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Prevalence and Determinants of QuantiFERON-Diagnosed Tuberculosis Infection in 9810 Mongolian Schoolchildren

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Background. There is controversy regarding the potential influence of vitamin D deficiency, exposure to environmental tobacco smoke, BCG vaccination, season, and body habitus on susceptibility to *Mycobacterium tuberculosis* (MTB) infection.

Methods. We conducted a cross-sectional analysis to identify determinants of a positive QuantiFERON-TB Gold (QFT) assay result in children aged 6–13 years attending 18 schools in Ulaanbaatar, Mongolia. Data relating to potential risk factors for MTB infection were collected by questionnaire, physical examination, and determination of serum 25-hydroxyvitamin D (25[OH]D) concentrations. Risk ratios (RRs) were calculated with adjustment for potential confounders, and population attributable fractions (PAFs) were calculated for modifiable risk factors identified.

Results. Nine hundred forty-six of 9810 (9.6%) participants had a positive QFT result. QFT positivity was independently associated with household exposure to pulmonary tuberculosis (adjusted RR [aRR], 4.75 [95% confidence interval {CI}, 4.13–5.46, $P < .001$]; PAF, 13.1% [95% CI, 11.1%–15.0%]), vitamin D deficiency (aRR, 1.23 [95% CI, 1.08–1.40], $P = .002$; PAF, 5.7% [95% CI, 1.9%–9.3%]), exposure to environmental tobacco smoke (1 indoor smoker, aRR, 1.19 [95% CI, 1.04–1.35]; ≥2 indoor smokers, aRR, 1.30 [95% CI, 1.02–1.64]; P for trend = .006; PAF, 7.2% [95% CI, 2.2%–12.0%]), and increasing age (aRR per additional year, 1.14 [95% CI, 1.10–1.19], $P < .001$). No statistically significant independent association was seen for presence of a BCG scar, season of sampling, or body mass index.

Conclusions. Vitamin D deficiency and exposure to environmental tobacco smoke are potentially modifiable risk factors for MTB infection.

Keywords. latent tuberculosis; environmental tobacco smoke; BCG vaccine; vitamin D standardization program; VDSP.

Mycobacterium tuberculosis (MTB) infection in children necessarily arises from recent transmission: this group therefore represents a sentinel population for infectious tuberculosis. Population-based cross-sectional studies to estimate the prevalence and determinants of MTB infection in children living in high-incidence settings can inform tuberculosis (TB) control programs by allowing estimates of ongoing transmission and identifying risk factors for infection that are potentially amenable to intervention.

Exposure to an infectious index case and increasing age are well-recognized risk factors for MTB infection in children and have been demonstrated in numerous settings [1]. The evidence for other potential risk factors is less consistent, however. Specifically, some studies have reported associations with lack of BCG vaccination [2, 3], exposure to environmental tobacco smoke [4], vitamin D deficiency [5], winter and spring season [6], and lower body mass index (BMI) [7], whereas others have found no such associations [8–10]. Existing studies in the literature are variously limited by lack of power, low participation rates, use of the tuberculin skin test to diagnose MTB infection (which may yield false-positive results in BCG-vaccinated individuals), restriction to household contacts, and insufficiently detailed information on potential confounders, all of which may underlie the heterogeneity of results seen when their results are meta-analyzed [11–13]. Additional studies addressing these limitations are therefore needed to clarify whether or not these factors influence risk of MTB infection.

An opportunity to undertake such a study recently arose in the context of screening for a community-based phase 3 clinical trial with very broad eligibility criteria that enrolled primary schoolchildren living in Ulaanbaatar, Mongolia [14], where BCG is administered at birth only. A total of 9814 children underwent screening for MTB infection using the QuantiFERON TB Gold test. Comprehensive data relating to potential susceptibility factors were collected, and multivariable analyses were performed to identify those that were independently associated

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with increased risk of MTB infection. Population attributable fractions (PAFs) were then calculated for modifiable risk factors identified.

