# Associations between *IL12B* Polymorphisms and Tuberculosis in the Hong Kong Chinese Population

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**Background.**    Interleukin (IL)–12 plays a vital role in regulating cell-mediated immunity against tuberculosis (TB).

**Methods.**    To test whether *IL12B* genetic polymorphisms might contribute to human TB susceptibility, we examined the genotype frequencies of 5 *IL12B* polymorphisms (at promoter, intron 2, intron 4, exon 5, and 3′ untranslated region [UTR]) in 516 patients with TB and 514 healthy control subjects from the Hong Kong Chinese population.

**Results.**    Individuals homozygous for the *IL12B* intron 2–repeat marker $(ATT)_8$ had a 2.1-fold increased risk of developing TB ($P < .001$) (odds ratio, 2.14 [95% confidence interval, 1.45–3.19]). Estimation of the frequencies of multiple-locus haplotypes composed of *IL12B* promoter, intron 2, intron 4, and 3′ UTR alleles revealed potential risk haplotypes (designated "A" and "K") and protective haplotypes (designated "B") for TB. Furthermore, combining the genotype data of the 4 informative *IL12B* loci revealed a strong association between a specific genotype pattern, termed "diplotype I" (heterozygous A and K haplotypes), and TB. In contrast, diplotype II (homozygous BB haplotypes) appeared protective against TB.

**Conclusions.**    These findings support the association between *IL12B* intron 2 polymorphism and TB and between specific *IL12B* haplotypes and TB.

One-third of the world's population is infected with *Mycobacterium tuberculosis.* Tuberculosis (TB) has re-emerged as a leading global health threat, accounting for 8.7 million new cases and 1.7 million deaths every year [1]. It is inferred that a role is played by host genetic factors in TB susceptibility because 10% of infected individuals develop clinical disease [2], there is higher disease concordance in monozygotic twins than in dizygotic twins [3, 4], and infection rates differ among racial groups [5].

The host genetic factors related to TB have been investigated via 3 different approaches. Linkage studies

have identified 3 regions, at chromosome 2q35 [6], 15q11-13 [7], and Xq27 [8], that have probable linkages to TB. Case-control association studies have found significant associations between gene polymorphisms in *NRAMP1* [9], HLA [10], vitamin D receptor [11], mannose-binding lectin [12], interleukin (IL)–1 [13], IL-1$\beta$, IL-1 receptor antagonist [14], surfactant proteins [15], $P2X_7$ [16], interferon (IFN)–$\gamma$ [17], IL-10 [18], IL-12 receptor [19], and IL-8 [20] and TB. Mendelian susceptibility to mycobacterial infections, which is caused by a mutation in a single major gene, indicates that a critical role is played by the IL-12–IFN-$\gamma$ cytokine axis in host immunity against the disease [21]. In knockout murine models, *IL-12*–deficient mice are more susceptible to infection with *M. bovis* bacille Calmette-Guérin [22] and *M. tuberculosis* [23] than are wild-type mice. In addition, *IL12B*-deficient mice develop more severe mycobacterial infection than do *IL12A*-deficient mice [24].

IL-12 is a heterodimeric cytokine that is composed of 2 subunits, p35 and p40, and that plays an essential role in the induction and maintenance of Th1 responses [25]. Polymorphisms in *IL12B* have been reported at promoter [26], intron 2 [27], intron 4 [28], exon 5

**Table 1. Primers, probes, and genotyping methods for *IL12B* polymorphisms.**

| Polymorphic site[a] | Sequence (5′→3′) | | Genotyping method[b] |
|---|---|---|---|
| | Forward | Reverse | |
| Promoter[c] [26] | GGTCATTGGCAGGTTGTTCT | CGCCCATAGGGTAAGCAATA | The polymorphism was a 7-bp deletion and 3-bp insertion, leading to an allelic difference of 4 bp. The shorter allele (1) and the longer allele (2) were genotyped by use of the ABI PRISM 700 Sequence Detection System (Applied Biosystems), with TaqMan probes (Applied Biosystems). |
| Intron 2 [27] | **FAM**-GACTGAGGAGTCAGCCAACTG | TTGGCCTCAGTACGCTTCTT | Allele 1 $(ATT)_8$ and allele 2 $(ATT)_9$ were amplified with fluorescence-labeled forward primers and were analyzed by use of ABI PRISM 377 DNA Sequencers and GeneScan software (version 3.1) (Applied Biosystems). |
| Intron 4 [28] | **FAM**-GTCATGGAGAGCTCCCCATA | TGGCCAGAACTTTTCAATG | Allele 1 $(TA)_{10}(TG)_{10}$ and allele 2 $(TA)_9(TG)_9$ were genotyped by the same method as that described for intron 2. |
| Exon 5 [29] | GAGGAGAGTCTGCCCATTGA | GATCTGGGGCAAGTTGCTTA | Allele serine (AGC) and allele asparagine (AAC) were distinguished by overnight digestion at 37°C with *Fnu*4HI (New England Biolab), because allele serine gained a *Fnu*4HI site. |
| 3′ UTR [28, 30] | TGATCCAGGATGAAAATTTGG | GGCAACTTGAGAGCTGGAAA | Allele 1 (A) and allele 2 (C) were genotyped by overnight digestion at 65°C with *Taq*I (Invitrogen), because allele 2 created a *Taq*I restriction site. |

