Polymorphisms in *TLR4* and *TNFA* and Risk of *Mycobacterium tuberculosis* Infection and Development of Active Disease in Contacts of Tuberculosis Cases in Brazil: A Prospective Cohort Study

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Summary of the article's main point: Immune-related genetic polymorphisms were screened in a large cohort of contacts of active tuberculosis patients from Brazil. Single nucleotide polymorphisms of *TLR4* and *TNFA* were independently associated with increased risk for tuberculin skin test conversion and development of active tuberculosis.

ABSTRACT

Background: The role of genetic polymorphisms in latent tuberculosis (TB) infection and progression to active TB is not fully understood.

Methods: We tested the single nucleotide polymorphisms (SNPs) rs5743708 (*TLR2*), rs4986791 (*TLR4*), rs361525 (*TNFA*), rs2430561 (*IFNG*) rs1143627 (*IL1B*) as risk factors for tuberculin skin test (TST) conversion or development of active TB in contacts of active TB cases. Contacts of microbiologically confirmed pulmonary TB cases were initially screened for longitudinal evaluation up to 24 months, with clinical examination and serial TST, between 1998 and 2004 at a referral center in Brazil. Data and biospecimens were collected from 526 individuals who were contacts of 177 active TB index cases. TST conversion was defined as induration ≥5mm after a negative TST result (0 mm) at baseline or month 4 visit. Independent associations were tested using logistic regression models.

Results: Among the 526 contacts, 60 had TST conversion and 44 developed active TB during follow-up. Multivariable regression analysis demonstrated that male sex (OR: 2.3, 95%CI: 1.1-4.6), as well as SNPs in *TLR4* genes (OR: 62.8, 95%CI: 7.5-525.3) and *TNFA* (OR: 4.2, 95%CI: 1.9-9.5) were independently associated with TST conversion. Moreover, a positive TST at baseline (OR: 4.7, 95%CI: 2.3-9.7) and SNPs in *TLR4* (OR: 6.5, 95%CI: 1.1-36.7) and *TNFA* (OR: 12.4, 95%CI:5.1-30.1) were independently associated with incident TB.

Conclusions: SNPs in *TLR4* and *TNFA* predicted both TST conversion and active TB among contacts of TB cases in Brazil.

Key words: single nucleotide polymorphism, tuberculin skin test, Mycobacterium tuberculosis, tumor necrosis factor, toll-like receptor.

INTRODUCTION

Approximately 1.7 billion individuals are infected with *Mycobacterium tuberculosis* (Mtb), representing one-quarter of the global population [1]. Because BCG vaccine does not protect either against infection or TB disease in adults, the only currently effective strategy to prevent active TB in adults is treatment of latent TB infection (LTBI). Treatment is efficacious in decreasing TB risk, but compliance is low, and effectiveness therefore decreased, particularly with longer-course regimens [2]. Although the World Health Organization has recently emphasized the need to treat LTBI, high burden countries are unable to implement full-scale contact investigations and LTBI treatment. Of note, if left untreated, only a small proportion (5-10%) of infected persons will develop active disease [3]. Although some risk factors for developing TB disease have been recognized, such as HIV co-infection, diabetes, young age and recently-acquired Mtb infection [4], many TB patients do not have any known risk factors. To identify those who would most benefit from LTBI treatment, biomarkers for susceptibility have been investigated. Interferon-gamma release assays have been widely tested as a marker of LTBI and to a lesser extent, susceptibility to TB disease [5]. However, these tests do not discriminate between active disease and LTBI and, more importantly, have a low predictive value for progression to TB [6].

In addition, not all contacts of pulmonary TB patients acquire Mtb infection. A meta-analysis reported great variability in the proportion of infected household contacts with a positive tuberculin skin test (TST) [7]. Transmission of Mtb depends on index case-related factors, such as bacillary burden and duration of cough [7] and on contact-related factors, such as degree of exposure and individual genetic susceptibility [8]. Mtb infection

and progression to TB disease may have distinct genetic influences that underlie the biological mechanisms involved in individual susceptibility [9]. Robust activation of the innate immune response is considered an essential prerequisite for protective immunity and vaccine efficacy. However, data published to date provide an incomplete view of the functional importance of innate immunity in TB [10].

Some key genetic components of protective immunity in human TB include toll-like receptor (TLR)2, TLR4, tumor necrosis factor (TNF)A, interferon (IFN)G and interleukin (IL)1B [11-14]. Indeed, immune-related SNPs such as *TLR2* rs5743708 [15], *TLR4* rs4986791 [13],*TNFA* rs361525 [16], IFNG rs2430561 [17] and IL1B rs1143627 [18], and many others, have all been suggested to influence susceptibility to TB, but, the functional immunologic correlates are still unclear. The objective of this study was to evaluate potential genetic biomarkers of susceptibility to Mtb infection and TB disease. We studied close contacts of microbiologically-confirmed pulmonary TB patients to estimate the risk of Mtb infection (TST conversion) and development of active TB according to the presence of five immune-related SNPs, while also accounting for clinical and epidemiological factors.

