Genome-Wide Meta-Analysis Identifies Genetic Associations with Resistance to *Mtb* Infection

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Study Aims

1. Identify individuals *resistant to Mtb infection* among close contacts to infectious TB index patients from Brazil and India
2. Identify genetic factors associated with resistance to *Mtb* infection by conducting a human GWAS study

Background

- Tuberculosis (TB) is the leading infectious disease cause of death worldwide.
  - TB causes 30% of all deaths among people with HIV.
- Development of a TB vaccine is hampered by a lack of knowledge of host mechanisms that mediate susceptibility to *Mtb* infection.
- 5% to 20% of individuals *resist* becoming infected with *Mtb*.
  - Individuals who remain uninfected despite heavy exposure to an infectious TB patient are considered "resisters."
- Understanding of biologic mechanisms that allow *resistance to Mtb infection* would inform the development of new TB vaccines.
- A genetic basis that confers *resistance to Mtb infection* has been postulated but not yet identified.
- Genome-wide association studies (GWAS) provide an unbiased approach to uncovering genetic variants associated with rare and common diseases.

Methods

Study Population: 3,158 household and close contacts of microbiologically-confirmed pulmonary TB patients from RePORT India and Brazil cohorts **(Table 2).**

Identification of "*resisters*" (Table 1):

- Examined index patient's infectiousness, contact's exposure to index case, and tested for *Mtb* infection with TST and/or IGRA.
- Created a resistance hierarchy to characterize participants' likelihood of being resistant to *Mtb* infection.

GWAS analysis: GWAS using a general linear model (GLM) in GENESIS aimed at identifying genetic variants associated with resistance to TB infection.

- **Model**: Resistance ~ SNP + Age + Sex + PC1 – PC10

Table 1. Resisters A – C identified as the highest likelihood of resistance to *Mtb* infection and compared to all other groups

Hierarchy	QFT/TST Results	Contact Exposure Level	Index infectiousness
Resister A	QFT- (>90 days) & TST- (>90 days)	High	High
Resister B	QFT- (>90 days) OR TST- (>90 days)	High	High
Resister C	QFT- (<90 days) OR TST- (<90 days)	High	High
Uninfected D	Any negative	Low	Medium/Low
Uninfected E	QFT- or TST- (any time)	Unknown	Unknown
Discordant	Discordant QFT and TST results	Any/Unknown	Any/Unknown
Infected B	QFT+ OR TST+	Any/Unknown	Any/Unknown
Infected A	QFT+ & TST+	Any/Unknown	Any/Unknown

We studied resistance to *Mtb* infection among 3,158 close contacts of active pulmonary TB patients in Brazil and India.

14% of close contacts were resistant to *Mtb* infection despite high exposure to a very infectious index patient.

GWAS identified a genetic locus significantly associated with resistance to *Mtb* infection on chromosome 13.

Results

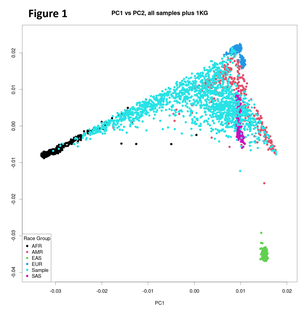


Figure 1. PCA plot comparing PC1 and PC2. Study samples from the Brazil cohort are represented in light blue. The remaining dots are from the 1000 Genome Project.

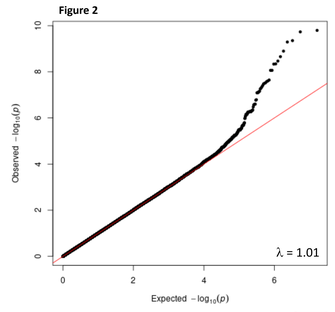


Figure 2. QQ-Plot with observed p-values against expected p-values scaled to -$\log_{10}$(p). The calculated inflation factor (λ) is 1.01.

