Human *SLC11A1* gene polymorphism has the propensity to confer susceptibility to *M. africanum* TB disease in Ghana

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Key Words

Tuberculosis; Host pathogen interactions; SNPs

Abstract:

Human tuberculosis (TB) is caused mainly by *Mycobacterium tuberculosis* (MTB) and *M. africanum* (MAF) remains a major global health threat. The varying response of different host to contact with the TB bacteria, indicates the importance of host genetics in susceptibility to TB disease. We explored the association among selected human/host genomic variants and disease caused by the two causative pathogens in Ghana through a case control study. MTBC isolates (323) recovered from pulmonary TB patients recruited between 2016 and 2018 were genotyped using spoligotyping. A selection of 29 SNPs from MTB-related genes with high frequency among African populations were genotyped using a TaqMan® SNP Genotyping Assay and iPLEX Gold Sequenom Mass Genotyping Array. Associations between MTBC lineages and host variables were assessed using univariate and multivariate logistic regression. The prevalence of MTB and Maf among the participants were 79% and 21% respectively. Association analysis between the controls and MAF showed that rs2695342 variant on the *SIC11A1* gene have the propensity to confer susceptibility to MAF infections (P = 0.093, OR = 8.35, 95% CI = 0.70 – 99.24) whilst the rs17048476 (P = 0.088, OR = 1.57, 95% CI = 0.93 – 2.63) and rs1482868 (P = 0.095, OR = 0.60, 95% CI = 0.33 – 1.09) were observed to be only suggestive. Our findings implicate *SLC11A1* as a potential susceptibility gene of substantial interest for TB caused by MAF which is an important pathogen in West Africa and highlight the need for in-depth host pathogen studies in West Africa.

Keywords: Tuberculosis, *M. africanum*, *SLC11A1*

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1.INTRODUCTION

Pulmonary tuberculosis (TB), is a significant public health burden worldwide, with 10 million cases and an estimated 1.5 million deaths in 2018(1). As a disease transmitted by the inhalation of aerosolized droplets, [1], TB is expected that susceptible individuals who come into contact with aerosol(s) containing viable bacteria will be equally infected. In about 90% of infected individuals, between 3 to 8 weeks after MTB contained in inhaled aerosols becomes implanted in alveoli (1), the host immune system comprising of both the innate and adaptive arm will wall off the site if infection in a granuloma (ghon complex), such individuals are asymptomatic and are latent TB infection (LTBI) [2-4]. Only 5% of infected immune competent individuals will potentially develop active TB disease within 2-3 years of infection while the remaining 5% develop TB later in their life [5-8]. This difference potentially depends on the interplay between the environment, the bacteria and most importantly the host genetic factors associated with TB pathogenesis.

For many years, the diverse impact of several important genes such as the major histocompatibility complex (MHC), and non-HLA genes like killer immunoglobulin-like receptor (KIR), toll-like receptors (TLRs), cytokine/chemokines and their receptors, vitamin D receptor (VDR), *SLC11A1* and C-type lectins on the susceptibility to TB or otherwise has been demonstrated by several studies (8-10). For instance, In 2012, Pydi et al reported Inhibitory genes *KIR3DL1* ,*KIR2DL3* conferring susceptibility towards TB in individuals and recently, Bartlelt et in 2020 observed significant interaction between a single nucleotide polymorphism (SNP) in *SLC11A1* and the L4-Ugandan lineage in TB household contact study in Kampala, Uganda [9-10]. Indeed, familial risk of tuberculosis has been recognized for centuries, largely through studies of mono- and dizygotic twin concordance rates, studies of families with Mendelian susceptibility to mycobacterial disease, and candidate gene studies performed in the 20th century leading to the realization that susceptibility to TB disease has a substantial host genetic component [2-8].

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Although anatomical modern humans evolved from Africa, very few genetic studies have been conducted in ancestral Africans with regards to TB host genetic susceptibility. Therefore, our knowledge of even the most fundamental information on the genetic basis of susceptibility or otherwise to TB disease in Africa is quite limited. Moreover, only few studies have taken into consideration the potential influence of different genotypes of the TB pathogen on such interactions. Human TB is mainly caused by *Mycobacterium africanum* (MAF) and *M. tuberculosis* sensu stricto (MTB), both members of *M. tuberculosis* complex (MTBC), which also includes several sub-species adapted to a variety of wild and domestic animals [2-3]. Of special interest to Africa is MAF, which is restricted to West Africa and may cause up to 50% of human TB in some of the countries unlike the globally ubiquitous MTB. In Ghana for instance, MAF causes about 20% of all TB cases [2]. Thus, host genetics and susceptibility to distinct MTBC lineages cannot be overlooked. Findings from two independent molecular epidemiological studies by our group showed a strong association between MAF (driven by lineage 5) and a native West African ethnic group [11-12]. This current study thence explored potential host and pathogen interactions towards understanding MAF and MTB infections to enhance our understanding of TB pathogenesis

2.MATERIALS AND METHODS

2.1. Ethical Approval

The study was performed in accordance with the Declaration of Helsinki. Ethical approval for the study was obtained from the Noguchi Memorial Institute for Medical Research Institutional Review Board (NMIMR-IRB 097/15-16). Written and signed informed consent was obtained from participants before enrolment into the study

2.2. Study population

Our study population were in two categories: Diagnosed TB patients' group and non-TB patients serving as control group (NTB). For the TB group, only newly diagnosed sputum smear-positive adult TB cases registered at the Department of chest disease, Korle-bu teaching Hospital in Ghana were recruited into the study before the commencement of TB treatment from 1st July 2016- 31st July 2018. All patients were unrelated. Ethnicity was defined in line with Ghana demographic data 2010 [13].

For the NTB group, 5 communities' sites by the National TB control programme were selected. These sites included Korle-gonno (Ablekuma south sub metro), Bukom (Ashiedu Keteke sub metro), Abossey-okai (Okaikoi south), Glefe (Ablekuma West) and Amanfrom (Ga West District). Medical outreaches were conducted in these sites and with a new structured questionnaire, clinical characteristics (diabetes, HIV, hypertension) as well demography and epidemiological data were obtained from each participant. Only patients with no history of TB were recruited into the study. In accordance with ethics, participants with elevated stage 1 or 2 hypertension were transported to the nearest hospital for immediate medical attention and were excluded from the study.