MATERIALS AND METHODS

Study Design, Setting, and Ethical Approval

We conducted a cross-sectional analysis of baseline data from children attending 18 public schools located in 6 districts of Ulaanbaatar, Mongolia (Bayanzurkh, Songino-Kharkhan, Bayangol, Khan-Uul, Chingeltei, and Sukhbaatar) who were being screened for participation in a randomized controlled trial of vitamin D supplementation for the prevention of MTB infection [14]. Mongolia is an East Asian country situated between China and Russia with a population of approximately 3.1 million people, of whom 1.2 million (39%) reside in the capital city, Ulaanbaatar. School attendance is mandatory for children aged 6–16 years. Incidence of active TB in Mongolia is estimated at 428 cases per 100 000 population per annum [15], and prevalence of human immunodeficiency virus (HIV) infection is very low at 0.02% [16]. The study was approved by institutional review boards (IRBs) at the Mongolian Ministry of Health, the Mongolian National University, and the Harvard T. H. Chan School of Public Health (IRB reference number 14-0513).

Participants

Eligibility criteria were as for the clinical trial for which children were being screened [14]. Inclusion criteria were age 6–13 years at screening; provision of written informed assent to participate by the child; and provision of written informed consent for the child to participate from his/her parent/guardian. Exclusion criteria were known HIV seropositivity, primary hyperparathyroidism, sarcoidosis, or previous active or latent TB; taking cytotoxic therapy or other immunosuppressant medication, enzyme-inducing anticonvulsant therapy, cardiac glycoside, any preparation containing 1-α-hydroxylated vitamin D, or vitamin D supplementation of >10 μg/day; planning to move away from Ulaanbaatar within 4 years of enrollment; and presence of clinical signs of rickets, assessed by school doctors who checked for leg bowing, knock knees, pectus carinatum, and thickened wrists and ankles.

Data Collection and Measurements

Fieldworkers collected information from each child's parent for the following variables using an electronic questionnaire on the RedCAP database: age, sex, highest education level attained by either parent, type of residence, monthly household income, home ownership, number of people per room, indoor tobacco smoking in the household, active smoking by the child themselves, presence of an index case of pulmonary TB (PTB) living in the household during the child's lifetime, and the average amount of time the child spends outdoors per day. Height was measured to the nearest 0.1 cm using a portable stadiometer (SECA, Hamburg, Germany). Weight was measured to the nearest 0.1 kg using a Digital Floor Scale (SECA). BMI was calculated using the formula BMI = weight (kg) / (height $[m]^2$). Percentage body fat was estimated using a body composition analyzer (SC-331S, Tanita, Tokyo, Japan). School doctors ascertained the BCG status of participating children by clinical examination for a vaccination scar. One milliliter of venous blood was drawn into nil, TB antigen, and mitogen QuantiFERON-TB Gold High Altitude tubes (Qiagen, Hilden, Germany), which were processed as described below. Children with positive QuantiFERON-TB Gold results were referred to the Mongolia National Centre for Communicable Disease for clinical and radiographic screening for active TB. QuantiFERON-positive children in whom active TB was excluded were not preventively treated for latent tuberculosis, in line with World Health Organization recommendations [17].

The QuantiFERON-TB Gold assay was performed according to the manufacturer's instructions at the Global Laboratory, Ulaanbaatar, Mongolia, which participates in the QuantiFERON Quality Assurance Program of the Royal College of Pathologists of Australasia. Serum 25(OH)D concentrations were determined using an enzyme-linked fluorescent assay (VIDAS 25OH Vitamin D total, bioMérieux, Marcy-l'Étoile, France). The coefficient of variation was 7.9%, mean bias was 7.7%, and the limit of quantitation was 8.1 ng/mL. Nonzero 25(OH)D values were standardized using a set of 40 serum samples provided by the Vitamin D External Quality Assessment Scheme as previously described by the Vitamin D Standardization Program [18]. Values below the limit of quantitation were classified as <8.1 ng/mL. Season-adjusted (deseasonalized) values were then calculated for each participant from their individual standardized 25(OH)D concentration and date of blood sample collection, using a sinusoidal model with values derived from standardized values for all participants [19].