	Meta- Analysis							Brazil		India	
SNP ID	CHR	Position	Nearest Gene	Genomic Region	Effect:Ref Allele	OR (95%CI)	P-value	OR (95%CI)	EAF	OR (95%CI)	EAF
rs1295104126	13	108506112	MYO16	intergenic	CT:C	0.47 (0.37-0.59)	6.73×10^{-11}	0.55 (0.39-0.75)	0.13	0.40 (0.29-0.55)	0.13

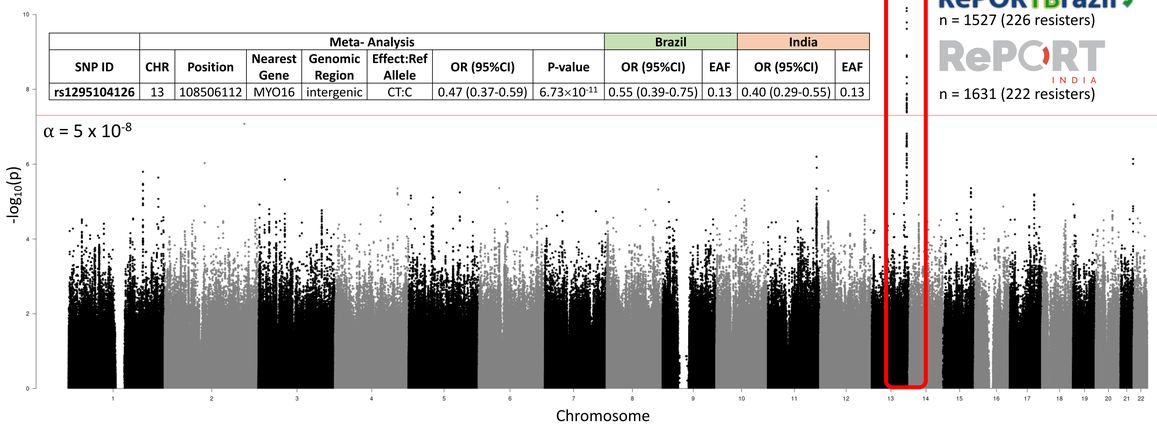


Figure 3. Manhattan plot of all p-values logarithmically scaled on the y-axis; chromosomes 1 through 22 on the x-axis (in alternating colors). Statistical significance threshold ($p<5\times10^{-8}$) is represented by the red line. SNP rs1295104126 on chromosome 13 achieves significance with a p-value of 6.73×10^{-11}.

Results

Principal Component Analysis – Brazilian Cohort

- We found strong evidence of an ancestrally diverse study population using a reference panel from 1000 Genomes Project (**Figure 1**).

GWAS – Meta-Analysis

- We identified one locus strongly associated with resistance to TB infection ($p=6.73\times10^{-11}$) on chromosome 13 (**Figure 3**).
- Those with a C allele at the top SNP are twice as likely to be resisters.

P-Value Inflation

- We calculated an inflation factor (λ) of 1.01, indicating that there is no inflation present in our meta-analysis results.
- We used PC-AiR's matrix calibration approach to account for the relatedness present within each cohort (**Figure 2**).

Table 2.	Brazil and India Participants		
	Resistors A-C N (%)	All other participants N (%)	Total N (%)
n	448 (14)	2710 (86)	3158
Age (median (IQR))	28 (14-42)	29 (16-43)	29 (16-43)
Sex = Female (%)	264 (59)	1573 (58)	1837 (58)
Index Infectiousness (%)	---	---	---
High: Cavities and/or AFB smear 2/3+	448 (100)	1541 (57)	1989 (63)
Medium: AFB smear scanty/1+ and no cavity	0 (0)	796 (29)	796 (25)
Low: AFB smear-negative and no cavity	0 (0)	365 (13)	365 (12)
Exposure	---	---	---
Slept in same bed	88 (20)	322 (12)	410 (13)
Slept in same room	170 (38)	589 (22)	759 (24)
Spent ≥ 5 hours indoors together	190 (42)	576 (21)	766 (24)
None of the above	0 (0)	516 (19)	516 (16)
Missing	0 (0)	554 (20)	554 (18)

Conclusions

- We assembled a study population rich in ancestral diversity across Brazil and India.
- With rigorous characterization of contact exposure, index patient infectiousness, and TST/IGRA testing, we identified household contacts with a high likelihood of resistance to *Mtb* infection.
- A locus with a strong association with resistance to *Mtb* infection was identified and must be replicated in other cohorts.
- The nearest gene to the locus is MYO16, which encodes for the myosin XVI protein postulated to regulate cell cycle progression.
- Metabolomic studies may provide further insight into the genomic role of the identified locus.

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