Clinical Infectious Diseases

MAJOR ARTICLE

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

Juan Manuel Cubillos-Angulo,[1,2,3,a] María B. Arriaga,[1,2,3,a] Elisângela C. Silva,[4,5,a] Beatriz L. A. Müller,[4,6] Daniela M. P. Ramalho,[4] Kiyoshi F. Fukutani,[1,3] Pryscila F. C. Miranda,[4] Adriana S. R. Moreira,[4] Antonio Ruffino-Netto,[7] Jose R. Lapa e Silva,[4] Timothy R. Sterling,[8] Afrânio L. Kritski,[4] Martha M. Oliveira,[9] and Bruno B. Andrade[1,3,8,10,11,12]

[1]Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, [2]Faculdade de Medicina, Universidade Federal da Bahia, and [3]Multinational Organization Network Sponsoring Translational and Epidemiological Research (MONSTER) Initiative, Fundação José Silveira, Salvador, Bahia, [4]Programa Acadêmico de Tuberculose, Faculdade de Medicina e Complexo Hospitalar HUCFF-IDT, Universidade Federal do Rio de Janeiro, [5]Recognize the Biology Laboratory, Center of Bioscience and Biotechnology, State University of North Fluminense Darcy Ribeiro, and [6]Laboratório de Genômica Funcional e Bioinformática, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, and [7]Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brazil; [8]Division of Infectious Diseases, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee; [9]Centro de Desenvolvimento Tecnológico em Saúde, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil; [10]Wellcome Centre for Infectious Disease Research in Africa, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, South Africa; [11]Universidade Salvador (UNIFACS), Laureate University, and [12]Escola Bahiana de Medicina e Saúde Pública, Salvador, Bahia, Brazil

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 ≥5 mm 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 (odds ratio [OR]: 2.3, 95% confidence interval [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.

Keywords. single-nucleotide polymorphism; tuberculin skin test; *Mycobacterium tuberculosis*; tumor necrosis factor; Toll-like receptor.

Approximately 1.7 billion individuals are infected with *Mycobacterium tuberculosis* (Mtb), representing one-quarter of the global population [1]. Because bacille Calmette-Guerin (BCG) vaccine does not protect either against infection or tuberculosis (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; however, 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 human immunodeficiency virus (HIV) coinfection, 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

Received 10 August 2018; editorial decision 12 November 2018; accepted 20 November 2018; published online November 24, 2018.

[a]J.M. C.-A., M. B. A., and E. C. S. equally contributed to the work.

Correspondence: B. B. Andrade, Laboratório de Inflamação e Biomarcadores, Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Rua Waldemar Falcão, No. 121, Candeal, Salvador, Bahia 40269-710, Brazil (bruno.andrade@bahia.fiocruz.br).

Clinical Infectious Diseases® 2019;69(6):1027–35

DOI: 10.1093/cid/ciy1001

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 single-nucleotide polymorphisms (SNPs) such as *TLR2* rs5743708 [15], *TLR4* rs4986791 [13],*TNFA* rs361525 [16], IFNG rs2430561 [17], 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 5 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 ≥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 also 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 (eg, 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 ≥5 mm 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 retested 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 healthcare 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 acid-fast bacilli (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 (PCR) 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 vs variant) for each polymorphism were compared. The Fisher and χ^2 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 ≤ .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 sociodemographic, clinical, laboratory, and genetic parameters, with 100× bootstrapping. Only associations which remained statistically significant in >20 of 100× bootstraps were considered significant. A *P*-value < .05 was considered statistically significant.

RESULTS

Characteristics of the Study Participants

We approached 1458 contacts of 1191 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