2.2. Sample collection

Diagnosed TB patients' group

To confirm the initial diagnosis at the health facility and to identify the infecting mycobacterial species, sputum specimen was collected from each TB study participant, following the National Tuberculosis Control Program guidelines. Samples were taken only after a detailed explanation of the study and written, or thumb-printed consent have been obtained for participation. Clinical characteristics (previous history of TB, HIV) as well demography and epidemiological data including age, sex and ethnic origin were obtained from each participant. In addition, each patient was screened for Diabetes mellitus (DM) using random blood glucose level. Based on the American Diabetes Association (ADA) criteria, a glucometer (ACCU-CHEK) Active Glucose Monitoring System, Roche Diabetes Care Limited, Burgess Hill, UK which uses finger prick test OneTouch Ultra test strips was used to screen all patients for DM irrespective of known DM status. If the blood glucose level was less than 7mmol/L, no further action was taken, however if blood glucose level was above 13 mmol/L, test was confirmed as having Diabetes. 5ml of blood was collected from each TB patient for host genetics analysis.

Non-TB patients control group (NTB) and chest X-ray screening.

The screening of eligible control individuals was done in a stepwise manner**.** Firstly, Adults (>18 years) presenting at any of the outreach centers were verbally screened for fever, diabetes and hypertension. Individuals with no evidence of fever, diabetes or elevated hypertension were then taken through chest X-ray (CXR) screening using CAD4TB (version 3.07, Diagnostic Image Analysis Group, The Netherlands) for abnormalities suggestive of pulmonary TB. The software has two abnormality detection systems that is textural abnormality and shape abnormality systems, which analyze the abnormalities in the unobscured lung fields that have been segmented automatically [14]. A higher score is suggestive of TB. A CAD4TB threshold score of 60 was used for this population determined using previously collected CXR data in a similar population. Sputum samples was collected from all individuals with high CAD4TB scores (60 or greater) and transported to the laboratory for further analysis. Individuals with high CAD4TB scores were referred to the hospitals for further clinical evaluation. 5ml of blood was collected from each TB patient for host genetics analysis.

141 **2.3. Laboratory analysis**

142 *Isolation and characterization of Mycobacterium spp.*

143 Sputum samples obtained were decontaminated using 5% oxalic acid [15-16] and inoculated on two pairs of Lowenstein Jensen (LJ)
144 slants; one supplemented with 0.4% sodium pyruvate to enhance the isolation of MAF and *M. bovis*, and the other with glycerol for
145 the growth of *MTB*. The cultures were incubated at 37 °C and were observed weekly for growth for a maximal duration of 16 weeks.
146 MTBC strains were identified by PCR detection of insertion sequence IS*6110* as previously described [16]. Colonies from positive
147 cultures were purified and stored at -80 °C in 2 ml Middlebrook 7H9 supplemented with ADC enrichment media until use. Pure
148 Bacteria DNA was extracted for genotyping using a modified protocol [17] and stored at -20 °C until further use.
149 All MTBC isolates were further typed by spoligotyping [18]. This was performed according to the manufacturer's instructions, using
150 commercially available kits (Isogen Bioscience BV Maarssen, The Netherlands). Briefly, The DR containing region was amplified by
151 PCR using primers, DRa and DRb (GGTTTTGGGTCTGACGAC, and CCGAGGGGACGGAAAC). The amplified products were hybridized to
152 set of 43 oligonucleotides each corresponding to one spacer, immobilized on a nylon membrane. Detection of hybridization was
153 achieved using chemiluminescent ECL (Amersham) liquid followed by X-ray exposure. The Spoligotyping patterns obtained were
154 defined according to SITVITWEB database (http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE). SITVITWEB assigned shared
155 types numbers were used whenever a spoligotyping pattern was found in the database while families and subfamilies were assigned
156 based on the MIRU-VNTRplus database (http://www.miru-vntrplus) (http://www. miru-vntrplus.org). Shared types were defined as
157 patterns common to at least two or more isolates. All patterns that could not be assigned were considered orphan spoligotypes.
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Host DNA Isolation

For all enrolled participants 5 ml of whole blood was drawn into EDTA-coated tubes (BD Biosciences) and immediately stored at 4 ºC. DNA was extracted within 24 hours from peripheral blood, following instructions on the available commercial kit Gentra Puregene Blood Kit (QIAGEN) in accordance with the manufacturer's recommendations. All DNA samples were stored at 80 ºC prior to genotyping.

DNA quality was evaluated according to the 260/280 ratio with a NanoDrop 2000 spectrophotometer (Thermo Scientific). In total 792 samples with concentrations of 5 ug/ul-851.87 ug/ul were selected and sent to the whilst single nucleotide polymorphisms (SNPs) typing.

Host genetics analysis: Genotyping of targeted SNPs in MTB-related host genes

Targeted sequencing was conducted in the Division of Human Genetics Laboratory, Faculty of Health Sciences, at the University of Cape Town, South Africa. Genetic variants that are potentially associated with MTB were identified from the recent literature. Once the SNPs of interests were identified, we investigated their allele frequencies in African populations present in the 1000 Genomes project (http://www.internationalgenome.org/home), and further narrowed the selection to SNPs that showed high frequency among African populations. This resulted in the selection of 29 SNPs from MTB-related genes that were investigated in the present study (Table 1). SNPs (Table 1) were genotyped using a TaqMan® SNP Genotyping Assay and TaqMan® Universal Master Mix (Life Technologies, Carlsbad, CA, USA), at the Division of Human Genetics, Faculty of Health Sciences, University of Cape Town; and by iPLEX Gold Sequenom Mass Genotyping Array (Inqaba Biotec, Pretoria, South Africa). Validation was done in a subset of samples (10%), by Sanger sequencing using BigDye terminator mix (Promega, Madison, WI, USA) (Supplementary figure 1).