MATERIALS AND METHODS

Ethics Statement

Written informed consent was obtained from all participants or their legally responsible guardians, and all clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the Clementino Fraga Filho University Hospital (HUCFF), Federal University of Rio de Janeiro Ethics Review Board. The anonymity of study subjects was preserved and all study specimens were de-identified.

Study design

We performed a longitudinal study of contacts of pulmonary TB patients at the time of diagnosis from November 1998 through March 2004. TB was diagnosed by acid-fast bacilli (AFB) smear and/or culture, according to Brazilian Ministry of Health Guidelines [19]. All TB index cases diagnosed at HUCFF >18 years old. Investigation of TB cases included data on cough, AFB sputum grade, and chest radiographs. After identifying TB cases, we searched for their close contacts. TB contacts were defined as living in the same household or reporting contact with the TB index case for $\geq$20 hours weekly for 2 months. All individuals identified who were ≥9 years-old were invited to participate in the study and were evaluated and screened for active TB following Brazilian guidelines [19]. Prevalent TB cases among close contacts were excluded from analyses.

Procedures

Close contacts were evaluated at baseline, and 4 and 12 months after identification of the TB index case. At first visit, a standardized questionnaire was administered to obtain demographic and clinical data, including type and duration of contact with the index case, and history of risk factors for TB (e.g., HIV, diabetes, hematologic malignancies, and use of immunosuppressant drugs). If a contact was a grandparent, parent or sibling of the index case, they were considered to have consanguinity (this definition extended to children with the index case), whereas spouses or other relationships did not. At study baseline, a medical visit and chest radiograph were scheduled. BCG scar was assessed and TST performed by a trained nurse using the Mantoux technique [19], with 2 tuberculin units of the purified protein derivative RT 23 (Statens Serum Institute, Copenhagen, Denmark). TST reading was performed 48-72 hours after administration. Additional TST screening was performed at months 4 and 12 to evaluate for possible TST conversion.

TST interpretation and TB diagnosis

A positive TST was defined as ≥5mm induration, according to the Brazilian Ministry of Health [19]. A positive TST at the first visit was considered to represent LTBI. Contacts with any TST ≥5 mm were not re-tested with TST. During the study period, the Brazilian National TB Guidelines indicated that treatment of TST-positive individuals was not mandatory, and assessment of cost-benefit of therapy with isoniazid for 6 months was performed by health care workers prior to a decision to treat [19]. If treatment was not initiated, individuals were followed-up with periodic examinations to identify development of active TB disease. Twenty-nine participants received isoniazid.

Contacts with signs or symptoms suggestive of active TB underwent medical visits and investigation for TB disease by AFB smear and culture in Lowenstein Jensen (LJ) medium. Active TB was diagnosed when ≥1 specimen yielded a positive microbiologic (AFB smear or culture) result. An incident active TB case was defined as TB diagnosed after baseline study assessment. All patients (n=526) were contacted after 12 additional months (24 months after study enrollment) to assess for incident TB disease. Data on TB incidence from all individuals who could not be contacted at month 24 (n=168) were collected by searching the Brazil's Information System for Notifiable Diseases (SINAN). Of 44 incident TB cases, 8 (18%) had TB diagnosis extracted from SINAN rather than at month 24 interview.

Genotyping

Genomic DNA was extracted from peripheral blood collected from TB contacts at study enrollment. DNA extraction and genotyping were performed using the FlexiGene kit (Qiagen, Germany). Genotypes of 5 gene polymorphisms *TLR2* (rs5743708), *TLR4* (rs4986791), *TNFA* (rs361525), *IFNG* (rs2430561) and *IL1B* (rs1143627) were detected using polymerase chain reaction restriction fragment length polymorphism (RFLP) method [20, 21]. The primer sequences are in Supplementary Table 1. The PCR products were digested by the enzymes *Msp I* for *TLR2*, *Hinf I* for *TLR4*, *BamHI* for *TNFA*, *AvaII* for *IFNG* and *AluI* for *IL1B*.

