temeke District Hospital in Dar es Salaam, Tanzania when they
360 first presented for care. Sputum and blood samples were collected from each patient to
361 extract DNA for sequencing of the MTBC strain, and genotyping or whole-genome
362 sequencing (WGS) the patient. Additionally, clinical and sociodemographic information was
363 obtained from every patient. In total, there was either human or bacterial data available for
364 1,906 patients. Of those 1,444 patients had human genetic data and 1,471 had bacterial
365 genetic data available, respectively. A total of 1,000 patients had both types of data available
366 after quality-based filtering. The geographical locations of the self-indicated ethnic group of
367 each patient were retrieved by searching for the original region of the respective ethnic group,
368 and if they originated from a single region, the geographic coordinates according to
369 Wikipedia were taken. If two neighboring regions were among the origins, then a random
370 location between the two regions was taken as surrogate (Supplementary Table S1).

371 **Bacterial and human sample processing**

372 The MTBC bacteria were cultured on solid Löwenstein-Jensen media at the TB laboratory of
373 the Ifakara Health Institue in Bagamoyo, Tanzania. Before 2018, the MTBC isolates were
374 transferred to Switzerland for DNA extraction and later, DNA extraction was carried out in
375 Bagamoyo. Bacterial WGS was done using the Illumina short-read technology at the
376 Department of Biosystems Science and Engineering of ETH Zurich in Basel (DBSSE).
377 Human WGS was done at the Health 2030 Genome Center in Geneva, Switzerland using an

Illumina NovaSeq 6000 sequencer. The human genotyping was done at the iGE3 Genomics platform at the University of Geneva in Switzerland using the Illumina Infinium H3Africa genotyping microarray (Version 2; https://chipinfo.h3abionet.org) plus custom Tanzanian-specific SNP add-ons (69).

Human genetic data

The processing of the human genetic data has been described in detail by Xu et al (46). Briefly, we used the GRCh38 as a human reference genome to map the WGS reads of 118 patients using BWA aligner (v0.7.17) (70). Duplicate reads were then marked with the markduplicates module of Picard (v2.8.14) (71). Variant calling was first done for each sample individually following GATK best practices for germline short variant discovery. Samples with a coverage below 5 were excluded followed by a joint calling of the variants. A Quality Score Recalibration (VQSR) based filter was applied (real sensitivity of 99.7, excess heterozygosity of 54.69) and samples with more than 50% missing genotype calls were removed.

For the genotyping data, we used the Illumina GenomeStudio software (v2.0.5, https://support.illumina.com/array/array_software/genomestudio/downloads.html) to analyze the raw microarray data. Samples with a low quality, that were badly clustered, or that had a call rate below 0.97, were excluded. The PLINK format was then converted to VCF format using PLINK (v1.9) (72). The first round of imputation was performed with the African Genome Resources (AFGR, https://www.apcdr.org/) reference panel on the sanger imputation server with EAGLE (73) for phasing and Positional Burrows-Wheeler transform (PBWT) (74) for imputing. The second round was performed with a reference panel created in house that was based on 118 patients with available whole-genome sequences (69) with SHAPEIT4 for phasing and Minimac3 for imputing. For each SNP, the reference panel with

402 the highest imputation quality score was used to determine the final genotype call. SNPs with
403 an INFO score below 0.8 were discarded.

404 Bcftools (v1.15) was used to merge the WGS and genotyping samples after identifying SNPs
405 shared between the two methods that were missing in fewer than 10 samples.

406 **Whole-genome sequence analysis of the MTBC bacteria**

407 We analyzed all FASTQ files using the WGS analysis pipeline described previously (75). In
408 summary, Trimmomatic (76) v. 0.33 (SLIDINGWINDOW:5:20) was used to remove the
409 Illumina adaptors and to trim low quality reads. Only reads with a length of at least 20 bp
410 were kept for further analysis. Overlapping paired-end reads were mergend using SeqPrep v.
411 1.2 (77) (overlap size = 15). We then mapped the resulting reads to a reconstructed ancestral
412 sequence of the MTBC (78) with BWA v. 0.7.13 (mem algorithm) (70). Picard v. 2.9.1 (79)
413 was then applied to mark and exclude duplicated reads. Furthermore, the
414 RealignerTargetCreator and IndelRealigner modules of GATK v. 3.4.0 (80) were used to
415 perform local realignment of reads around INDELs. Reads having an alignment score lower
416 than $(0.93 \times read\ length) - (read\ length \times 4 \times 0.07)$, corresponding to more than 7 miss-
417 matches per 100bp, were excluded using Pysam v. 0.9.0 (81). SNP calling was then
418 performed with SAMtools v. 1.2 mpileup (82) and VarScan v. 2.4.1 (83) with the following
419 thresholds: a minimum mapping quality of 20, a minimum base quality at a position of 20,
420 minimum read depth at a position of 7 and no strand bias. Positions in repetitive regions such
421 as PE, PPE, and PGRS genes or phages were excluded, as described in (84). A whole-
422 genome Fasta file was created from the resulting VCF file. We applied some additional
423 filters; genomes were excluded from downstream analysis if they had a sequencing coverage
424 of lower than 15 or if they contained SNPs suggestive of different MTBC lineages (i.e. mixed