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190 Table 1: Allele frequencies of SNPs used in the study

			Current study		Ensembl					
Gene	rs number	Allele	Frequency	Ratio	All	AFR	AMR	EAS	EUR	YRI
CALN1	rs844669	A	593	0,74	0,68	0,72	0,57	0,75	0,61	0,74
		C	206	0,26	0,32	0,28	0,43	0,25	0,39	0,26
SLC11A1	rs17235409	A	146	0,18	0,07	0,06	0,08	0,14	0,01	0,06
		G	653	0,82	0,93	0,94	0,92	0,86	0,99	0,94
LOC107984706	rs857063	A	599,5	0,75	0,66	0,67	0,57	0,47	0,82	0,66
		C	199,5	0,25	0,34	0,33	0,43	0,53	0,18	0,34
F13A1	rs1482868	C	664,5	0,83	0,69	0,75	0,74	0,76	0,66	0,75
		T	134,5	0,17	0,31	0,25	0,26	0,24	0,34	0,25
PLCD4	rs3731869	G	553	0,69	0,08	0,12	0,02	0,12	0,03	0,14
		T	246	0,31	0,92	0,88	0,98	0,88	0,97	0,86
MBL2	rs5030737	A	43,5	0,05	0,03	0	0,03	0	0,06	0
		G	755,5	0,95	0,97	100	0,97	100	0,94	100
ZPLD1b	rs12636260	G	295	0,73	0,54	0,73	0,53	0,54	0,45	0,76
		T	107	0,27	0,46	0,27	0,47	0,46	0,55	0,24
F13A1	rs1482868	C	277,5	0,70	0,69	0,75	0,74	0,76	0,66	0,52
		T	119,5	0,30	0,31	0,25	0,26	0,24	0,34	0,48
DAB1	rs1524713	C	295,5	0,72	0,69	0,75	0,74	0,76	0,66	0,75
		T	115,5	0,28	0,31	0,25	0,26	0,24	0,34	0,25
Bsm-I	rs1544410	C	312,5	0,74	0,7	0,73	0,74	0,94	0,6	0,7
		T	108,5	0,26	0,3	0,27	0,26	0,06	0,4	0,3
GLO1	rs1616723	C	54,5	0,17	0,12	0,18	0,05	0,18	0,08	0,19

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		T	271,5	0,83	0,88	0,82	0,95	0,82	0,92	0,81
AK124857	rs17048476	A	165	0,40	0,24	0,4	0,18	0,08	0,21	0,37
		G	245	0,60	0,76	0,6	0,82	0,92	0,79	0,63
SLC11A1	rs17235409	A	23	0,05	0,07	0,06	0,08	0,14	0,01	0,06
		G	408	0,95	0,93	0,94	0,92	0,86	0,99	0,94
RBFOX1	rs2346943	G	88,5	0,21	0,33	0,3	0,38	0,26	0,42	0,29
		T	331,5	0,79	0,67	0,7	0,62	0,74	0,58	0,71
ADAMTS14	rs2587469	A	225,5	0,58	0,62	0,56	0,5	0,7	0,62	0,6
		G	165,5	0,42	0,38	0,44	0,5	0,3	0,38	0,4
THSD7A	rs2681052	A	155	0,38	0,5	0,38	0,64	0,41	0,64	0,39
		C	248	0,62	0,5	0,62	0,36	0,59	0,36	0,61
SLC11A1	rs2695342	G	422	0,97	0,98	0,94	100	100	100	0,96
		A	12	0,03	0,02	0,06	0	0	0	0,04
	rs2807348	A	330	0,78	0,45	0,72	0,41	0,33	0,26	0,77
		G	92	0,22	0,55	0,28	0,59	0,67	0,74	0,23
RGS3	rs3860173	C	234	0,57	0,63	0,65	0,62	0,39	0,75	0,59
		T	177	0,43	0,37	0,35	0,38	0,61	0,25	0,41
SFRP1	rs4236914	C	282,5	0,72	0,79	0,73	0,79	0,72	0,95	0,68
		T	111,5	0,28	0,21	0,27	0,21	0,28	0,05	0,32
SLC38A4	rs4768760	A	243,5	0,58	0,61	0,59	0,61	0,61	0,66	0,59
		C	173,5	0,42	0,39	0,41	0,39	0,39	0,34	0,41
ADGRL3	rs4860106	A	102	0,40	0,49	0,63	0,61	0,21	0,59	0,63
		G	156	0,60	0,51	0,37	0,39	0,79	0,41	0,37

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UBLCPI, IL12B	rs4921437	C	282,5	0,68	0,86	0,74	0,89	100	0,83	0,76
		T	130,5	0,32	0,14	0,26	0,11	0	0,17	0,24
WNT4	rs557438	A	117	0,30	0,23	0,33	0,12	0,15	0,23	0,29
		G	279	0,70	0,77	0,67	0,88	0,85	0,77	0,71
GRIN2B	rs7297313	A	250,5	0,64	0,78	0,64	0,87	0,8	0,86	0,61
		C	142,5	0,36	0,22	0,36	0,13	0,2	0,14	0,39
Taq-1	rs731236	A	306,5	0,76	0,72	0,71	0,74	0,93	0,6	0,69
		G	98,5	0,24	0,28	0,29	0,26	0,07	0,4	0,31
VPS13C	rs8028149	C	166	0,43	0,65	0,44	0,77	0,84	0,67	0,44
		T	224	0,57	0,35	0,56	0,23	0,16	0,33	0,56
SH3TC2	rs930205	C	170	0,41	0,51	0,37	0,49	0,65	0,58	0,39
		T	242	0,59	0,49	0,63	0,51	0,35	0,42	0,61
SORBS2	rs955263	A	187	0,45	0,42	0,43	0,47	0,5	0,23	0,41
		G	226	0,55	0,58	0,57	0,53	0,5	0,77	0,59
ABCA8	rs9893385	A	231	0,56	0,52	0,53	0,57	0,51	0,41	0,58
		G	183	0,44	0,48	0,47	0,43	0,49	0,59	0,42

2.4. Data Analysis

Epidemiological Data for this study was double entered using Microsoft© Access and validated to remove duplicates. To analyze the population structure, we computed principal component (PC) using PLINK version 1.9. Ten (10) principal components were computed using PLINK1.9's --*pca* command and the top two principal components (PC1 vs PC2) were plotted using R. A generalized linear model (glm) was also performed using the R statistical package to evaluate correlation between the PCs. The quality of the variants was assessed, and association analysis was run with 28 high-quality variants using PLINK version 1.9 for the controls

against all the cases put together. A total of 333 participants (113 cases; 181 controls), of which 161 were males and 172 were females were included in the association analysis. What was the P values for statistical significance?

