A substantial proportion of subjects exposed to a contagious tuberculosis case display lack of tuberculin skin test (TST) reactivity. We previously mapped a major locus (TST1) controlling the TST reactivity in families from an area in South Africa where tuberculosis is hyperendemic. Here, we conducted a household tuberculosis contact study in a French area where the endemicity of tuberculosis is low. A genome-wide analysis of TST negativity identified a significant linkage signal \( (P < 3 \times 10^{-5}) \) in close vicinity of TST1. Combined analysis of the 2 samples increased evidence of linkage \( (P = 2.4 \times 10^{-6}) \), further implicating genetic factors located on 11p14-15. This region overlaps the TNF1 locus controlling mycobacteria-driven tumor necrosis factor \( \alpha \) production.

### Keywords
- tuberculosis infection
- tuberculin skin test
- genome-wide linkage
- human genetics

*Mycobacterium tuberculosis* is the main cause of human tuberculosis, a disease that remains a major public health concern worldwide. Despite the widespread use of BCG vaccine and specific control programs based on combinations of potent antibiotics, tuberculosis kills more individuals per year than any other infectious agent. Progression to clinical tuberculosis is far from inevitable, as only an estimated 10% of individuals infected by *M. tuberculosis* develop clinical disease [1], whether during primary infection or later in life by reactivation of latent or secondary infection. There is accumulating evidence that human genetics play an important role in explaining this large clinical variability [1-3].

There is also interindividual variability at an earlier stage of the infectious process, as approximately 30%-50% of subjects with heavy exposure to *M. tuberculosis* in household studies do not become infected [4]. This estimate is based on the diagnosis of *M. tuberculosis*-infected and uninfected persons by means of the tuberculin skin test (TST), which measures induction on the skin after intradermal inoculation of tuberculin (ie, *M. tuberculosis* purified protein derivative). Several family studies have provided consistent evidence for an important role of human genetics in the control of *M. tuberculosis* infection or TST reactivity [5, 6]. Indeed, by focusing on TST negativity (TST induration diameter, 0 mm vs >0 mm) in an area hyperendemic for tuberculosis in South Africa, we recently mapped TST1, located on chromosomal region 11p14, as a major locus controlling this phenotype [7].

We report here results of a genome-wide linkage analysis (GWLA) of TST negativity in a household tuberculosis contact study conducted in a suburb of Paris, France. We identified a significant linkage signal \( (P \text{ value, approximately } 2 \times 10^{-5}) \) in close vicinity of TST1. Combined analysis of the French and South African samples increased evidence of linkage at the TST1 locus \( (P \text{ value, approximately } 2 \times 10^{-6}) \), consistent with the hypothesis that TST1 is a major locus controlling TST negativity.

### MATERIAL AND METHODS

A prospective study of household tuberculosis contacts was conducted in the Val-de-Marne suburb of Paris, as detailed in [8]. Briefly, Val-de-Marne is an area of low tuberculosis endemicity with an annual tuberculosis incidence of 22.1 cases per
100,000 inhabitants. From April 2004 to January 2009, household contacts exposed to a patient with culture-confirmed pulmonary tuberculosis were enrolled in the context of a general screening procedure. A household contact was defined as any person sharing the residence of a tuberculosis index case during the 3 months preceding diagnosis of the case. The first screening visit (V1) included clinical examination, performance of a TST (ie, intradermal injection of 0.1 mL [5 tuberculin units] of tuberculin obtained from a human-pathogenic M. tuberculosis strain [Tubertest; Sanofi Pasteur, Lyons, France]), chest radiography, and a completion of standardized questionnaire to assess risk factors of M. tuberculosis infection, as detailed elsewhere [8]. These investigations were repeated 8–12 weeks later (V2) in household contacts for whom no infection was diagnosed at V1 [8].