Data analysis

Categorical data were presented as proportions and continuous data as medians and interquartile ranges (IQR). The frequency distributions of alleles (wild type versus variant) for each polymorphism were compared. The Fisher's and Chi-square tests were used to compare categorical variables between study groups. Continuous variables were compared using the Mann-Whitney *U* test. A multivariable regression model using variables with univariate p-value ≤ 0.2 was performed to assess the odds ratios (OR) and 95% confidence intervals (CIs) of the associations with TST conversion and incident active TB. For analysis of *TLR4* in the multivariable model, there was no event among participants who remained TST negative, thus for OR calculation we added "1" to the group without detected events. In addition, we employed Bayesian Network modeling [22] to infer causal relationships between TST conversion and active TB disease and socio-demographic, clinical, laboratory and genetic parameters, with 100X bootstrapping. Only associations which remained statistically significant in >20 of 100X bootstraps were considered significant. A p-value < 0.05 was considered statistically significant.

RESULTS

Characteristics of the study participants

We approached 1,458 contacts of 1,191 microbiologically-confirmed TB index cases who attended HUCFF between 1998 and 2004. Of those, 932 persons were excluded for the reasons listed in Figure 1. The final study population, from which we collected data and samples, included 526 contacts of 177 TB index cases. The description of the study population is in Table 1. The study population was mostly female, household contacts, and consanguineous with the index case. Indeed, 474 persons (90.5%) were household contacts, with a high rate of consanguinity with the index case (62.5%). There were low frequencies of HIV infection, alcohol use, illicit drug use and use of immunosuppressant drugs. Only 8 persons (1.8%) had a history of TB. At baseline, few reported cough for more than four weeks, and of these, only three had a positive AFB smear and were then treated for TB. During the evaluation of the index cases associated with the contacts, almost all had TB diagnosis confirmed by culture and cough for more than 4 weeks. TB index cases frequently exhibited high bacterial loads in sputum (41.1% had AFB grade ≥ +2;). In addition, 84 index TB patients had cavitary lesions on chest radiograph.

Variant alleles of *IFNG* were the most common polymorphism in the study population, present in 82.9% of the participants (Table 2). Variations in the *IL1B* gene were also common (47%), whereas polymorphisms in *TLR2*, *TLR4*, and *TNFA* genes were less common (Table 2).

Association between polymorphisms and TST conversion

Exposure to Mtb at the time of study enrollment was examined by TST screening of the 526 individuals; 237 (45.1%) had a positive TST (Figure 1). There were 135 individuals who missed the month 4 visit and 154 (53.3% of those TST-negative at baseline) were re-tested. A positive TST was detected in 48 individuals, representing 16.6% of the participants with an initially negative TST. At month 12, a third TST was performed in TB contacts who remained TST-negative at month 4. In addition, 26 participants who missed TST testing at month 4 were tested at month 12. A total of 99 individuals were tested. Twelve individuals had a positive TST at this time point. Thus, during the study period, 60 persons converted to a positive TST, suggesting recent Mtb infection.

TST converters were more commonly male and more frequently household contacts than non-converters (Table 3). Other characteristics were similar between converters and non-converters. Univariate analyses indicated that variant alleles in *TLR2* (p=0.03), *TLR4* (p<0.01), and *TNFA* (p=0.001) were associated with TST conversion, whereas mutant *IL1B* (p=0.006) alleles were more common in those who did not convert (Table 4). Multivariable regression analysis confirmed that male sex and genetic variants in *TLR4 and TNFA* were all independently associated with increased odds of TST conversion (Figure 2A), whereas *IL1B* SNP was not significant (adjusted OR: 0.6, 95% CI: 0.28-1.29, p=0.191).

Furthermore, we applied Bayesian network modeling to infer causal relationships between the presence of polymorphisms and TST conversion, and all recorded statistically relevant demographic, epidemiologic and behavioral information from univariate analyses cited above. This approach confirmed the strong direct associations between male sex, polymorphisms in *TLR4* and *TNFA*, in addition to *IL1B*, with TST conversion (Figure 2B). The *TLR2* polymorphism was not directly connected to TST conversion but was associated with *TLR4* SNP using the Bayesian network approach. In fact, 10 out of 11 individuals with TST conversion and the *TLR4* SNP also had the *TLR2* polymorphism.

Individuals who were TST positive at study baseline (n=203) were similar to those who were TST negative and did not convert nor develop active TB during study follow up (n=224) with regard to most of the characteristics evaluated, including the SNPs (Supplementary Table 2). Cavitary lesions as well as cough in the index TB cases were more frequent in participants who were TST positive at the first visit compared to those who remained TST-negative (p=0.005 and p=0.009, respectively).

Association between polymorphisms and incident TB

Incident TB was higher in those who were TST positive at baseline (Table 5). Only two of the 29 individuals who received isoniazid therapy developed incident TB. In addition, index cases from participants who developed active TB more frequently

had cavitary lung lesions identified on chest x-ray compared to index cases of contacts who did not develop TB (Table 5). Lastly, incident TB was more frequent in participants who had allelic variants in both *TLR4* and *TNFA* genes (Table 6).