3.0.Results

3.1.Characteristics of studied cohorts

The study population consisted of 793 individuals (323 TB individuals and 470 controls). The male gender was associated with active TB in univariate logistics regression (odds ratio (OR) 1.78, 95% confidence interval (CI). 1.23-2.57, p = 0.002). Young patients (less than 35 years; n = 158) and patients older than 65 years (n = 11), were significantly associated with active TB (p= 0.001) (Table 2). Analysis of risk factors revealed that 34%, 11.6% and 5.8% of the patients were registered as alcohol abusers, cigarette users and substances abusers respectively

Information on the TB isolates (n = 323) was available for 300 patients. The vast majority had pulmonary TB(97.0%) followed by Pulmonary TB with anemia (2.0%), TB with lobber pneumonia (1.0%) (Table 2). At the time of sampling, 10.0% (n = 32) of the patients were hospitalized and 6.5% of the patients (n = 21) had previous history of TB. Analysis of risk factors revealed that 7.1% of the patients were registered TB-Diabetes patients. Moreover, HIV-positive patients accounted for 5.8% of the study population and 9.4% were notified as suffering from malnutrition. Sputum bacillary load at presentation was significantly higher in the MTBss group n = 90 patients having sputum grades of 2+ compared to (n =28) patients in the MAF group, p<0.001.).

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236 Table 2: Demographic characteristics of patients

Characteristics	(TB patients) N=323	(NTB) N=469)	P-value	OR	95%CI
Gender					
Male	253	124	<0.001	10.05	7.19 – 14.06
Female	70	345		Ref	
Age					
13-24	67	56	<0.001	2.44	1.51 – 3.94
25-34	91	78	<0.001	2.37	1.53 – 3.70
35-44	75	83	0.008	1.84	1.17 – 2.88
45-54	55	112		Ref	
55-64	24	66	0.300	0.74	0.42 – 1.31
65+	11	74	0.001	0.30	0.15 – 0.62
Risk factors					
BCG					
Yes	200	313	0.163	0.81	0.60 – 1.09
No	123	156		Ref	
Substance abuse (Cigarate , alcohol etc)					
Yes	143	144	<0.001	1.79	1.33 – 2.41
No	180	325		Ref	
Diabetes					
Yes	20	3	<0.001	10.25	3.02 – 34.80
No	303	466		Ref	
HIV					
Yes	19	0		NA	
No	304	469			
Education					
Primary	51	132	<0.001	0.47	0.32 – 0.69
Secondary	185	227		Ref	
Tertiary	42	15	<0.001	3.43	1.85 – 6.39
None	45	95	0.009	0.58	0.39 – 0.87

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Ethnicity					
Akan	125	113		Ref	
Ewe	47	71	0.025	0.60	0.38 – 0.93
Ga	95	264	<0.001	0.32	0.23 – 0.46
others	56	21	0.002	2.4	1.37 – 4.23

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3.2.Identified MTBC Phylogenetic Lineages

The 323 TB isolates after spoligotyping (S1) were identified as 79% (n = 255) MTBss and 21.1% (n = 68) MAF. Stratifying by Lineage, 87% (n = 222/255) of the MTBss strains belonged to the Euro-American lineage, with sublineages Cameroon (50%), Ghana (10.0%) and Haarlem (5.0%) being the most prevalent and among Maf Lineage 5 (west African 1) (56%) (n = 38/68. We detected a total of 66 spoligotypes, 286/323 isolates (88.2%) had previously defined shared type number (SIT). The remaining 37 isolates could not be defined by the SITVIT database and thus were defined as 'orphan' [S1]

3.3. Host genotype population structure and its association with Pathogen Lineages

The SNPs investigated in our current study had similar allele frequencies compared to the global and African populations (Table 1). The principal component analysis (PCA) plots coloured by case-control status, and by ethnicity show that the samples are homogeneous based on the typed SNPs (Figure 1 and 2). Furthermore, the generalized linear model (glm) showed no significant correlation between the PCs and the case-control phenotype (Table 3). However, a glm for case-control status against age, weight, and height revealed that the variables were significantly correlated with the phenotype based on the typed SNPs (Table 5) and were thus included as covariates in the analysis [S2].

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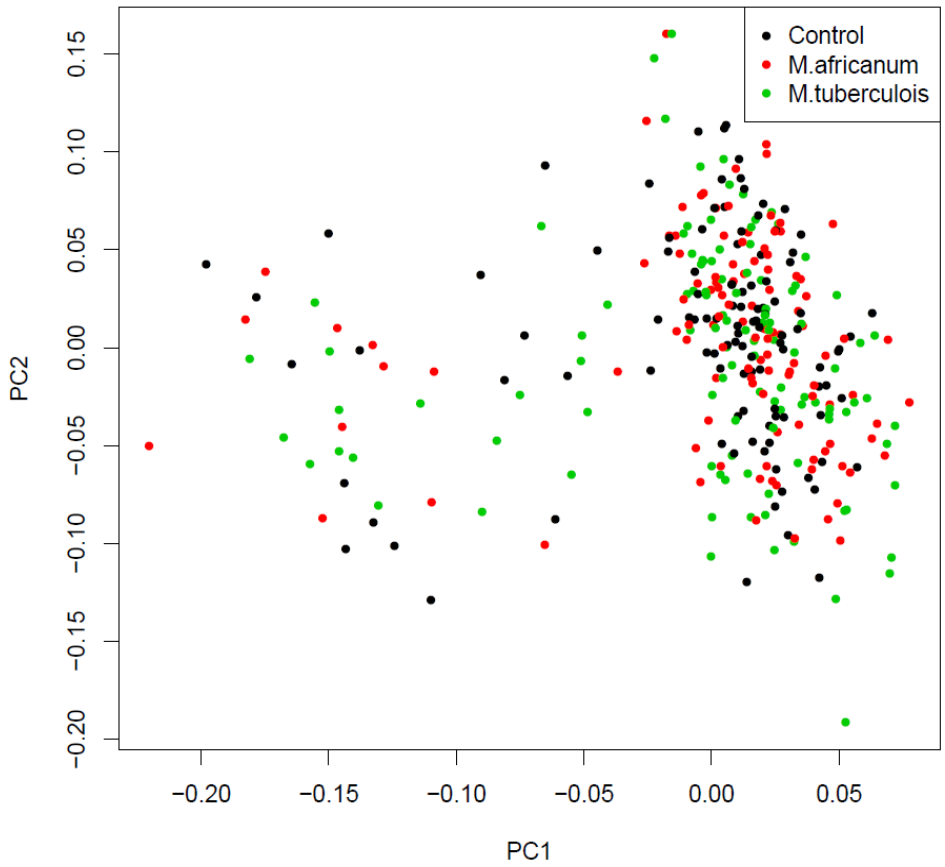