**NOTE.** UTR, untranslated region.

[a] The original reference paper(s) describing each *IL12B* polymorphism is provided.
[b] The 2 alleles at each polymorphic locus are designated "1" and "2."
[c] TaqMan probes used for the promoter allele were **FAM**-CCACATTAGAGCCTCTC and **VIC**-CCACAGCCCTCTCT.

[29], and 3′ untranslated region (UTR) [28, 30]. The allelic variants at 3′ UTR and promoter have different effects on *IL12B* mRNA [31] and IL-12p70 protein expression levels [26, 32].

Because IL-12 is a key Th1 cytokine that protects individuals from mycobacterial infection, we hypothesized that genetic polymorphisms in *IL12B* might contribute to TB susceptibility in the general population. To test this hypothesis, we first investigated whether any associations between 5 reported *IL12B* polymorphisms and TB could be found in 516 patients with TB and 514 healthy control subjects. Second, we performed functional analysis to correlate the associated polymorphisms with IL-12p70 protein expression.

## PATIENTS, MATERIALS, AND METHODS

***Study populations.*** The present study was approved by the ethics committees of the Faculty of Medicine, the University of Hong Kong, and of the Department of Health, Hong Kong Special Administrative Region (HKSAR), China. The study participants included 516 Hong Kong Chinese patients with TB (mean age, 48 years [SD, 18.0 years]; number of men, 328; number of women, 188), who provided informed consent and were recruited over a 2-year period (2000–2002) from territory-wide chest clinics that operate under the Department of Health of the HKSAR. All patients had established TB. Sixty percent were either culture or smear positive for *M. tuberculosis*. The remaining patients satisfied the TB diagnostic criteria of the International Union against Tuberculosis and Lung Diseases [33] and were diagnosed on the basis of clinical-radiological and histological grounds and of positive response to antimycobacterial treatment. None of the patients was infected with HIV. The control subjects included 514 healthy, unrelated, and ethnically matched Hong Kong Chinese blood donors from the

Hong Kong Red Cross, who were recruited during 2001–2003 (mean age, 28 years [SD, 10.4 years]; number of men, 356; number of women, 158).

***DNA extraction.*** Genomic DNA was isolated from whole blood that had been collected from patients with TB and from buffy-coat lymphocytes from control subjects, according to a standard procedure [34] or by use of a DNA extraction kit (Qiagen).

***IL12B genotyping.*** The sequences of all primers and probes and the genotyping methods for 5 *IL12B* polymorphisms (at promoter, intron 2, intron 4, exon 5, and 3′ UTR) are described in table 1. Each polymorphic locus consists of 2 alleles (designated "1" and "2") except for intron 4, where other minor alleles are also present (table 1). Polymerase chain reaction (PCR) was performed in a 50-μL reaction mixture containing 50 ng genomic DNA, 0.2 μmol/L primers, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L dNTPs, and 0.5 U *Taq* DNA polymerase (Amersham). The PCR mixture was incubated initially for 2 min at 95°C and was followed by 35 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 60°C, and extension for 1 min at 72°C; final extension was for 5 min at 72°C.

***Genotype analyses.*** The genotype frequencies of each of the *IL12B* polymorphisms were determined in the 516 patients with TB and the 514 control subjects (table 2). Because the allele frequency of asparagine (AAC) in exon 5 was <1%, this allele was considered an uncommon variant in the Hong Kong Chinese population, and exon 5 was excluded from further analyses. At each polymorphic locus, the genotype frequencies of the patients with TB and the control subjects were compared, and odds ratios (OR) were calculated by assigning the reference value (1.0) to the homozygous genotype that was more frequent in the control subjects than in the patients with TB. The OR and *P* values were calculated by multiple logistic regression