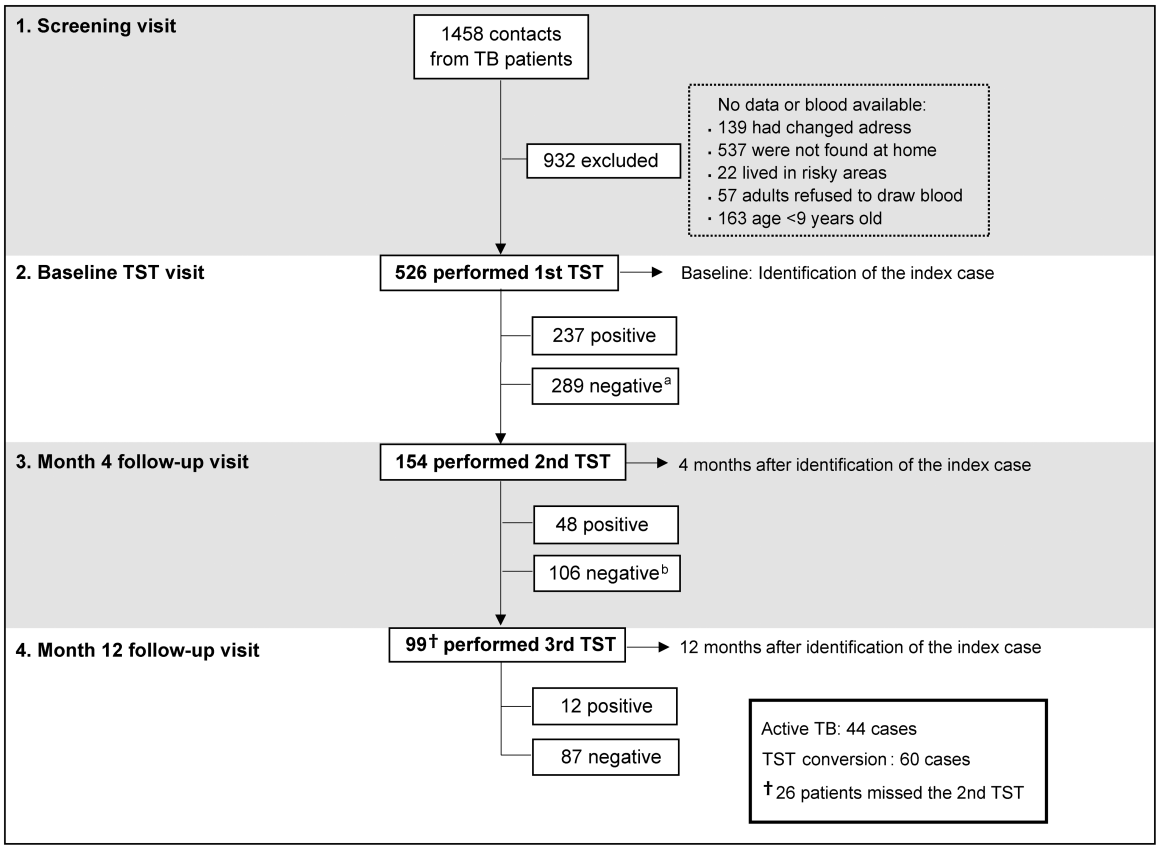

Figure 1. Study flow chart. Index case: first tuberculosis case identified in the household. [a]Missing 2nd TST: 135 cases; [b]Missing 3rd TST: 33 cases and 26 people who missed the 2nd TST showed up. Abbreviation: TST, tuberculin skin test.

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 4 weeks, and of these, only 3 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).

Table 1. Clinical and Demographic Characteristics of the Study Population

Characteristics of Contact	n/N	n	(%)
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)

Abbreviations: AFB, acid fast bacilli; BCG, bacille Calmette-Guerin; HIV, human immunodeficiency virus; IDU, illicit drug use; IQR, interquartile range; n, number of persons for whom such data were available; N, number total that participants from the study available; TB, tuberculosis.

[a]Household contact is defined 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 Polymorphisms of the Study Participants

SNP	n	(%)
rs5743708 (*TLR2*)		
GG	365	(83.9)
GA+AA[a]	70	(16.1)
rs4986791 (*TLR4*)		
CC	410	(96.7)
CT+TT[a]	14	(3.3)
rs361525 (*TNFA*)		
GG	447	(85.8)
GA+AA[a]	74	(14.2)
rs2430561 (*IFNG*)		
TT	69	(17.1)
TA+AA[a]	335	(82.9)
rs1143627 (*IL1B*)		
TT	254	(53.0)
TC+CC[a]	225	(47.0)

Data on 526 individuals are shown.