Statistical Analysis

Data were analyzed using SAS software version 9.4 (SAS Institute, Cary, North Carolina) and Stata software version 15 (StataCorp, College Station, Texas). Annual risk of TB infection (R) was estimated using the formula $R = 1 - (1 - \text{prevalence})^{1/(\text{mean age})}$ [20]. The following factors were investigated as risk factors for QuantiFERON positivity, and handled as independent variables in the analysis: sex, age, parental education, type of residence, monthly household income, home ownership, number of people per room, month of sampling, number of people smoking cigarettes in the home, active smoking by the child, presence of BCG scar, BMI, percentage body fat, household exposure to an index case of PTB,

time spent outdoors, and vitamin D deficiency, defined as serum 25(OH)D concentration <10 ng/mL; this threshold was prespecified, based on findings of a previous study that reported susceptibility to *M. tuberculosis* infection to be increased below this cutoff [5]. Risk ratios (RRs) for the association between these independent variables and the dependent variable of MTB infection (categorized as QuantiFERON positive vs negative) were estimated using generalized estimating equations with the binary distribution, log link function, and exchangeable working correlation structure [21]. When the log-binomial model failed to converge, log-Poisson models, which provide consistent but not fully efficient estimates of the RR and its confidence interval (CI), were used [22]. We conducted 2 multivariable analyses to identify factors that were independently associated with risk of MTB infection: one adjusted for age and sex only, and the other additionally adjusted for all covariates that were associated with QuantiFERON positivity with $P < .20$ in the age- and sex-adjusted analysis. PAFs and their 95% CI were calculated for modifiable risk factors for MTB infection using Stata software as previously described [23]. Participants with indeterminate QuantiFERON results (n = 4) were excluded from analyses of risk factors for MTB infection.

RESULTS

A total of 11 475 children were invited to participate in the study from July 2015 to January 2017, of whom 1065 (9.3%) declined and 596 (5.2%) were ineligible; reasons for ineligibility are presented in Supplementary Table 1. Sociodemographic characteristics of the remaining 9814 children who participated in the study are presented in Table 1. Males and females were equally represented; mean age was 9.4 years and mean household income was US$840 per month. The BCG strains in use over the period of participants' birth were Japan BCG (2001–2003), Intervax Toronto (2003–2006), and Serum Institute of India (2007–2009). Among participants, 2365 (24.1%) lived in a centrally heated house or apartment, 3774 (38.5%) lived in a house or apartment without central heating, and 3675 (37.4%) lived in a *ger* (traditional Mongolian yurt); 3562 (36.3%) participants lived in a household where at least 1 person smoked tobacco indoors, and 374 (3.8%) participants had a history of household exposure to a case of PTB. Deseasonalized 25(OH)D concentrations were available for 9760 of 9814 (99.4%) participants. They ranged from undetectable to 41.9 ng/mL, with a mean value of 12.1 ng/mL and standard deviation of 4.1 ng/mL; 2432 (24.9%) were vitamin D deficient (25[OH]D <10 ng/mL).

Of the 9814 children who underwent QuantiFERON testing, 8864 (90.3%) had a negative result, 946 (9.6%) had a positive result, and 4 (0.04%) had an indeterminate result and were excluded from analyses. Based on a mean participant age of 9.4 years, the annual risk of TB infection (R) estimated using the formula $R = 1 - (1 - \text{prevalence})^{1/(\text{mean age})}$ was 1.1% [20].