Figure 1: Principal component association plot between MTB cases and control

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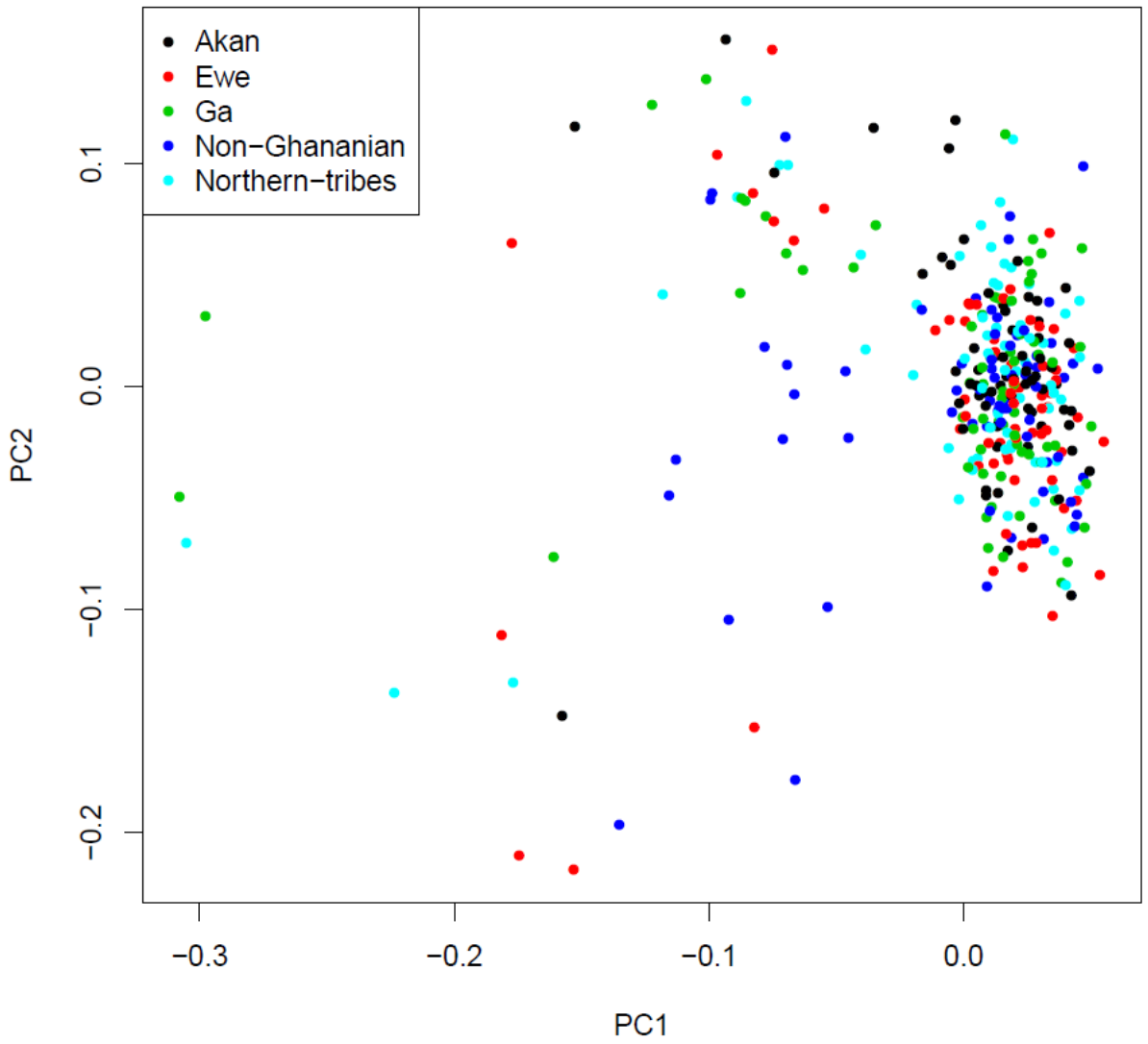

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265 **Figure 2: Principal component association plot between MTBC strains and patient ethnicity**

The variables that were correlated with the case-control phenotypes as earlier mentioned were included as covariates analysing the association between the controls and all cases (MTBss and MAF). Two variants (rs17048476 and rs1482868) emerged after adjusting for covariates. The variant rs17048476 is an intron variant on the AK124857 gene that has been previously found to be associated with resistance to TB in highly susceptible individuals (Sobota et al 2016:https://www.ncbi.nlm.nih.gov/pubmed/26942285) (18). However, in our work, the rs17048476 (*P* = 0.088, OR = 1.57, 95% CI = 0.93 – 2.63) and rs1482868 (*P* = 0.095, OR = 0.60, 95% CI = 0.33 – 1.09) were observed to be only suggestive compare to the high significance observed by Sobota et al in their study (https://www.ncbi.nlm.nih.gov/pubmed/26942285) (Table 3).