Abbreviations: *IFNG*, interferon gamma; *IL1B*, interleukin-1 beta; SNP, single-nucleotide polymorphism, TLR, Toll-like receptor, *TNFA*, tumor necrosis factor α.

[a]Variant alleles of SNP.

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 retested. 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 nonconverters (Table 3). Other characteristics were similar between converters and nonconverters. Univariate analyses indicated that variant alleles in *TLR2* ($P = .03$), *TLR4* ($P < .01$), and *TNFA* ($P = .001$) were associated with TST conversion, whereas mutant *IL1B* ($P = .006$) 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, $P = .191$).

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 were 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

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)		.85
Male	284/284	28 (46.7)	76 (33.9)	1.7 (1.0–3.0)	.072
Consanguinity with index case	284/284	36 (60.0)	142 (63.4)	0.9 (0.5–1.6)	.65
BCG vaccination	281/284	17 (28.8)	74 (33.3)	0.8 (0.4–1.5)	.54
HIV infection	20/284	1 (5.9)	2 (66.7)	0.03 (0.0–0.7)	.05
Nonwhite race	275/284	28 (48.3)	117 (53.9)	0.8 (0.4–1.4)	.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)	.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)	.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)	.64
Positive AFB	231/284	0	1 (0.2)	…	.92
Characteristics of TB index case					
Cavities on chest x-ray	276/284	4 (7.1)	24 (10.9)	0.6 (0.2–1.9)	.62
Cough (> 4 weeks)	283/284	30 (50.0)	88 (39.5)	1.5 (0.9–2.7)	.18
≥2 AFB	256/284	16 (30.8)	79 (38.7)	0.7 (0.4–1.4)	.37
Positive culture	201/284	39 (95.1)	151 (94.4)	1.2 (0.2–5.6)	1

Data represent no. (%). Comorbidities: diabetes, heart failure, and/or hypertension, chronic obstructive pulmonary disease, neoplasia, systemic lupus erythematous, and hepatitis.

Abbreviations: AFB, acid fast bacilli; CI, confidence interval; IDU, illicit drug use; n, number of persons for whom such data were available; N, number total that participants from the study available; OR, odds ratio; TB, tuberculosis; TST, tuberculin skin test.

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 ($P = .005$ and $P = .009$, respectively).

Association Between Polymorphisms and Incident TB

Incident TB was higher in those who were TST positive at baseline (Table 5). Only 2 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

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)	.03
rs4986791–*TLR4*	11 (21.6)	0 (0)	…	…	<.01
rs361525–*TNFA*	18 (30.0)	24 (10.9)	3.5	(1.7–7.0)	.001
rs2430561–*IFNG*	36 (78.3)	140 (83.8)	0.6	(0.3–1.6)	.38
rs1143627–*IL1B*	14 (25.5)	94 (46.3)	0.4	(0.2–0.8)	.006

Data represent no. (%).

Abbreviations: CI, confidence interval; *IFNG*, interferon gamma; *IL1B*, interleukin-1beta; OR, odds ratio; SNP: single-nucleotide polymorphism; TLR, Toll-like receptor; *TNFA*, tumor necrosis factor α; TST, tuberculin skin test.