Table 1. Characteristics of Study Participants (N = 9814)

Characteristic	No. (%)
Sex	
Female	4868 (49.6)
Male	4946 (50.4)
Mean age, year (SD)	9.4 (1.6)
Parental education[a]	
University/polytechnic	1881 (19.2)
Secondary school or lower	7933 (80.8)
Type of residence	
Centrally heated	2365 (24.1)
Not centrally heated	3774 (38.5)
Ger (yurt)	3675 (37.4)
Mean monthly household income, US dollars (SD)[b]	840 (580)
Home ownership	
No	2114 (21.5)
Yes	7700 (78.5)
No. of people per room, mean (SD)	4.7 (1.3)
No. of people in household smoking indoors	
0	6252 (63.7)
1	3141 (32.0)
≥2	421 (4.3)
Child actively smoking	
No	9765 (99.5)
Yes	49 (0.5)
Household PTB contact	
No	9440 (96.2)
Yes	374 (3.8)
Deseasonalized serum 25(OH)D[b]	
<10 ng/mL	2432 (24.9)
≥10 ng/mL	7328 (75.1)

Data are presented as No. (%) otherwise indicated.

Abbreviations: PTB, pulmonary tuberculosis; QFT, QuantiFERON-TB Gold; SD, standard deviation; US, United States.

[a]Highest educational level attained by either parent.

[b]Data missing for mean monthly household income (n = 10) and serum 25(OH)D (n = 54).

Nine hundred thirty-eight of the 946 QuantiFERON-positive children (99.2%) were screened for active TB; of these, 129 (13.8%) were diagnosed with active TB.

Table 2 presents results of univariable and multivariable analyses evaluating potential determinants of QuantiFERON positivity in the 9810 participants who had a positive or negative result. After adjustment for age, sex, parental education, type of residence, monthly household income, home ownership, number of people per room, month of sampling, exposure to household environmental tobacco smoke, child active smoking status, household PTB contact, and deseasonalized serum 25(OH)D concentration, the following factors were found to be associated with increased risk of QFT positivity: household exposure to PTB (adjusted risk ratio [aRR], 4.75 [95% CI, 4.13–5.46]), vitamin D deficiency (aRR, 1.23 [95% CI, 1.08–1.40]), number of people smoking indoors (aRR for 1 indoor smoker, 1.19 [95% CI, 1.04–1.35]; aRR for ≥2 indoor smokers, 1.30 [95% CI, 1.02–1.64]; P for trend = .006), and increasing age (aRR per additional year, 1.14 [95% CI, 1.10–1.19]). Population attributable risk fractions for modifiable risk

Table 2. Risk factors for QuantiFERON®-TB Gold-positivity, all participants with non-indeterminate result (n=9,810)