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275 **Table 3: Association analysis of ccontrols vs all cases (MTBss + MAF)**

CHR	POS	ID	REF	ALT	A1	TEST	OBS_CT	OR	LOG(OR)_SE	L95	U95	Z_STAT	P
1	22206613	rs2807348	A	G	G	ADD	333	1,08	0,29	0,61	1,90	0,25	0,8022
1	57537459	rs1524713	C	T	T	ADD	333	1,04	0,26	0,62	1,73	0,14	0,8865
2	218389899	rs2695342	G	A	A	ADD	333	2,13	0,78	0,46	9,77	0,97	0,3310
2	218395009	rs17235409	G	A	A	ADD	333	0,95	0,44	0,40	2,25	-0,12	0,9064
2	218395009	rs17235409	G	A	A	ADD	332	1,26	0,57	0,41	3,85	0,40	0,6870
3	7987199	rs17048476	G	A	A	ADD	331	1,57	0,26	0,93	2,63	1,70	0,0883
3	103407750	rs12636260	G	T	T	ADD	326	0,78	0,29	0,44	1,36	-0,88	0,3775
4	61984804	rs4860106	A	G	G	ADD	329	1,30	0,26	0,79	2,15	1,03	0,3033
4	185697150	rs955263	G	A	A	ADD	328	0,74	0,26	0,44	1,24	-1,14	0,2528
5	113228247	rs557438	G	A	A	ADD	321	0,76	0,28	0,44	1,31	-0,99	0,3215
5	148985671	rs930205	T	C	C	ADD	331	1,02	0,24	0,63	1,65	0,08	0,9359
5	159263943	rs4921437	C	T	T	ADD	331	1,11	0,26	0,67	1,85	0,42	0,6729
6	6202501	rs1482868	C	T	T	ADD	319	0,60	0,31	0,33	1,09	-1,67	0,0958
7	11609312	rs2681052	C	A	A	ADD	331	1,22	0,25	0,75	1,99	0,80	0,4238
7	72039275	rs844669	A	C	C	ADD	333	1,01	0,29	0,56	1,80	0,02	0,9806
8	41260502	rs4236914	C	T	T	ADD	315	0,98	0,26	0,59	1,64	-0,06	0,9492
9	113674597	rs3860173	C	T	T	ADD	330	1,11	0,25	0,68	1,82	0,42	0,6745
10	52771466	rs18004511	C	T	T	ADD	333	1,02	0,26	0,61	1,72	0,08	0,9347
10	52771482	rs5030737	G	A	A	ADD	333	0,66	0,38	0,31	1,39	-1,10	0,2722
10	70761399	rs2587469	A	G	G	ADD	319	1,11	0,27	0,65	1,89	0,39	0,6986
12	13855572	rs7297313	A	C	C	ADD	312	1,10	0,24	0,68	1,76	0,39	0,6995
12	46843681	rs4768760	A	C	C	ADD	330	1,19	0,26	0,71	1,97	0,66	0,5124
12	47844974	rs731236	A	G	G	ADD	327	0,81	0,29	0,46	1,43	-0,72	0,4708
12	47846052	rs1544410	C	T	T	ADD	332	1,20	0,29	0,68	2,11	0,64	0,5201
14	98046406	rs857063	A	C	C	ADD	333	0,71	0,24	0,45	1,12	-1,46	0,1437
15	61724906	rs8028149	T	C	C	ADD	320	1,26	0,27	0,74	2,14	0,86	0,3898
16	7445185	rs2346943	T	G	G	ADD	332	0,96	0,31	0,53	1,75	-0,12	0,9006
17	68822194	rs9893385	A	G	G	ADD	331	1,06	0,25	0,66	1,71	0,24	0,8108

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The association analysis between the controls and all MTB cases identified the rs1482868 variant with a suggestive association to MTBss (*P* = 0.0681) This suggestive association was however absent when the two species were analyzed together (Table 4). It appears that possession of this variants confers protection to being infected with MTBss (OR 0.55)

Table 4: Association analysis of controls vs all MTB cases

CHROM	POS	ID	REF	ALT	A1	TEST	OBS_CT	OR	LOG(OR)_SE	L95	U95	Z_STAT	P
1	22206613	rs2807348	A	G	G	ADD	294	1,16	0,31	0,64	2,12	0,48	0,6278
1	57537459	rs1524713	C	T	T	ADD	294	1,02	0,28	0,59	1,77	0,07	0,9452
2	218389899	rs2695342	G	A	A	ADD	294	1,57	0,90	0,27	9,11	0,51	0,6124
2	218395009	rs17235409	G	A	A	ADD	294	0,93	0,48	0,36	2,38	-0,16	0,8753
2	218395009	rs17235409	G	A	A	ADD	293	1,53	0,58	0,49	4,76	0,73	0,4668
3	7987199	rs17048476	G	A	A	ADD	292	1,44	0,28	0,84	2,49	1,32	0,1862
3	103407750	rs12636260	G	T	T	ADD	287	0,66	0,32	0,35	1,24	-1,29	0,1983
4	61984804	rs4860106	A	G	G	ADD	290	1,28	0,27	0,75	2,18	0,91	0,3648
4	185697150	rs955263	G	A	A	ADD	290	0,73	0,27	0,43	1,25	-1,13	0,2565
5	113228247	rs557438	G	A	A	ADD	284	0,75	0,30	0,41	1,35	-0,97	0,3314
5	148985671	rs930205	T	C	C	ADD	292	0,94	0,26	0,56	1,56	-0,25	0,8010
5	159263943	rs4921437	C	T	T	ADD	292	1,01	0,28	0,59	1,74	0,04	0,9663
6	6202501	rs1482868[x]	C	T	T	ADD	281	0,55	0,33	0,28	1,05	-1,82	0,0681
7	11609312	rs2681052	C	A	A	ADD	292	1,35	0,28	0,79	2,32	1,09	0,2746
7	72039275	rs844669	A	C	C	ADD	294	0,96	0,32	0,52	1,79	-0,13	0,9005
8	41260502	rs4236914	C	T	T	ADD	276	1,05	0,28	0,60	1,81	0,16	0,8716
9	113674597	rs3860173	C	T	T	ADD	291	1,02	0,27	0,60	1,72	0,06	0,9535
10	52771466	rs18004511	C	T	T	ADD	294	1,02	0,29	0,57	1,80	0,06	0,9554
10	52771482	rs5030737	G	A	A	ADD	294	0,61	0,42	0,27	1,39	-1,17	0,2425
10	70761399	rs2587469	A	G	G	ADD	281	0,94	0,29	0,53	1,64	-0,23	0,8192
12	13855572	rs7297313	A	C	C	ADD	275	1,05	0,26	0,63	1,74	0,18	0,8572
12	46843681	rs4768760	A	C	C	ADD	292	1,22	0,28	0,70	2,12	0,70	0,4854
12	47844974	rs731236	A	G	G	ADD	288	0,83	0,31	0,45	1,52	-0,61	0,5405
12	47846052	rs1544410	C	T	T	ADD	293	1,23	0,31	0,67	2,26	0,65	0,5145
14	98046406	rs857063	A	C	C	ADD	294	0,72	0,25	0,44	1,18	-1,32	0,1880