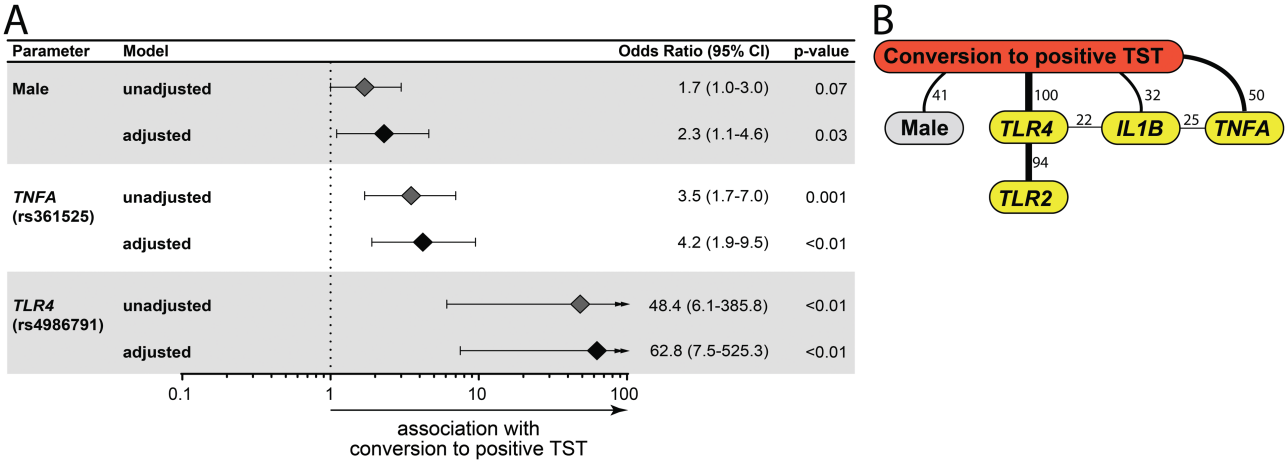

Figure 2. Factors associated with TST conversion. *A*, Multivariable regression model of variables shown in Tables 3 and 4, which displayed univariate *P*-value ≤ .2. *B*, Bayesian network with bootstrap (100×) was used to illustrate the statistically significant associations between the parameters and 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. Abbreviation: TST, tuberculin skin test.

(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 3 were TST converters, of whom 2 also developed active TB. A total of 5 TST converters developed TB disease. Of these, 2 had 2 SNPs, *TLR4* and *TNFA*, 1 had only the TLR4 variants and 1 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

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)	…	.04
Male	526/526	18 (40.9)	163 (33.8)	1.4 (0.7–2.5)	.4
Consanguinity with index case	526/526	31 (70.5)	299 (62.0)	1.5 (0.8–2.6)	.3
BCG vaccination	521/526	14 (31.8)	163 (34.1)	0.9 (0.5–1.7)	.8
HIV infection	31/526	2 (22.2)	2 (9.1)	2.9 (0.3–24.3)	.6
Nonwhite race	505/526	21 (46.9)	258 (55.8)	0.8 (0.4–1.4)	.4
IDU	439/526	1 (3.4)	6 (1.5)	2.4 (0.3–20.7)	.4
Smoking	524/526	12 (27.3)	119 (24.8)	1.1 (0.6–2.3)	.7
Alcohol use	444/526	1 (3.1)	0	…	.07
Prior TB	440/526	6 (19.4)	2 (0.5)	48.8 (9.4–254.4)	<.01
Household contact	523/526	41 (93.2)	433 (92.9)	1.5 (0.4–5.0)	.8
Frequency of contact (>20 hours)	526/526	42 (95.5)	448 (92.9)	1.6 (0.4–6.9)	.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)	.2
Cough (> 4 weeks)	518/526	8 (18.2)	7 (1.5)	15.0 (5.2–43.8)	<.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)	<.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)	.04
Cough (> 4 weeks)	518/526	43 (97.7)	427 (90.1)	4.7 (0.6–35.2)	.1
≥2+ AFB	444/526	21 (47.7)	179 (40.4)	1.3 (0.7–2.5)	.4
Positive culture	367/526	29 (96.7)	323 (95.8)	1.3 (0.2–9.9)	1.0

Data represent no. (%). Comorbidities: diabetes, heart failure, and/or hypertension, chronic obstructive pulmonary disease, neoplasia, systemic lupus erythematous, and hepatitis.

Abbreviations: AFB, acid fast bacilli, CI, confidence interval; HIV, human immunodeficiency virus; IDU, illicit drug use; n, number of persons for whom such data were available; N, number total that participants from the study available; OR, odds ratio; TB, tuberculosis.

Table 6. Gene Polymorphisms of Contacts of Pulmonary TB Cases Evaluated for Development of Active TB Infection

SNP	Active TB n = 44		No Active TB n = 482		OR	95% CI	*P*-Value
rs5743708–*TLR2*	8	(23.5)	62	(15.5)	1.7	(0.7–3.9)	.2
rs4986791–*TLR4*	5	(14.7)	9	(2.3)	7.3	(2.3–23.2)	<.01
rs361525–*TNFA*	23	(52.3)	51	(10.7)	9.0	(4.7–17.7)	<.01
rs2430561–*IFNG*	25	(78.1)	310	(83.3)	0.7	(0.3–1.7)	.5
rs1143627–*IL1B*	17	(44.7)	208	(47.2)	0.9	(0.5–1.8)	.8

Data represent no. (%).