425 infections). We identified lineages and sublineages using the SNP-based classification by
426 Steiner et al. (85), and Coll et al. (86), respectively.

427 **Identification of bacterial SNPs diagnostic for the successful MTBC introductions**

428 We previously identified several successful MTBC introductions into Dar es Salaam (49).
429 For these, we aimed to obtain a set of diagnostic SNPs that would allow assigning MTBC
430 strains not included in our previous study to these genotypes. For that, we merged the VCF
431 files from the 1,082 MTBC genomes included in that previous dataset by using BCFtools
432 (v1.9). We then used VCFtools (v0.1.16) to remove Indels and positions that were variable in
433 less than 12 genomes (12 was the minimal threshold selected when identifying the successful
434 introductions in our previous publication (50)). By using the R package VariantAnnotation
435 (87) and a customized Python script, SNPs specific to one of the most successful
436 introductions were extracted. To ensure the SNPs identified as markers for the introductions
437 were specific, we also identified phylogenetic SNPs on a bigger and global dataset
438 representing the human-adapted MTBC diversity (75), and tested whether any of the
439 phylogenetic SNPs identified for any of the successful Introductions was present in any of the
440 MTBC lineages or sublineages. We compiled a subset of 25 SNPs (Error: Reference source
441 not found) that we used as phylogenetic markers for the different MTBC introductions and
442 identified strains belonging to one of the four most successful MTBC introductions
443 (Introduction 1, Introduction 5, Introduction 9, and Introduction 10) in the expanded MTBC
444 dataset based on these SNPs by using a customized script.

445 **Measures of TB disease severity**

446 We used three different proxies for TB disease severity. The first one was the TB-score,
447 which is a clinical score adapted from Wejse et al. (88) that consists of several signs and
448 symptoms including the presence of fever and the Body Mass Index (BMI). A point was

449 given for each of the following symptoms or clinical measures if present: BMI below 18,
450 BMI below 16, mid upper arm circumference (MUAC) below 220, MUAC below 200, body
451 temperature higher than 37°C, cough, hemoptysis, dyspnea, chest pain, night sweat, abnormal
452 auscultation, anaemia. A maximum of 12 points could be achieved, and a TB-score below 6
453 was considered as mild and everything above as severe. As a second proxy, we assessed the
454 amount of lung damage. Two independent radiologists assessed chest x-ray pictures of the
455 patients and gave a Ralph score (89). X-ray scores above 71 were considered as severe and
456 everything below as mild. As a third proxy we used the bacterial load in the sputum
457 represented by the difference between the first (early cycle) and the last (late cycle) threshold
458 during quantitative PCR (Ct-value). For each sputum sample, we took five probes, run a
459 quantitative PCR each and reported the lowest Ct-value.

460 **Genetic ancestry analysis of TB patients**

461 To investigate the genetic ancestry of the TB patients, we combined our dataset with the data
462 from ten other projects: The Gambian Genome Variation Project (GGVP) (90), the 1000
463 Genomes Project (1000G) (91), the Human Genome Diversity Project (HGDP) (92), Simons
464 Genome Diversity Project (93), as well as data generated by Patin et al. 2014 and 2017 (4,
465 94), Hollfelder et al. 2017 (95), Semo et al. 2019 (8), Schlebusch et al. 2012 (96), and Fortes-
466 Lima et al. 2022 (97). We used the GRCh37 version of all datasets. The dataset of HGDP was
467 in GRCh38 version and we thus did a lift over to GRCh37 using the picard (v2.26.10) (71)
468 tool LiftoverVcf. For all the datasets including only populations from one single continent ,
469 we excluded variants with a missingness of more than 10% and only included variants that
470 did not deviate from Hardy-Weinberg equilibrium ($p < 1e-5$) using PLINK (version 1.9b,
471 www.cog-genomics.org/plink/1.9/) (72). For the 1000G, SGDP, and HGDP data, we first
472 identified variants that deviated from the Hardy-Weinberg equilibrium ($p < 1e-5$) in each
473 superpopulation using PLINK (version 2.0a, www.cog-genomics.org/plink/2.0/) and removed