We dichotomized the TST distribution, using a 0-mm induration as the threshold, to define a binary TST status (TST-bin). Individuals with a discordant TST-bin status between V1 and V2 were excluded. Prior to linkage analysis, we assessed the effect of relevant covariates on TST-bin by determining the infectivity of the index case, based on the presence of cavitation on chest radiography or the presence of bacilli in sputum smears, age, sex, and estimated total exposure of the contact to the index case (quantified as the number of days the contact spent with the index case during the 3 months preceding the diagnosis of tuberculosis). To account for the relatedness of individuals, the impact of these covariates was assessed by estimating equation methods, as implemented in the R package GEEPACK (http://cran.r-project.org/web/packages/geepack/index.html), using a logit link function and an identity working correlation matrix.

Genotyping of children and parents for the GWLA was performed at the Centre National de Génotypage (Paris) with the Illumina linkage V panel. Nonpolymorphic single-nucleotide polymorphisms (SNPs) and SNPs with a call rate of <90% were removed from the analysis. Linkage analysis was performed on the quantitative Pearson residuals, obtained by adjustment of TST-bin on sex, age, level of exposure, and index case infectivity. Model-free GWLA was performed by means of the new maximum-likelihood binomial (MLB) method for quantitative traits (nMLB-QTL v.3.0) [9, 10]. The MLB approach considers the sibship as a whole and does not make any assumption about the distribution of the phenotype. The test of linkage is a maximum-likelihood ratio test asymptotically distributed as a 50:50 mixture of χ² distributions with 0 and 1 degree of freedom and can be expressed as a classic LOD score. We used LOD scores of 3.6 and 2.2 as genome-wide significant and suggestive thresholds, respectively, as described in [7].

RESULTS AND DISCUSSION

First, the analysis of covariates in the French cohort was performed on 540 household tuberculosis contacts belonging to 155 pedigrees. Among them, 84 (15.6%) had a TST induration size of 0 mm at both visits and were classified as having a negative TST-bin. The mean age (±SD) at the time of TST was 24.1 ± 18.0 years, and the male to female ratio was 1.05. BCG vaccine coverage was very high, with 13 individuals (2.6%) not vaccinated with BCG vaccine. The mean duration of exposure to the index case (±SD) was 19.25 ± 21.6 days, and 449 household contacts (83.1%) were exposed to an index case presenting cavitation on chest radiography or bacilli in sputum smears. Univariate and multivariate (ie, testing all covariates simultaneously) analysis showed that age had the strongest impact on TST positivity (Table 1). Index case infectivity and total exposure time to the index case were also significantly associated with a positive TST result (Table 1).

Second, from the French sample, 97 nuclear families, including 237 offspring, were informative for linkage analysis (ie, composed of at least 2 genotyped sibs with a TST value). Characteristics of the 237 offspring are shown in Supplementary Table 1. Among them, only 4 had not received BCG vaccine, eliminating BCG vaccination as a potential confounding factor in our study. Results of the GWLA of TST-bin adjusted on the covariates are presented in Figure 1A. Information content was high across all autosomes, with mean genome-wide information of approximately 90%. We identified 1 major locus, at position 14.3 Mb on chromosomal region 11p15, that reached the threshold commonly used to claim genome-wide significance (LOD score, 3.65; P = 2.08 × 10⁻⁵; Figure 1A). In addition, we identified 18 weaker linkage peaks with a P value of <0.01 (Supplementary Table 2).

This major locus mapped at approximately 12 Mb from the TST1 locus previously identified as controlling TST negativity in South Africa [7]. Considering the precision of location estimates in linkage studies of complex traits [11], this result is consistent with replication of the South African TST1 locus in the French cohort. We therefore refined the linkage signal at chromosomal region 11p14-15 by performing a GWLA on the pooled samples (Figure 1B). Evidence of linkage for TST-bin increased in the analysis of the combined sample, compared with the analysis of each sample separately (Figure 1A; [7]), with a maximum LOD score of 4.54 at position 23.44 Mb (P = 2.40 × 10⁻⁶; Figure 1C). This linkage peak was preceded by a plateau from 11 to 20 Mb with LOD scores varying between 2.5 and 2.8. It is therefore difficult to precisely define the bounds of the confidence interval for the true location of TST1. Of note, we also observed suggestive evidence of linkage in the combined sample on chromosomal region 19q13 (LOD score, 2.85 at position 55.55 Mb), whereas only moderate evidence of linkage was observed in the analysis of each sample separately.