			Univariable analysis		Adjusted for age and sex only		Adjusted for age, sex and other covariates[1]	
Risk factors		Proportion QFT-positive (%)	Risk ratio (95% CI)	P	Adjusted risk ratio (95% CI)	P	Adjusted risk ratio (95% CI)	P
Sex	Female	494/4867 (10.2%)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	
	Male	452/4943 (9.1%)	0.90 (0.80, 1.02)	0.09	0.89 (0.79, 1.01)	0.07	0.92 (0.82, 1.04)	0.18
Age, years		-	1.17 (1.13, 1.22)	<0.001	1.17 (1.13, 1.22)	<0.001	1.14 (1.10, 1.19)	<0.001
Parental education[2]	University / polytechnic	128/1881 (6.8%)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	
	Secondary school or lower	818/7929 (10.3%)	1.52 (1.27, 1.81)	<0.001	1.43 (1.20, 1.71)	<0.001	1.16 (0.95, 1.42)	0.14
Type of residence	Centrally heated	170/2365 (7.2%)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	
	Not centrally heated	377/3772 (10.0%)	1.39 (1.17, 1.65)	<0.001	1.36 (1.14, 1.62)	<0.001	1.10 (0.92, 1.33)	0.29
	Ger (Yurt)	399/3673 (10.9%)	1.51 (1.27, 1.79)	<0.001	1.45 (1.22, 1.72)	<0.001	1.09 (0.88, 1.35)	0.41
Monthly household income[3], 100 US dollars [4]		-	0.97 (0.95, 0.99)	<0.001	0.97 (0.96, 0.99)	0.003	0.99 (0.98, 1.01)	0.49
Home ownership	No	223/2113 (10.6%)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	
	Yes	723/7697 (9.4%)	0.89 (0.77, 1.03)	0.11	0.90 (0.78, 1.03)	0.13	0.95 (0.83, 1.09)	0.48
Number of people/room[3]		-	1.06 (1.03, 1.10)	<0.001	1.05 (1.02, 1.09)	0.003	1.01 (0.97, 1.05)	0.63
Month of sampling	Jun-Nov	247/3259 (7.6%)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	
	Dec-Feb	381/3671 (10.4%)	1.37 (1.18, 1.60)	<0.001	1.31 (1.12, 1.53)	<0.001	1.19 (1.00, 1.41)	0.06
	Mar-May	318/2880 (11.0%)	1.46 (1.24, 1.71)	<0.001	1.45 (1.23, 1.69)	<0.001	1.31 (1.10, 1.56)	0.003
No. of people in household smoking indoors	0	534/6249 (8.5%)	1.00 (ref)	<0.001[5]	1.00 (ref)	<0.001[5]	1.00 (ref)	0.006[5]
	1	347/3140 (11.1%)	1.29 (1.14, 1.47)	<0.001	1.26 (1.11, 1.44)	<0.001	1.19 (1.04, 1.35)	0.009
	2 or more	65/421 (15.4%)	1.81 (1.42, 2.29)	<0.001	1.68 (1.33, 2.13)	<0.001	1.30 (1.02, 1.64)	0.03
Child actively smoking[3]	No	944/9761 (9.7%)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	-
	Yes	2/49 (4.1%)	0.42 (0.11, 1.64)	0.21	0.40 (0.10, 1.54)	0.18	0.40 (0.10, 1.57)	0.19
BCG scar	Absent	195/1958 (10.0%)	1.00 (ref)	-	1.00 (ref)	-		
	Present	751/7852 (9.6%)	0.96 (0.83, 1.12)	0.60	0.98 (0.84, 1.14)	0.78		
Body mass index[3], kg/m^2		-	1.02 (0.99, 1.04)	0.17	1.00 (0.97, 1.02)	0.84	-	
Body fat[3], %		-	0.99 (0.98, 1.00)	0.24	1.00 (0.99, 1.01)	0.56	1.00 (ref)	-
Household PTB contact	No	788/9437 (8.4%)	1.00 (ref)	-	1.00 (ref)	-	4.75 (4.13, 5.46)	<0.001
	Yes	158/373 (42.4%)	5.07 (4.43, 5.81)	<0.001	4.96 (4.33, 5.68)	<0.001		
Time spent outdoors, hours / day[3]	<1	414/4120 (10.1%)	1.00 (ref)	0.17[4]	1.00 (ref)	0.13[4]	1.00 (ref)	0.56[4]
	1-2	290/3013 (9.6%)	0.96 (0.83, 1.10)	0.55	0.95 (0.83, 1.10)	0.49	0.96 (0.83, 1.10)	0.53
	>2	242/2677 (9.0%)	0.90 (0.77, 1.05)	0.17	0.89 (0.76, 1.03)	0.13	0.95 (0.80, 1.12)	0.52
Deseasonalized serum 25(OH) D[3], ng/ml	≥10	656/7325 (9.0%)	1.00 (ref)	-	1.00 (ref)	-		
	<10	285/2431 (11.7%)	1.32 (1.16, 1.50)	<0.001	1.20 (1.05, 1.37)	0.01	1.23 (1.08, 1.40)	0.002

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BCG, Bacille Calmette–Guérin vaccine; CI, confidence interval; PTB, pulmonary tuberculosis; QFT, QuantiFERON®-TB Gold; US, United States.

[1] Adjusted for age, sex and the following covariates with P<0.2 in age/sex adjusted analysis: parental education, type of residence, monthly household income, home ownership, number of people per room, month of sampling, exposure to household environmental tobacco smoke, child active smoking status, household PTB contact and serum 25(OH)D concentration; 9,745 participants with non-missing data for all covariates included in this multivariable analysis.