them from the whole dataset. We additionally removed variants with a high missingness (> 10%) from the full datasets using PLINK (version 1.9b). After extraction of 103,262 nucleotide positions common to all datasets, we merged the datasets using PLINK (version 1.9b). From the merged dataset, we removed second-degree relatives using PLINK (version 2.0a, king cutoff of 0.088) (N=369) and patients from our cohort where the sex according to the genetic data did not correspond with the sex indicated in the clinical data, patients who were genetic outliers based on principal component analysis (PCA) or who did not cluster with any other African samples (N=83). In addition, we removed regions of high linkage disequilibrium (https://genome.sph.umich.edu/wiki/Regions_of_high_linkage_disequilibrium_(LD)) and applied additional filters to the merged dataset (missingness > 10%, minimum allele frequency of 5%, removal of sex chromosomes, variant pruning with –indep-pairwise 50 10 0.1, only biallelic positions) ending up with 53,255 positions and 7,479 individuals from 249 populations.

To infer the ancestry proportions of the Tanzanian TB patients, we used ADMIXTURE (version 1.3.0) (48). We estimated the number of ancestral populations (K) by running ADMIXTURE 15 times for each value for K from K=2 until K=29 with the option --cv. The --cv option performs 5-fold cross-validation and allows to identify the value for K resulting in the lowest cross-validation error (Error: Reference source not found). The cross-validation error was lowest for K = 24. From the 15 runs performed with K = 24, we selected a representative of an output that was supported by most of the 15 runs (6/15) to extract the ancestry proportions of each individual. A PCA was performed using PLINK (version 1.9b) on all African populations included.

497 **Spatial interpolation of human genetic ancestry proportions**

498 To visualize the distributions of the different patient ancestries, we performed spatial
499 interpolation using the OrdinaryKriging function from the pykrige module in Python
500 (variogram_model = "linear", grid space of 500). For the Eastern and Southern Bantu
501 ancestries, we included all African populations for the interpolation. For the Western Bantu,
502 the interpolation failed when using all African populations with an insufficient slope,
503 suggesting little spatial variability. Since many populations were sampled in the region with
504 the highest proportions of Western Bantu genetic ancestry and among them many hunter-
505 gatherer populations containing little to no Western Bantu genetic ancestry, we repeated the
506 interpolation with only a subset of the non-hunter-gatherer populations.

507 **Correlation between distance matrices**

508 To assess, whether MTBC genotypes that are more closely related tend to infect people that
509 are also more closely related genetically which would be compatible with co-evolution, we
510 investigated the correlation between the human and bacterial distance matrices. To calculate
511 the pairwise bacterial genetic distances, alignments of variable positions where data was
512 missing in less than 10% of the genomes were generated and used to create SNP distance
513 matrices according to the Hamming distance (https://git.scicore.unibas.ch/TBRU/tacos).
514 Insertions and deletions were considered as missing data. To get the human pairwise genetic
515 distances, we calculated the Euclidean distance based on the first two principal components.
516 When only looking at the Tanzanian TB patients, we calculated the principal components for
517 the Tanzanians only while for the continental dataset we included all available African
518 populations.

519 To investigate, whether human populations that are geographically more distant are also
520 genetically more distant, we calculated the correlation between the geographical and the

521 genetic distance on an African level as well as on the level of Tanzania. For the geographical
522 distance matrix, we calculated the Euclidean distance based on the latitude and the longitude.
523 At the level of Tanzania, the broad geographic location of the original area of the ethnic
524 group was considered (Supplementary Table S1) and for the continental level, the coordinates
525 of the hospital in Temeke were taken for the Tanzanian TB patients considering that for the
526 other studies only the sampling locations were known. The human and bacterial genetic
527 distance matrices as well as the human genetic and geographic distance matrices were tested
528 for correlations using the mantel.test() function from the mantel module in Python (options:
529 perms = 10000, method = "pearson", tail = "upper").