To further explore and refine the region of interest on chromosome 11p14-15, we restricted our analysis to French and South African families showing evidence of linkage (ie, those with a LOD score of >0) in the region extending from 10 Mb to approximately 12 Mb.
to 34 Mb. Our goal was to narrow the size of the region of interest by removing the noise randomly generated by unlinked families (e.g., because of genetic heterogeneity). Results are shown in Figure 1D. While the height of the linkage peak has no more formal interpretation in that situation, its shape shows that the plateau observed between 11 and 20 Mb in Figure 1C is likely part of the region of interest. This region of interest, defined as displaying a LOD score of >2.2 in Figure 1C, extends from 11.6 Mb to 33.1 Mb and contains 194 genes (Supplementary Table 3). Of note, WT1 located at 32.4 Mb is the closest gene to rs2057178, a SNP found to be associated with pulmonary tuberculosis in several genome-wide association studies performed in different populations [12, 13]. Identification of genetic variant(s) underlying the linkage peak is ongoing following the same strategy (based on ultra-fine linkage disequilibrium mapping) as the one successfully used for pulmonary tuberculosis [3].

While the phenotype of interest was similar in the South African and French samples (i.e., TST negativity, defined using a stringent cutoff of a 0-mm induration), the 2 studies were otherwise remarkably different. The South African study took place in an area hyperendemic for tuberculosis [7], where the transmission of *M. tuberculosis* occurs preferentially in the community, and with no requirement for the enrolled individuals to be household contacts of tuberculosis cases. By contrast, France is a country with low tuberculosis endemicity, and the study design was a household tuberculosis contact study, which enabled us to take into account and quantify both the level of exposure and the index case infectivity. Beside the exposure context, the 2 studies also differed in terms of genetic background, as captured by the difference in ethnicities of subjects in each study. All the individuals studied in [7] belonged to the South African Coloured ethnic group, a unique highly admixed population resulting from the encounter of different founders from Africa, Europe, and Asia. By contrast, individuals in the present study belonged to several ethnic groups (white [48%], African [33%], Asian [6%], and mixed and other [13%]). Thus, replication of TST1 in such different settings suggests an important role of this locus in the control of TST negativity in humans.

The risk of developing tuberculosis for immunocompetent persons without TST reactivity despite sustained exposure to *M. tuberculosis* was previously shown to be extremely small [14]. Thus, persons with a TST induration of 0 mm are more likely not infected by *M. tuberculosis* than intrinsically deficient in mounting a delayed type hypersensitivity response. In the French cohort, the individual level of exposure to *M. tuberculosis* was variable. Some individuals were constantly exposed to a highly infectious case and are very likely naturally resistant to *M. tuberculosis* infection. Others were occasionally exposed to a moderately infectious case, and in this instance it is not...
possible to distinguish between lack of exposure and resistance to infection. However, since lack of exposure is difficult to reconcile with a genetic cause, the most parsimonious explanation is that the 11p14-15 locus reflects an innate mechanism of resistance to *M. tuberculosis* infection. Such a mechanism is an attractive working avenue for prevention as it could
be an efficient target to protect persons from infection with highly drug resistant *M. tuberculosis* strains or those with a weakened acquired immunity arm, such as human immunodeficiency virus–infected subjects.

Interestingly, the major locus at chromosomal region 11p14-15 overlaps the TNF1 locus identified for mycobacteria-driven tumor necrosis factor (TNF-α) production by leukocytes in the South African family sample (Figure 1C and 1D) [15]. TNF is a key effector molecule of tuberculosis resistance that is critical for the sequestration of *M. tuberculosis* in infectious granuloma during latent *M. tuberculosis* infection [1]. Hence, the overlap of TST1 with TNF1 provides additional support for the interpretation of the TST1 locus as conferring resistance to *M. tuberculosis* infection. In addition, the observation that the suggestive locus on chromosomal region 19q13 mapped in the vicinity of a locus also found to be linked to TNF production in the South African family sample (P < .01 from 58.1 to 59.1 Mb [15]) reinforces the hypothesis of a genetic connection between TST negativity and TNF production.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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