[2] Highest educational level attained by either parent

[3] Missing values (income, 10 missing; number per room, 1 missing; body mass index, 2 missing; body fat, 42 missing; 25(OH)D, 54 missing)

[4] 1$=1000MNT

[5] P-value for trend

factors for MTB infection were 13.1% for household TB contact (95% CI, 11.1%–15.0%), 5.7% for vitamin D deficiency (95% CI, 1.9%–9.3%), and 7.2% for passive smoking (95% CI, 2.2%–12.0%). No independent associations were seen for sex, socioeconomic indices, presence of a BCG scar, BMI, percentage body fat, or season of sampling. A sensitivity analysis excluding children diagnosed with active TB yielded similar results (Supplementary Table 2).

In a subset of 373 children with a history of household exposure to an index case of PTB, risk of QFT positivity was independently associated with the total number of index cases to whom the child had been exposed (aRR per additional index case, 1.72 [95% CI, 1.33–2.23], $P < .001$) and increasing age (aRR per additional year, 1.08 [95% CI, 1.01–1.16], $P = .04$), but not with any other potential risk factor investigated (Table 3).

DISCUSSION

We present results of the largest and most comprehensive study investigating risk factors for MTB infection in children conducted to date, and the first such investigation to be done in Mongolia. In a representative population of schoolchildren aged 6–13 years living in the capital city, Ulaanbaatar, we found that household contact with a case of PTB, vitamin D deficiency, household exposure to environmental tobacco smoke, and increasing age were independent risk factors for infection. We found no association between risk of MTB infection and gender, socioeconomic factors, presence of BCG scar, season, or BMI.

Our study has several positive findings. The observation that MTB infection risk is associated with household contact and increasing age is consistent with results from community-based studies and household contact studies in the literature [1]. The former finding emphasizes the importance of efforts to protect children from MTB infection in the home, and highlights the potential for a policy of household contact tracing with provision of preventive therapy to reduce the population-level burden of TB in low- and middle-income countries [17, 24, 25]. Demonstration of an independent association between household exposure to environmental tobacco smoke and increased risk of MTB infection suggests that this

Table 3. Risk factors for QuantiFERON®-TB Gold-positivity, sub-set of household pulmonary tuberculosis contacts (n=373)