Multivariable regression analysis revealed that contacts who were TST-positive at baseline had 7 times greater odds of developing active TB than those who remained TST-negative (Figure 3A). Occurrence of allelic variants in either *TLR4* or *TNFA* genes was independently associated with odds of incident TB. Bayesian networks confirmed the associations between *TNFA* and *TLR4* polymorphisms and incident TB (Figure 3B). Three participants had both SNPs: all three were TST converters, of whom two also developed active TB. A total of five TST converters developed TB disease. Of these, two had two SNPs, *TLR4* and *TNFA*, one had only the TLR4 variants and one had only the *TNFA* polymorphism. In addition, prior TB and being TST-positive at baseline were robustly associated with development of active TB (Figure 3B). Interestingly, this model indicated that *TLR2* SNPs were again indirectly associated with incident TB through *TLR4* polymorphisms, suggesting that the combination of allelic variants in these genes may be associated with increased risk of Mtb infection and development of active TB.

DISCUSSION

In this study we tested associations between SNPs from immune related genes in a large cohort of TB contacts from a highly endemic region in Brazil. The most important finding was that *TLR4* Thr399Ile (rs4986791) and *TNFA*-238 (rs361525) were independently associated with both TST conversion and subsequently developing TB disease. These findings highlight the importance of innate immunity, particularly of these molecules, in the pathogenesis of human Mtb infection and TB disease.

Our results are consistent with our current understanding of TB pathogenesis, in which TLRs are considered critical for host immunity against Mtb in both experimental and clinical settings. Indeed, several groups have shown that polymorphisms in TLR genes are associated with increased susceptibility to TB disease [13]. The *TLR4* ectodomain plays a key role in recognition of pathogen-associated molecular patterns. Interestingly, *TLR4* Thr399Ile has been associated with hypo-responsiveness to ligand interaction due its location near the central ectodomain region [23]. This polymorphism has been associated with more severe forms of pulmonary TB as quantified by sputum bacillary loads and chest radiographs [24]. Our findings on TB contacts provide additional evidence for the critical role of TLR4 in susceptibility to TB. Upon activation through interaction between Mtb ligands and TLR4, myeloid cells produce IL-12 among other proinflammatory mediators [25], which are important to drive T helper 1 (Th1) responses. Exposure to mycobacteria also triggers production of TNF-α and IL-1β [26]. Thus, TLR4 may be critical to drive the protective Th1 responses in the context of Mtb infection and hypo-responsiveness may drive increased susceptibility to TB.

TNF-α has a central role both in the host immune response to Mtb infection and in the immunopathology of TB. TNF-α is produced by many cell types and has cytotoxic synergy with human interferon [27]. Experimental studies have shown that TNF-α is required for the formation and maintenance of granulomas [28]. In humans, anti-TNF drugs are associated with heightened risk of a number of severe respiratory infections including TB [29] In a Chinese population, the *TNFA*-308 allele was associated with elevated odds of pulmonary TB [21]. To our knowledge, no previous study has tested the *TNFA* SNP in the context of TB in Brazil. While examining a Brazilian population, Rocha et al. reported that *TNFA*-238 (rs361525) was associated with spondylarthritis [30]. Our results argue that screening for *TNFA* SNPs could serve as a tool to guide implementation of preventive therapy in TB contacts.

In the present study, the LTBI cases identified at baseline may reflect a cumulative risk for infection before the programmatic contact tracing. Initial LTBI was associated with non-white ethnicity and with the presence of cavity on chest radiograph of the index case. Non-white ethnicity has been found as a risk factor for extrapulmonary TB [31], but in our study, this characteristic may be a proxy variable for socioeconomic conditions in Brazil, reflecting crowding and higher community exposure.

Both logistic regression and Bayesian network analyses demonstrated that male sex was associated with TST conversion. This relationship has been reported previously [25, 32]. Other direct associations with TST conversion found here included *TLR4* and *TNFA* SNPs. The Bayesian network analyses refined these relationships while suggesting that *TLR2* and *TLR4* SNPs may sometimes act combined to increase odds of TST

conversion. Both *TLR2* and *TLR4* are expressed on cell surface and share common intracellular signaling adaptors [33]. Our findings are intriguing and deserve additional investigations to validate the results and narrow down potential interdependency between TLR2 and TLR4 in the immune response against Mtb.