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15	61724906	rs8028149	T	C	C	ADD	283	1,31	0,29	0,75	2,30	0,94	0,3464
16	7445185	rs2346943	T	G	G	ADD	293	1,04	0,33	0,55	1,96	0,11	0,9141
17	68822194	rs9893385	A	G	G	ADD	293	1,03	0,26	0,62	1,73	0,12	0,9053

The association analysis between the controls and *M. africanum* cases (MAF) showed that the rs2695342 variant among those infected with MAF appeared to have the propensity to confer susceptibility to MAF infections ((*P* = 0.093, OR = 8.35, 95% CI = 0.70 – 99.24) in this population.). This is different from the previous two variants with marginal significance. rs2695342 is a synonymous variant (http://www.ensembl.org/Homo_sapiens/Variation/Mappings?db=core;r=2:218389399-218390399;v=rs2695342;vdb=variation;vf=55370148) on the SLC11A1 gene and it has been previously associated with cutaneous leishmaniasis.

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300 Table 5: Association analysis of controls vs all MAF cases

CHR	POS	ID	REF	ALT	A1	TEST	OBS_CT	OR	LOG(OR)_SE	L95	U95	Z_STAT	P
1	22206613	rs2807348	A	G	G	ADD	220	0,71	0,49	0,27	1,84	-0,71	0,4790
1	57537459	rs1524713	C	T	T	ADD	220	1,04	0,38	0,50	2,19	0,11	0,9136
2x	218389899	rs2695342	G	A	A	ADD	220	8,35	1,26	0,70	99,24	1,68	0,0927
2	218395009	rs17235409	G	A	A	ADD	220	0,56	0,58	0,18	1,74	-1,00	0,3168
2	218395009	rs17235409	G	A	A	ADD	220	0,38	1,24	0,03	4,32	-0,78	0,4368
3	7987199	rs17048476	G	A	A	ADD	219	1,48	0,39	0,69	3,18	1,01	0,3127
3	103407750	rs12636260	G	T	T	ADD	215	1,90	0,47	0,76	4,77	1,37	0,1695
4	61984804	rs4860106	A	G	G	ADD	217	1,09	0,38	0,52	2,29	0,24	0,8127
4	185697150	rs955263	G	A	A	ADD	216	0,73	0,40	0,33	1,59	-0,80	0,4242
5	113228247	rs557438	G	A	A	ADD	214	0,60	0,44	0,25	1,43	-1,15	0,2493
5	148985671	rs930205	T	C	C	ADD	218	1,17	0,38	0,56	2,46	0,43	0,6691
5	159263943	rs4921437	C	T	T	ADD	218	1,95	0,42	0,85	4,48	1,57	0,1153
6	6202501	rs1482868	C	T	T	ADD	214	0,77	0,47	0,31	1,93	-0,55	0,5819
7	11609312	rs2681052	C	A	A	ADD	218	0,93	0,36	0,46	1,90	-0,20	0,8451
7	72039275	rs844669	A	C	C	ADD	220	1,06	0,45	0,43	2,58	0,12	0,9025
8	41260502	rs4236914	C	T	T	ADD	210	0,86	0,43	0,37	1,98	-0,35	0,7235
9	113674597	rs3860173	C	T	T	ADD	218	1,48	0,39	0,68	3,20	0,99	0,3221
10	52771466	rs18004511	C	T	T	ADD	220	1,02	0,41	0,46	2,27	0,06	0,9527
10	52771482	rs5030737	G	A	A	ADD	220	0,67	0,55	0,23	1,98	-0,72	0,4688
10	70761399	rs2587469	A	G	G	ADD	210	1,21	0,42	0,53	2,75	0,46	0,6468
12	13855572	rs7297313	A	C	C	ADD	204	1,84	0,39	0,85	3,97	1,56	0,1195
12	46843681	rs4768760	A	C	C	ADD	218	0,95	0,39	0,45	2,04	-0,12	0,9052
12	47844974	rs731236	A	G	G	ADD	215	0,85	0,43	0,37	2,00	-0,36	0,7180
12	47846052	rs1544410	C	T	T	ADD	220	1,20	0,45	0,50	2,90	0,41	0,6807
14	98046406	rs857063	A	C	C	ADD	220	0,64	0,35	0,32	1,28	-1,27	0,2039
15	61724906	rs8028149	T	C	C	ADD	207	0,76	0,49	0,29	1,99	-0,55	0,5808
16	7445185	rs2346943	T	G	G	ADD	220	0,99	0,49	0,38	2,60	-0,01	0,9884
17	68822194	rs9893385	A	G	G	ADD	219	1,02	0,41	0,46	2,27	0,06	0,9558

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4.0.Discussion

Host factors are increasingly being recognized as critical for TB control considering the diversity of the outcome of interaction between the MTBC and distinct human host populations. This study sought to explore potential host genetic factor (s) that may confer susceptibility or protection to distinct MTBC lineages, towards understanding crucial mechanisms of host-pathogen interaction. The main findings from this study were: 1) SNP rs2695342 *on SIC11A1* gene has the propensity to confer susceptibility to MAF; 2) In addition, we also found that 7.1% of 393 adult TB patients studied had DM, three- old higher than the general population average of 2.0% and finally young patients less than 35 years and patients older than 65 years are associated with active TB in Ghana