			Univariable analysis		Adjusted for age and sex only		Adjusted for age, sex and other covariates[1]	
Risk factors		Proportion QFT-positive (%)	Risk ratio (95% CI)	P	Adjusted risk ratio (95% CI)	P	Adjusted risk ratio (95% CI)	P
Sex	Female	79/185 (42.7%)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	-
	Male	79/188 (42.0%)	0.98 (0.78, 1.25)	0.89	1.00 (0.79, 1.26)	0.98	1.01 (0.80, 1.28)	0.92
Age, years				0.02	1.09 (1.01, 1.17)	0.02	1.08 (1.01, 1.16)	0.04
Parental education[2]	University / polytechnic	20/52 (38.5%)	1.00 (ref)	-	1.00 (ref)	-	-	-
	Secondary school or lower	138/321 (43.0%)	1.12 (0.78, 1.61)	0.55	1.09 (0.76, 1.55)	0.66	-	-
Type of residence	Centrally heated	27/74 (36.5%)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	-
	Not centrally heated	51/140 (36.4%)	1.00 (0.69, 1.45)	0.99	1.00 (0.70, 1.45)	0.98	0.94 (0.65, 1.37)	0.76
	Ger (Yurt)	80/159 (50.3%)	1.38 (0.98, 1.93)	0.06	1.40 (1.00, 1.94)	0.05	1.36 (0.91, 2.03)	0.14
Household income[3], 100 US dollars[4]		-	-	0.71	1.00 (0.97, 1.02)	0.85	-	-
Home ownership	No	45/103 (43.7%)	1.00 (ref)	-	1.00 (ref)	-	-	-
	Yes	113/270 (41.9%)	0.96 (0.74, 1.24)	0.75	0.95 (0.74, 1.24)	0.72	-	-
Number of people/room[3]		-	-	0.10	1.05 (0.99, 1.11)	0.09	0.99 (0.92, 1.07)	0.85
Month of sampling	Jun-Nov	40/115 (34.8%)	1.00 (ref)	-	1.00 (ref)	-	1.00 (ref)	-
	Dec-Feb	67/147 (45.6%)	1.31 (0.96, 1.78)	0.08	1.28 (0.94, 1.74)	0.11	1.23 (0.90, 1.67)	0.19
	Mar-May	51/111 (45.9%)	1.32 (0.96, 1.82)	0.09	1.33 (0.96, 1.83)	0.08	1.22 (0.87, 1.70)	0.25
No. of people in household smoking indoors	0	79/207 (38.2%)	1.00 (ref)	0.09[6]	1.00 (ref)	0.20[6]	1.00 (ref)	0.17[6]
	1	60/126 (47.6%)	1.25 (0.97, 1.61)	0.09	1.23 (0.96, 1.59)	0.10	1.16 (0.90, 1.50)	0.24
	2 or more	19/40 (47.5%)	1.24 (0.86, 1.80)	0.25	1.19 (0.82, 1.73)	0.35	1.19 (0.83, 1.73)	0.35
BCG scar	Absent	24/66 (36.4%)	1.00 (ref)	-	1.00 (ref)	-	-	-
	Present	134/307 (43.6%)	1.20 (0.85, 1.69)	0.30	1.19 (0.85, 1.68)	0.31	-	-
Body mass index, kg/m^2		-	1.01 (0.97, 1.05)	0.73	0.99 (0.95, 1.04)	0.78	-	-
Body fat, %[3]		-	0.99 (0.96, 1.01)	0.32	0.99 (0.96, 1.01)	0.34	-	-
Number of PTB index cases		-	1.62 (1.32, 2.00)	<0.001	1.58 (1.27, 1.96)	<0.001	1.72 (1.33, 2.23)	<0.001
Deseasonalized serum 25(OH) D, ng/ml[3]	≥10	113/280 (40.4%)	1.00 (ref)	-	1.00 (ref)	-		-
	<10	44/91 (48.4%)	1.20 (0.93, 1.55)	0.17	1.14 (0.87, 1.48)	0.35		

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BCG, Bacille Calmette–Guérin vaccine; CI, confidence interval; PTB, pulmonary tuberculosis; QFT, QuantiFERON®-TB Gold; US, United State

[1] Adjusted for age, sex and the following covariates with P<0.2 in age/sex adjusted analysis: type of residence, number of people per room, month of sampling, household environmental tobacco smoke, number of PTB index cases and serum 25(OH)D; 370 participants with non-missing data for all covariates were included in this multivariable analysis.

[2] Highest educational level attained by either parent

[3] Missing values (income, 1 missing; number per room, 1 missing; body fat, 2 missing; 25(OH)D, 2 missing)

[4] 1$=1000MNT

[5] Defined as the presence of at least one person other than the participating child smoking indoors

[6] P-value for trend

association is not explained by confounding due to socioeconomic factors, as has previously been suggested [12]. The case for a causal interpretation is further supported by our demonstration of a dose-response relationship between increasing number of people per household smoking indoors and increasing risk of QuantiFERON positivity and by results of mechanistic studies showing that tobacco smoke attenuates innate immune responses to MTB both in vitro and in vivo [26–29]. Vitamin D metabolites have also been shown to support innate immune responses to MTB in vitro [30, 31], and our finding of an independent association between vitamin D deficiency and QuantiFERON positivity supports the case for conducting clinical trials of vitamin D supplementation to prevent acquisition of MTB infection, 2 of which are currently in progress [14, 32].