Abbreviations: CI, confidence interval; *IFNG*, interferon gamma; *IL1B*, interleukin-1β; OR, odds ratio; SNP, single-nucleotide polymorphism; TLR, Toll-like receptor; *TNFA*, tumor necrosis factor α.

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

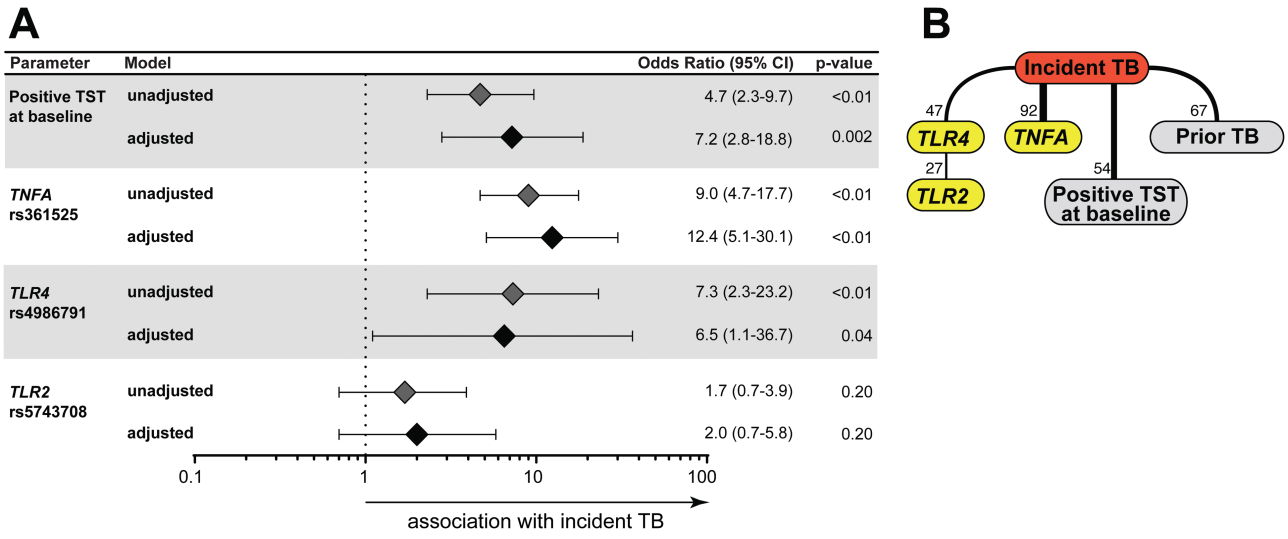

Figure 3. Variables associated with development of active TB among contacts of pulmonary TB. *A*, Multivariable regression model of variables shown in Tables 5 and 6 which displayed univariate *P*-value ≤ .2. *B*, Bayesian network with bootstrap (100×) was used to illustrate the statistically significant 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. Abbreviation: TB, tuberculosis.

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 nonwhite ethnicity and with the presence of cavity on chest radiograph of the index case. Nonwhite 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.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. The authors acknowledge study participants and also the staff of the Clementino Fraga Filho University Hospital of the Federal University of Rio de Janeiro.

Disclaimer. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Funding. This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) / Instituto Nacional de Ciência e Tecnologia (INCT, grant number: 573548/2008-0) and Fundação de Amparo à Pesquisa do Rio de Janeiro (FAPERJ, grant E-26/110.974/2011). A. K. is the recipient of a career award from CNPq (produtividade em pesquisa) and FAPERJ (Cientistas do Nosso Estado). The work from B. B. A. and K. F. F. was supported by an intramural research program from FIOCRUZ and from the National Institutes of Health (U01AI115940). J. M. C.-A. was supported by the Organization of American States - Partnerships Program for Education and Training (OAS-PAEC) and the Coordenação de Aperfeiçoamento de pessoal de Nível Superior Brasil (CAPES, Finance Code 001). M. B. A. receives a fellowship from the Fundação de Amparo à Pesquisa da Bahia.