530 **Statistical analyses**

531 Clinical and sociodemographic characteristics, MTBC genotype as well as human genetic
532 ancestry were summarized by the different disease severity measures using proportions or
533 means and standard deviations. Similarly, the human genetic ancestries were summarized by
534 MTBC genotype.

535 To estimate the associations between disease severity and the explanatory variables bacterial
536 genotype (binary, belonging to Introduction 10 or not) and human ancestries (Southeastern
537 Bantu, Eastern Bantu, Western Bantu), we used a logistic regression model. We included
538 three variables as proxies for disease severity: Lung damage based on X-ray score (mild
539 versus severe), TB-score (mild versus severe), bacterial load (continuous, log10 transformed).
540 We tested only for Introduction 10 because there were few observations of the other MTBC
541 genotypes. To account for the compositional nature of the human ancestries (i.e. that they
542 sum up to 1), we used the additive log transformation from the R package 'compositions'
543 (98). The ancestry proportions were transformed and categorized with category 1 comprising
544 the lowest amount of the respective ancestry and category 3 (4 in the case of Western Bantu)

545 the highest. The categories were chosen to have roughly equal numbers of patients in each.
546 We used categories to allow a non-linear relationship without specifying polynomials,
547 needing a large sample size or difficulty in interpretation but we recognize that a small
548 amount of information is lost. Similar results were also obtained with other
549 parameterizations. We assessed whether there was an interaction between ancestry and
550 genotype on TB severity using the likelihood ratio test. For that, we compared a model
551 including the interaction between the MTBC genotype and human genetic ancestry to a
552 model without the interaction using the 'lmtest' package (99). The estimates were adjusted
553 for: age, sex, smoking, and the number of weeks with cough. Only HIV-negative patients
554 were included in the analysis. All statistical analyses were carried out in R (version 4.1.2).
555 Code for the statistical analysis can be found (https://github.com/mzwyer/TB-Dar_Mtb).

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Data availability

The bacterial WGS data can be found under the bioprojects PRJEB49562 and PRJNA670836 on ENA and the human WGS and genotyping data under EGAS00001005850 and EGAS00001007216, respectively. Clinical data available under request.

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815 **Supporting information captions**

816 Supplementary Figure S1 - The first two PCA components for all African populations
817 included in this study (n=116).

818 Supplementary Figure S2 - Populations included in the Admixture analysis. Populations are
819 coloured according to the study from which the data was obtained (4, 8, 44, 91-93, 95-98).

820 Supplementary Figure S3 - Boxplots of cross-validation errors for values of K between 2 and
821 29 resulting from 15 runs. The lowest cross-validation error was obtained for K = 24.

822 Supplementary Figure S4 - Boxplots of the proportions of the 24 genetic ancestries among
823 the Tanzanian TB patients. The ancestries were named after the population(s) where they
824 were most abundant.

825 Supplementary Figure S5 - Ancestry plots for all African populations included (K = 24).

826 Supplementary Figure S6 - Heatmap showing the correlations between the genetic ancestries
827 and geographical location. A) Correlations at the country level of Tanzania between the
828 latitude and longitude of the ethnic group the patients belong to. B) Correlations on a
829 continental level including all African populations. For the latitude, a negative correlation
830 indicates higher genetic ancestry proportions towards the South and for the longitude a
831 negative correlation indicates lower genetic ancestry proportions towards the East.

832 Supplementary Figure S7 – Association between the Bantu genetic ancestries and TB-score
833 and X-ray score for each of the most successful introductions. A, C, E) Southeastern, Eastern,
834 and Western Bantu on mild or severe TB-score, B, D, E) Southeastern, Eastern, and Western
835 Bantu genetic ancestry lung damage assessed by lung X-ray.

836 Supplementary Table S1 - The different ethnic groups with at least 10 members in our cohort
837 and the region and broad geographic location of the original area of the ethnic group. The
838 latitude and longitude are given in decimal degrees. In the case of two associated regions, a
839 location close to the border of the two regions was selected.

840 Supplementary Table S2 - Phylogenetic markers selected to identify the Introductions. The
841 position is based on the reconstructed reference of the ancestor (79) and the derived base
842 indicates the base present in the respective Introduction. Intro1 refers to Introduction 1 within
843 L2.2.1, Intro5 to Introduction 5 within L4.3.4, Intro9 to Introduction 9 within L1.1.2, and
844 Intro10 to Introduction 10 within L3.1.1.

845