We examined the characteristics associated with development of active TB in our study population and found that polymorphisms in *TLR4* and *TNFA* were independent risk factors. Importantly, such SNPs were also associated with TST conversion, reinforcing the idea that TLR4 signaling and TNF-α production are critically involved in TB pathogenesis. As TNF-α is important for maintenance of granulomas [34], it is possible that the SNP reported here could affect this process and favor development of active TB. The *TLR4* polymorphism was also directly associated with development of active TB as well as with the *TLR2* polymorphism, which although not significantly linked to this clinical outcome in logistic regression, was identified by the Bayesian network and indirectly linked through *TLR4*, reinforcing the idea of interdependency between these TLRs. The same analyses revealed that a prior history of TB was also a risk factor, which has already been demonstrated previously [35].

Our study has several strengths such as serial TST testing (currently recommended as the diagnostic test for LTBI in most resource-restrained countries), microbiologically confirmed TB, and SNPs closely related to immune responses against TB. This study had some limitations. Approximately 20% (n=109) of the study population were lost to follow up, but this proportion was lower than the average reported by studies of TB contacts [36]. In addition, most contacts were consanguineous with the index TB case, but there

was no impact on the outcomes evaluated. Furthermore, we assumed that within a household all were infected by a common Mtb strain, which may not have always been true and might influence the host immune response.

In conclusion, our study provides strong evidence for associations between polymorphisms in innate immune genes and the risk of Mtb infection and development of active TB in Brazil. Further translational studies are warranted to delineate the molecular events behind these associations.

NOTES

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Tables

Table 1.

Characteristics of contact	n/N	n (%)

Clinical and demographic characteristics of the study population

Age -median (IQR)	526/526	35	(33-38)
Male	526/526	181	(34.4)
Consanguinity with index case	526/526	329	(62.5)
BCG vaccination	521/526	177	(33.9)
HIV infection	31/526	4	(12.9)
IDU	439/526	7	(1.6)
Smoking	524/526	131	(25.0)
Alcohol use	444/526	1	(0.2)
Prior tuberculosis	440/526	8	(1.8)
Household contact[a]	523/526	474	(90.5)
Frequency of contact (>20 hours)	526/526	489	(93.0)
Comorbid conditions[b]	500/526	127	(25.4)
Immunosuppressant drugs	444/526	3	(0.7)
Cough (> 4 weeks)	518/526	19	(3.6)
Positive AFB screening	429/526	3	(0.7)
Characteristics of TB index case			
Cavities on chest x-ray	517/526	84	(16.2)
Cough (> 4 weeks)	518/526	470	(90.7)
≥2+ AFB	444/526	200	(41.1)
Positive culture	367/526	352	(95.9)

"n" is the number of persons for whom such data were available. "N" is the number total that participants from the study available. IDU: Illicit drug use AFB: acid fast bacilli.

a. Household contact: as living in the same household or reporting contact with the TB index case for >20 hours weekly for 2 months.
b. Comorbidities: renal failure, diabetes, heart failure, and/or hypertension, chronic obstructive pulmonary disease, neoplasia, systemic lupus erythematous and hepatitis

Table 2. Gene

SNP	n	(%)
rs5743708 (*TLR2*)		
GG	365	(83.9)
GA+AA*	70	(16.1)
rs4986791 (*TLR4*)		
CC	410	(96.7)

polymorphisms of the study participants

CT+TT*	14	(3.3)
rs361525 (*TNFA*)		
GG	447	(85.8)
GA+AA*	74	(14.2)
rs2430561 (*IFNG*)		
TT	69	(17.1)
TA+AA*	335	(82.9)
rs1143627 (*IL1B*)		
TT	254	(53.0)
TC+CC*	225	(47.0)

Data on 526 individuals are shown. SNP: single-nucleotide polymorphism, *variant alleles of SNP, TLR: toll like receptor, *TNFA*: Tumor Necrosis Factor alpha, *IFNG*: Interferon gamma, *IL1B*: Interleukin-1.

Table 3. Characteristics of the study participants evaluated for conversion from TST negative to TST positive.