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Houben RM, Dodd PJ. The global burden of latent tuberculosis infection: a re-estimation using mathematical modelling. PLoS Med **2016**; 13:e1002152.
2. Zenner D, Beer N, Harris RJ, Lipman MC, Stagg HR, van der Werf MJ. Treatment of latent tuberculosis infection: an updated network meta-analysis. Ann Intern Med **2017**; 167:248–55.
3. Sutherland I. Recent studies in the epidemiology of tuberculosis, based on the risk of being infected with tubercle bacilli. Adv Tuberc Res **1976**; 19:1–63.
4. Ai JW, Ruan QL, Liu QH, Zhang WH. Updates on the risk factors for latent tuberculosis reactivation and their managements. Emerg Microbes Infect **2016**; 5:e10.
5. Trajman A, Steffen RE, Menzies D. Interferon-gamma release assays versus tuberculin skin testing for the diagnosis of latent tuberculosis infection: an overview of the evidence. Pulm Med **2013**; 2013:601737.
6. Rangaka MX, Wilkinson KA, Glynn JR, et al. Predictive value of interferon-γ release assays for incident active tuberculosis: a systematic review and meta-analysis. Lancet Infect Dis **2012**; 12:45–55.

7. Morrison J, Pai M, Hopewell PC. Tuberculosis and latent tuberculosis infection in close contacts of people with pulmonary tuberculosis in low-income and middle-income countries: a systematic review and meta-analysis. Lancet Infect Dis **2008**; 8:359–68.
8. Lienhardt C, Fielding K, Sillah J, et al. Risk factors for tuberculosis infection in sub-Saharan Africa: a contact study in The Gambia. Am J Respir Crit Care Med **2003**; 168:448–55.
9. Commandeur S, van Meijgaarden KE, Prins C, et al. An unbiased genome-wide *Mycobacterium tuberculosis* gene expression approach to discover antigens targeted by human T cells expressed during pulmonary infection. J Immunol **2013**; 190:1659–71.
10. Azad AK, Sadee W, Schlesinger LS. Innate immune gene polymorphisms in tuberculosis. Infect Immun **2012**; 80:3343–59.
11. Milano M, Moraes MO, Rodenbusch R, et al. Single nucleotide polymorphisms in IL17A and IL6 are associated with decreased risk for pulmonary tuberculosis in Southern Brazilian population. PLoS One **2016**; 11:e0147814.
12. Zhou Y, Tan CY, Mo ZJ, et al. Polymorphisms in the SP110 and TNF-α gene and susceptibility to pulmonary and spinal tuberculosis among Southern Chinese population. Dis Markers **2017**; 2017:4590235.
13. Schurz H, Daya M, Möller M, Hoal EG, Salie M. TLR1, 2, 4, 6 and 9 variants associated with tuberculosis susceptibility: a systematic review and meta-analysis. PLoS One **2015**; 10:e0139711.
14. Cobat A, Hoal EG, Gallant CJ, et al. Identification of a major locus, TNF1, that controls BCG-triggered tumor necrosis factor production by leukocytes in an area hyperendemic for tuberculosis. Clin Infect Dis **2013**; 57:963–70.
15. Guo XG, Xia Y. The rs5743708 gene polymorphism in the TLR2 gene contributes to the risk of tuberculosis disease. Int J Clin Exp Pathol **2015**; 8:11921–8.
16. Pacheco AG, Cardoso CC, Moraes MO. IFNG +874T/A, IL10 -1082G/A and TNF -308G/A polymorphisms in association with tuberculosis susceptibility: a meta-analysis study. Hum Genet **2008**; 123:477–84.
17. Wei Z, Wenhao S, Yuanyuan M, et al. A single nucleotide polymorphism in the interferon-γ gene (IFNG +874 T/A) is associated with susceptibility to tuberculosis. Oncotarget **2017**; 8:50415–29.
18. Amaral EP, Riteau N, Moayeri M, et al. Lysosomal cathepsin release is required for NLRP3-inflammasome activation by *Mycobacterium tuberculosis* in infected macrophages. Front Immunol **2018**; 9:1427.