With regard to the magnitude of protective associations observed, we found that 13.1% of the risk of acquiring MTB infection was attributable to household TB contact. This figure is similar to that reported from a meta-analysis of 10 studies, which yielded an estimate of 14.1% (95% CI, 11.6%–16.3%) for the PAF for household transmission [33]. This relatively low figure reflects the fact that TB disease affects <1% of households at any time, even in high-incidence settings, making exposure opportunities between a person with TB and their social network outside the household more numerous [34]. Studies in South Africa have indicated that significant transmission occurs in public transportation [35] and in schools [36]. Further study to investigate sites of TB transmission in community settings in Mongolia is needed. The relatively high PAF for passive smoking (7.0%) demonstrated in our study is also striking: it highlights the importance of tobacco control for TB prevention [37]. The PAF for vitamin D deficiency (5.7%) echoes results of a recent ecological analysis, indicating that 6.3% of global variation in tuberculosis incidence is attributable to variations in exposure to ultraviolet-B radiation [38], which is a key determinant of vitamin D status.

Our study also has some important null findings. In contrast to others [2, 3], we did not find that presence of a BCG scar was associated with protection against MTB infection. In considering the significance of this observation, it is important to note that absence of a BCG scar does not necessarily signify that BCG vaccine was not given, as a proportion of BCG-vaccinated children do not develop a scar. Coverage of BCG vaccination has been estimated to be as high as 98.6% in Mongolia [39]; thus, children without BCG scars in this study may have been vaccinated. The fact that we found no statistically significant association between active smoking and risk of MTB infection may be explained by a lack of statistical power to detect such an association, reflecting the rarity of this practice: Just 49 of 9810 (0.5%) participants smoked cigarettes. Accordingly, the 95% CI for the RR for active smoking was very wide (.10–1.57).

Our study has a number of strengths. Our use of the QuantiFERON test (as opposed to tuberculin skin test) to detect MTB infection allowed for MTB infection status to be evaluated without confounding by sensitization to BCG or environmental mycobacteria. The sample size was very large, reducing the potential for type 2 error, and we recorded detailed information on a wide range of potential determinants of infection risk, allowing for comprehensive adjustment for confounders. We employed an objective assessment of BCG status (presence vs absence of BCG scar, evaluated by school doctors) rather than a subjective assessment such as eliciting a history of BCG vaccination. The laboratory performing QuantiFERON tests participated in an external quality assurance scheme performed by an ISO 9001-accredited laboratory, and rates of indeterminate results were extremely low (0.04%).

Our study also has some limitations. As with any observational study, associations observed may be due to residual and/or unmeasured confounding. However, the associations that we report are all biologically plausible and independent, withstanding adjustment for a wide range of potential confounders; moreover, a dose-response relationship is seen for some risk factors (eg, age, number of TB contacts, number of people per household smoking cigarettes indoors). All of these factors strengthen the case for causal inference. A second potential limitation is that the study was a cross-sectional analysis of baseline data from clinical trial participants: if this approach had resulted in exclusion of significant numbers of children, it could have compromised generalizability of our findings. However, participation rates in our study were high (85.5%), comparing favorably with those of cross-sectional studies investigating prevalence of MTB infection in other settings [40]. A third limitation is that we did not test for HIV infection; however, the prevalence of HIV infection in Mongolia is very low at 0.02% [16].

In conclusion, this very large, cross-sectional analysis identifies household contact with an index case of PTB, vitamin D deficiency, and passive smoking as potentially modifiable risk factors for QuantiFERON-diagnosed MTB infection among Mongolian schoolchildren.

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

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Authors' contributions. G. D. and A. R. M. designed the study. G. D., U. B., B. J., D. B., O. M., N. Y., S. Bo., E. L., B. O., S. Br., and B. G. participated in implementation of the study, data management, and data collection. B. J., D. B., and O. M. performed laboratory assays. C. T. S. implemented standardization of 25(OH)D levels. Z. W. calculated deseasonalized 25-hydroxyvitamin D levels. P. K., with input from G. D. and A. R. M., performed data analysis, A. R. M., D. G., and P. K. wrote the article; all other authors critically reviewed it and approved the final version.

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