Characteristic	n/N	Conversion n=60	TST negative n=224	OR (95%CI)	p-value
Age -median (IQR)	284/284	37 (15.59)	34 (21-53)		0.85
Male	284/284	28 (46.7)	76 (33.9)	1.7 (1.0-3.0)	0.072
Consanguinity with index case	284/284	36 (60.0)	142 (63.4)	0.9 (0.5-1.6)	0.65
BCG vaccination	281/284	17 (28.8)	74 (33.3)	0.8 (0.4-1.5)	0.54
HIV infection	20/284	1 (5.9)	2 (66.7)	0.03 (0.0-0.7)	0.05
Race/Ethnicity	275/284	28 (48.3)	117 (53.9)	0.8 (0.4-1.4)	0.46
IDU	230/284	0 (0)	3 (1.5)	-	1
Smoking	283/284	15 (25.0)	56 (25.1)	1 (0.5-1.9)	1
Alcohol consumption	231/284	0 (0)	0 (0)	-	-
Prior tuberculosis	229/284	1 (2.9)	0 (0)	-	-
Household contact	282/284	50 (83.3)	195 (87.8)	0.7 (0.3-1.5)	0.39
Frequency of contact (>20 hours)	284/284	56 (93.3)	206 (92.0)	1.2 (0.4-3.8)	1
Comorbid conditions	266/284	9 (16.1)	54 (25.7)	0.5 (0.3-1.2)	0.16
Immunosuppressant drugs	231/284	0 (0)	0 (0)	-	
Cough (> 4 weeks)	283/284	2 (3.3)	5 (2.2)	1.5 (0.3-7.9)	0.64
Positive AFB	231/284	0	1 (0.2)	-	0.92
Conversion	284/284	5 (11.4)	55 (11.4)	1.0 (0.4-1.1)	1.0
Positive TST at baseline	284/284	34 (77.3)	203 (42.1)	4.7 (2.3-9.7)	<0.01
Characteristics of TB index case					
Cavities on chest x-ray	276/284	4 (7.1)	24 (10.9)	0.6 (0.2-1.9)	0.62
Cough (> 4 weeks)	283/284	30 (50.0)	88 (39.5)	1.5 (0.9-2.7)	0.18
≥2 AFB	256/284	16 (30.8)	79 (38.7)	0.7 (0.4-1.4)	0.37
Positive culture	201/284	39 (95.1)	151 (94.4)	1.2 (0.2-5.6)	1

"n" is the number of persons for whom such data were available. "N" is the number total that participants from the study available. Data represent no. (%). TST: tuberculin skin test; AFB: acid fast bacilli, CI: confidence interval; IDU: Illicit drug use; OR: odds ratio; Comorbidities: diabetes, heart failure, and/or hypertension, chronic obstructive pulmonary disease, neoplasia, systemic lupus erythematous and hepatitis.

Table 4. Gene polymorphisms of the study participants evaluated for conversion from TST negative to TST positive.

SNP	Conversion n=60	TST negative n=224	OR	95% CI	p-value
rs5743708 - *TLR2*	15 (29.4)	27 (15.1)	2.3	(1.1-4.9)	0.03
rs4986791 - *TLR4*	11 (21.6)	0 (0)	-	-	<0.01
rs361525 - *TNFA*	18 (30.0)	24 (10.9)	3.5	(1.7-7.0)	0.001
rs2430561 - *IFNG*	36 (78.3)	140 (83.8)	0.6	(0.3-1.6)	0.38
rs1143627 - *IL1B*	14 (25.5)	94 (46.3)	0.4	(0.2-0.8)	0.006

Data represent no. (%). CI: confidence interval; OR: odds ratio; TST: tuberculin skin test; SNP: single-nucleotide polymorphism; TLR: toll like receptor; *TNFA*: Tumor Necrosis Factor alpha; *IFNG*: Interferon gamma; *IL1B*: Interleukin-1beta.

Table 5. Characteristics of contacts of pulmonary TB cases evaluated for development of active TB disease.

Characteristic	n/N	Active TB n=44	No active TB n=482	OR (95%CI)	p-value
Age –median (IQR)	526/526	32 (29-39)	39 (34-40)	-	0.04
Male	526/526	18 (40.9)	163 (33.8)	1.4 (0.7-2.5)	0.4
Consanguinity with index case	526/526	31 (70.5)	299 (62.0)	1.5 (0.8-2.6)	0.3
BCG vaccination	521/526	14 (31.8)	163 (34.1)	0.9 (0.5-1.7)	0.8
HIV infection	31/526	2 (22.2)	2 (9.1)	2.9 (0.3-24.3)	0.6
Race/Ethnicity	505/526	21 (46.9)	258 (55.8)	0.8 (0.4-1.4)	0.4
IDU	439/526	1 (3.4)	6 (1.5)	2.4 (0.3-20.7)	0.4
Smoking	524/526	12 (27.3)	119 (24.8)	1.1 (0.6-2.3)	0.7
Alcohol use	444/526	1 (3.1)	0	-	0.07
Prior tuberculosis	440/526	6 (19.4)	2 (0.5)	48.8 (9.4-254.4)	<0.01
Household contact	523/526	41 (93.2)	433 (92.9)	1.5 (0.4-5.0)	0.8
Frequency of contact (>20 hours)	526/526	42 (95.5)	448 (92.9)	1.6 (0.4-6.9)	0.4
Comorbid conditions	500/526	10 (25.0)	117 (25.4)	1.0 (0.4-2.1)	1.0
Immunosuppressant drugs	444/526	1 (3.1)	2 (0.5)	6.6 (0.6-75.0)	0.2
Cough (> 4 weeks)	518/526	8 (18.2)	7 (1.5)	15.0 (5.2-43.8)	<0.01
Conversion	526/526	5 (11.4)	55 (11.4)	1.0 (0.4-1.1)	1.0
Positive TST at baseline	526/526	34 (77.3)	203 (42.1)	4.7 (2.3-9.7)	<0.01
Characteristics of TB index case					
Cavities on chest x-ray	517/526	13 (29.5)	71 (15.0)	2.4 (1.2-4.8)	0.04
Cough (> 4 weeks)	518/526	43 (97.7)	427 (90.1)	4.7 (0.6-35.2)	0.1
≥2+ AFB	444/526	21 (47.7)	179 (40.4)	1.3 (0.7-2.5)	0.4