19. (Brasil) MdS. Manual de Recomendações para o Controle da Tuberculose no Brasil. Available at: http://www.crf-rj.org.br/crf/arquivos/manual_recomendacoes_controle_tb.pdf. Accessed 10 October 2018.
20. Saleh MA, Ramadan MM, Arram EO. Toll-like receptor-2 Arg753Gln and Arg677Trp polymorphisms and susceptibility to pulmonary and peritoneal tuberculosis. APMIS **2017**; 125:558–64.
21. Fan HM, Wang Z, Feng FM, et al. Association of TNF-alpha-238G/A and 308 G/A gene polymorphisms with pulmonary tuberculosis among patients with coal worker's pneumoconiosis. Biomed Environ Sci **2010**; 23:137–45.
22. Tien I, Der Kiureghian A. Algorithms for Bayesian network modeling and reliability assessment of infrastructure systems. Reliab Eng Syst Saf **2016**; 156:134–47.
23. Mucha R, Bhide MR, Chakurkar EB, Novak M, Mikula I Sr. Toll-like receptors TLR1, TLR2 and TLR4 gene mutations and natural resistance to *Mycobacterium avium* subsp. paratuberculosis infection in cattle. Vet Immunol Immunopathol **2009**; 128:381–8.
24. Najmi N, Kaur G, Sharma SK, Mehra NK. Human Toll-like receptor 4 polymorphisms TLR4 Asp299Gly and Thr399Ile influence susceptibility and severity of pulmonary tuberculosis in the Asian Indian population. Tissue Antigens **2010**; 76:102–9.
25. Barletta-Naveca RH, Naveca FG, de Almeida VA, et al. Toll-like receptor-1 single-nucleotide polymorphism 1805T/G is associated with predisposition to multibacillary tuberculosis. Front Immunol **2018**; 9:1455.
26. Zhang ZM, Zhang AR, Xu M, Lou J, Qiu WQ. TLR-4/miRNA-32-5p/FSTL1 signaling regulates mycobacterial survival and inflammatory responses in *Mycobacterium tuberculosis*-infected macrophages. Exp Cell Res **2017**; 352:313–21.
27. Gardam MA, Keystone EC, Menzies R, et al. Anti-tumour necrosis factor agents and tuberculosis risk: mechanisms of action and clinical management. Lancet Infect Dis **2003**; 3:148–55.
28. Bean AG, Roach DR, Briscoe H, et al. Structural deficiencies in granuloma formation in TNF gene-targeted mice underlie the heightened susceptibility to aerosol *Mycobacterium tuberculosis* infection, which is not compensated for by lymphotoxin. J Immunol **1999**; 162:3504–11.
29. Keane J, Gershon S, Wise RP, et al. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N Engl J Med **2001**; 345:1098–104.
30. Rocha Loures MA, Macedo LC, Reis DM, et al. Influence of TNF and IL17 gene polymorphisms on the spondyloarthritis immunopathogenesis, regardless of HLA-B27, in a Brazilian population. Mediators Inflamm **2018**; 2018:1395823.
31. Noppert GA, Wilson ML, Clarke P, Ye W, Davidson P, Yang Z. Race and nativity are major determinants of tuberculosis in the U.S.: evidence of health disparities in tuberculosis incidence in Michigan, 2004–2012. BMC Public Health **2017**; 17:538.
32. Diwan VK, Thorson A. Sex, gender, and tuberculosis. Lancet **1999**; 353:1000–1.
33. Cervantes JL. MyD88 in *Mycobacterium tuberculosis* infection. Med Microbiol Immunol **2017**; 206:187–93.
34. Algood HM, Lin PL, Yankura D, Jones A, Chan J, Flynn JL. TNF influences chemokine expression of macrophages in vitro and that of CD11b+ cells in vivo during *Mycobacterium tuberculosis* infection. J Immunol **2004**; 172:6846–57.
35. Chiang CY, Riley LW. Exogenous reinfection in tuberculosis. Lancet Infect Dis **2005**; 5:629–36.
36. Alsdurf H, Hill PC, Matteelli A, Getahun H, Menzies D. The cascade of care in diagnosis and treatment of latent tuberculosis infection: a systematic review and meta-analysis. Lancet Infect Dis **2016**; 16:1269–78.