In our study, rs2695342 (G/A) *SIC11A1* gene had the propensity to confer susceptibility to TB caused by Maf. This variant is in introns of *SIC11A1* gene (solute carrier family 11 member A1, previously known as natural resistance-associated macrophage protein 1 (Nramp1). This gene is a member of a family of metal ion-transport proteins whose cellular expression is restricted to phagocytic cells. *SIC11A1* is a bivalent antiporter located on chromosome 2q35 that delivers metal cations from the cytosol into acidic endosomal and lysosomal compartments where Fenon and Haber-Weiss reaction generates toxic antimicrobial radicals for direct antimicrobial activity against infectious microorganisms such as mycobacteria [19-21]. Basically, *SLC11A1* may influence the survival of the TB pathogen after phagocytosis. We therefore suspect that the intronic position of this polymorphism might affect post-transcriptional modification of the affected gene hence potentially affecting the resulting *SLC11A1* protein. Previous *SLC11A1* studies in humans with TB in West Africans have primarily focused on four or five polymorphisms distributed across the gene: a GT_n repeat in the 5' promoter region, a four base-pair (TGTG) insertion/deletion (rs17235416) in the 3' untranslated region (UTR), and two single nucleotide polymorphisms (SNPs) in intron 4 (rs3731865) and exon 15 (rs17235409, D543N). These mutations were found to be significantly associated with pulmonary TB. This association has been replicated in studies from Guinea-Conakry [21] and Gambia

[21]. Our analysis shows that rs2695342 might actually be a promotor/repression gene mutation which even though its synonymous could lead to significant phenotypic consequences. Mutations in this gene might eventually make the phagocytic cells less toxic thus making the patients more prone to infections by Maf.

Associations between particular MTBC lineages and human ethnicities have been observed before. Indeed, Lineage 1, 2 and 4 are reported to were strongly associated with Filipino, Chinese, and ‘‘white’’ ethnicities, respectively [22-23]. Likewise in China, Hui ethnicity was found to be associated with the Beijing family of MTBC [24]. Indeed, human genetic diversity has been linked to an increased or reduced susceptibility to TB. Recent studies have reported human genetic polymorphisms that influence the susceptibility to TB caused by Maf but MTBss or vice versa. In particular these studies indicate that human genetic susceptibility to TB is further influenced by the MTBC genotype. For example, a human polymorphism in 5-lipoxygenase (ALOX5) involved in the synthesis of leukotrienes and lipoxins and an important mediators of the inflammatory response has been associated with increased to TB risk caused by Maf [25]. Conversely, a human polymorphism reported recently in the Mannose Binding Lectin (MBL) was associated with protection against TB caused by Maf [26]. Moreover, this latter study also found that Maf bound human recombinant MBL more efficiently, perhaps leading to an improved uptake of Maf by macrophages and selection of deficient MBL variants among human populations exposed to Maf. Although our study did not find any significant association between ethnicity and MTBC lineages, our study suggests that host genetics play an important role in TB pathogenesis hence the need for newer approaches to TB therapy such as host directed immune-therapy, which have the potential to shorten the TB treatment and prevent resistance by promoting autophagy.

M. africanum is an important cause of human TB in West Africa, causing nearly 50% of all TB cases reported in West Africa. Our finding from this study that the MAF causes 21% of human TB in Ghana confirms our previous report from Ghana [27] and this finding is in agreement with agreement with existing findings in sub-Saharan Africa where MAF was previously found to cause 39%

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of human TB cases in Benin, Burkina Faso 18%, Cameroon 56%, The Gambia 39%, Guinea Bissau 47%, Ivory Coast 55%, Nigeria 8%, Senegal 20%, Sierra Leone 24%. Indeed, one potential reason for the stability of Maf in these countries is that the bacteria might have adapted to (some) human populations along the Gulf of Guinea. In addition, findings from our comparative genomics analysis of MAF from Ghana suggested potential adaptation of MAF L5 to a definitive host whereas MAF L6 exhibited traits of a pathogen with a wide host-range [28]. Therefore, the observation of MAF with these ethnic groups which mainly driven by L5 seems to suggest that L5 has indeed adapted to causing TB among the said ethnic groups. The Ga and the Ewe speaking ethnic groups traditionally form part of the Kwa people. The Kwa people of Africa includes the Ga-Adange, Ewe and Kwahu ethnic groups. Although the Ga, Ewe have different dialects of the same Kwa language family, members of theses individual groups are genetically interrelated]. Together they constitute the indigenous inhabitants of coastal West Africa.

Diabetes Mellitus (DM) is a known risk for tuberculosis (TB). Although our current report of 7.1% is lower than our previously reported figure of 9.4% [29], the prevalence observed in this study is still three-times higher than in the general Ghanaian population prevalence of 2% [30]. In Sub-Saharan Africa, study findings regarding prevalence of diabetes mellitus amongst tuberculosis patients have been inconsistent. highly variable and differ by geographical region. The highest prevalence of diabetes mellitus among tuberculosis patients (38%) (was reported in Nigeria [31], followed by Ethiopia 16% [32], Tanzania (11%) [33], Cameroon 9.5% [33]. Because of the frequent co-morbidity of these two diseases, focusing on signs of diabetes among patients with TB, particularly if the risk factors are present, could contribute to improved detection and early treatment of diabetes in this population. Therefore, based on our findings, we recommend routine screening of TB patients for DM in addition to HIV which is the norm.

5.0. CONCLUSION

In conclusion, although our analyses do not include the previously studied SNPs, our findings implicate *SLC11A1* as a potential susceptibility gene of substantial interest for TB caused by MAF which is an important pathogen in West Africa

Supplementary figure 1: Sanger sequencing validation of TaqMan genotyping. Representative chromatogram for (A) the GG genotype for rs5030737 (B) the GG genotype for rs17235409 (C) the GA genotype for rs17235409.

Author contributions:

"Conceptualization, A.A.P, D.Y.M and A.W; methodology, A.A.P, P.M, P.A, K.M.H, S.Y.A, I.D.O, S.O.W, S.M.A, and K.M; formal analysis, A.A.P, P.A, K.M.H, I.D.O, S.M.A, and K.M; investigation, A.A.P, P.M, K.M.H, S.Y.A, S.M.A, and K.M; data curation, A.A.P, K.M.H, S,M,A, and K.M; writing—original draft preparation, A.A.P, D.Y.M and A.W.; writing—review and editing, A.A.P, P.M, P.A, K.M.H, S.Y.A, I.D.O, S.O.W, S.M.A, K.M., D.Y.M and A.W; project administration, A.A.P, D.Y.M and A.W.; funding acquisition, A.A.P. All authors have read and agreed to the published version of the manuscript

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Conflict of interest statement: The authors declare no conflict of interest.

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