Positive culture	367/526	29 (96.7)	323 (95.8)	1.3 (0.2-9.9)	1.0

"n" is the number of persons for whom such data were available. "N" is the number total that participants from the study available. Data represent no. (%). AFB: acid fast bacilli, CI: confidence interval; IDU: Illicit drug use; OR: odds ratio; Comorbidities: diabetes, heart failure, and/or hypertension, chronic obstructive pulmonary disease, neoplasia, systemic lupus erythematous and hepatitis.

Table 6. Gene polymorphisms of contacts of pulmonary TB cases evaluated for development of active TB infection.

SNP	Active TB n = 44	No ative TB n = 482	OR	95% CI	p-value
rs5743708 - *TLR2*	8 (23.5)	62 (15.5)	1.7	(0.7-3.9)	0.2
rs4986791 - *TLR4*	5 (14.7)	9 (2.3)	7.3	(2.3-23.2)	<0.01
rs361525 - *TNFA*	23 (52.3)	51 (10.7)	9.0	(4.7-17.7)	<0.01
rs2430561 - *IFNG*	25 (78.1)	310 (83.3)	0.7	(0.3-1.7)	0.5
rs1143627 - *IL1B*	17 (44.7)	208 (47.2)	0.9	(0.5-1.8)	0.8

Data represent no. (%). CI: confidence interval; OR: odds ratio; SNP: single-nucleotide polymorphism; TLR: toll like receptor; *TNFA*: Tumor Necrosis Factor alpha; *IFNG*: Interferon gamma; *IL1B*: Interleukin-1beta.

Figure Legends

Figure 1. Study flow chart. TST: tuberculin skin test; Index case: first tuberculosis case identified in the household. [a]Missing 2nd TST: 135 cases; [b]Missing 3rd TST: 33 cases and 26 people who missed the 2nd TST showed up.

Figure 2. Factors associated with TST conversion.

(A) Multivariable regression model of variables shown in Table 3 and 4 which displayed univariate p-value ≤ 0.2. **(B)** Bayesian network with bootstrap (100x) was used to illustrate the statistically significant associations between the parameters and the presence of TST conversion in the study population. Lines represent direct associations. Associations that remained statistically significant on ≥20 of 100 bootstraps are plotted. Numbers of times each association persisted during bootstrap are shown. Bold lines highlight the strongest associations. All parameters from Table 3 were included. Only those displaying significant associations are shown.

Figure 3. Variables associated with development of active TB among contacts of pulmonary TB.

(A) Multivariable regression model of variables shown in Table 5 and 6 which displayed univariate p-value ≤ 0.2. **(B)**Bayesian network with bootstrap (100x) was used to illustrate the statistically significant associations between the parameters and the occurrence of incident TB in the study population. Lines represent direct associations. Associations that remained statistically significant on ≥20 of 100 bootstraps are plotted. Numbers of times each association persisted during bootstrap are shown. Bold lines highlight the strongest associations. All parameters from Table 5 were included. Only those displaying significant associations are shown.

Figure 1

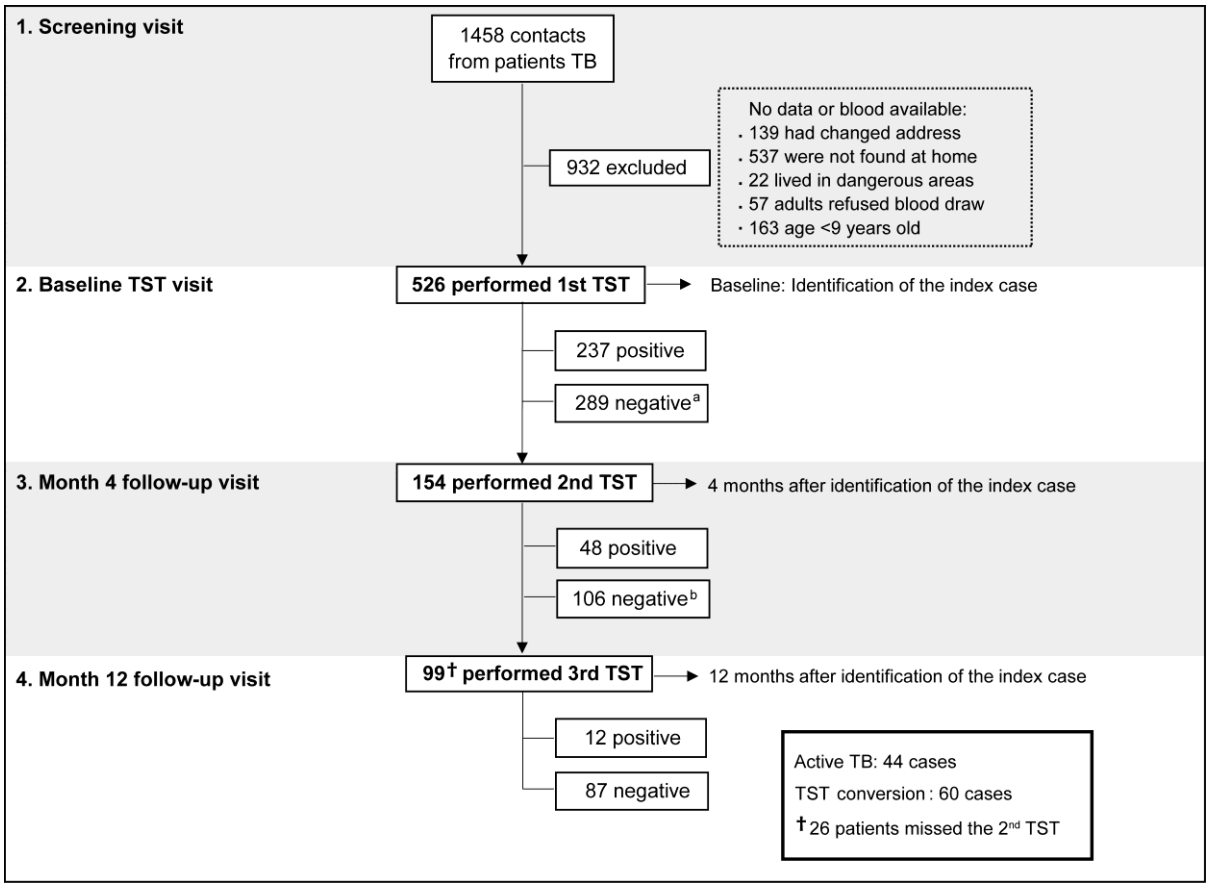

Figure 2

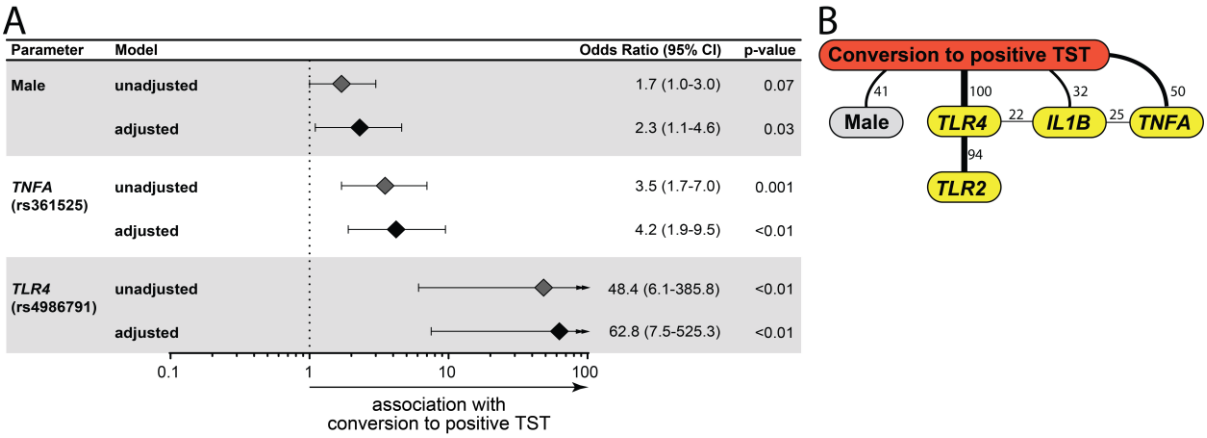

Figure 3