**Table 2.** *IL12B* genotype frequencies in patients with tuberculosis (TB) and in control subjects.

| *IL12B* genotype | Patients with TB (n = 516) | Control subjects (n = 514) | Multiple logistic regression[a] | |
|---|---|---|---|---|
| | | | OR (95% CI) | P |
| Promoter | | | | .038 |
| 1/1 | 147 (28) | 134 (26) | 1.46 (0.99–2.15) | … |
| 1/2 | 251 (49) | 225 (44) | 1.53 (1.08–2.16) | … |
| 2/2 | 118 (23) | 155 (30) | Reference | … |
| Intron 2 | | | | <.001 |
| 1/1 | 203 (39) | 149 (29) | 2.14 (1.45–3.19) | … |
| 1/2 | 224 (43) | 239 (46) | 1.40 (0.96–2.04) | … |
| 2/2 | 89 (17) | 126 (25) | Reference | … |
| Intron 4 | | | | .014 |
| 1/1 | 147 (28) | 139 (27) | 1.64 (1.09–2.50) | … |
| 1/2 | 238 (46) | 220 (43) | 1.05 (0.76–1.49) | … |
| 2/2 | 81 (16) | 121 (24) | Reference | … |
| Others[b] | 50 (10) | 34 (7) | 0.68 (0.39–1.19) | … |
| 3′ UTR | | | | .027 |
| 1/1 | 166 (32) | 143 (28) | 1.79 (1.16–2.77) | … |
| 1/2 | 280 (54) | 273 (53) | 1.56 (1.03–2.30) | … |
| 2/2 | 70 (14) | 98 (19) | Reference | … |

**NOTE.** Data are no. (%) of patients with TB or control subjects, unless otherwise noted. CI, confidence interval; OR, odds ratio; UTR, untranslated region.

[a] Genotype frequencies at each polymorphic locus were compared between the patients with TB and the control subjects, and ORs were calculated by assigning the reference value (1.0) to the homozygous genotype that was more frequent in the control subjects than in the patients with TB. The OR and P values were calculated by multiple logistic regression analysis, adjusted for the age and sex of the patients with TB and the control subjects. $P < .05$ was considered to be significant.

[b] Repeats other than $(TA)_{10}(TG)_{10}$ and $(TA)_9(TG)_9$ were grouped together.

analysis, adjusted for the age and sex of the patients with TB and the control subjects. A P value <.05 was considered to be significant. In addition, we analyzed, for genotype frequency, the 306 culture- or smear-positive patients with TB from the entire cohort of 516 patients and compared them with the 514 control subjects. The genotype distributions of all 4 informative polymorphisms (at promoter, intron 2, intron 4, and 3′ UTR) were tested for Hardy-Weinberg equilibrium (HWE) separately in the patients with TB and the control subjects, by $\chi^2$ test. Statistical analyses were conducted by use of SAS software (version 8.2; SAS Institute).

*Haplotype analyses.* The frequencies of multiple-locus haplotypes composed of the *IL12B* promoter, intron 2, intron 4, and 3′ UTR alleles were estimated by performing the haplotype procedure of the SAS/Genetics software (version 8.2; SAS Institute), which uses the expectation-maximization (EM) algorithm to generate maximum-likelihood estimates of haplotype frequencies given multiple genetic-marker genotypes under the assumption of HWE [35] (table 3). To test the overall difference in haplotype frequency between the patients with TB and the control subjects, the haplotype procedure was performed for the patients, for the control subjects, and for the patients and control subjects combined. The relevant test sta-

tistic was defined as $G = -2(\ln L_{PC} - \ln L_P - \ln L_C)$, where $\ln L_{PC}$ is the natural logarithm of the maximum-likelihood estimates for patients plus control subjects, $\ln L_P$ is the natural logarithm of the maximum-likelihood estimates for the patients with TB, and $\ln L_C$ is the natural logarithm of the maximum-likelihood estimates for the control subjects. With large sample sizes, the distribution of $G$ approximates a $\chi^2$ distribution, with $df$ equal to the number of haplotypes estimated [35].

*Diplotype analyses.* We combined the genotype data of the 4 *IL12B* polymorphic loci for each of the 516 patients with TB and the 514 control subjects. The frequencies of the multiple-locus genotypes (termed "diplotypes") in the patients and control subjects are cross-tabulated in table 4. The overall difference in diplotype frequency between patients with TB and control subjects was evaluated by $\chi^2$ test.

*IL-12p70 assay.* Peripheral-blood mononuclear cells (PBMCs) from 98 randomly selected control subjects were obtained, and the *IL12B* genotypes of each subject were determined. PBMCs were cultured at a concentration of $1 \times 10^6$ cells/mL in 24-well, round-bottomed tissue-culture plates (Corning Costar), in 1 mL of RPMI 1640 medium (Gibco) with 10% fetal bovine serum (Gibco). The cells were stimulated with 100 ng/mL IFN-$\gamma$ (R&D Systems) for 2 h at 37°C, followed by the addition of 10 ng/mL lipopolysaccharide (Sigma). Culture supernatants were harvested at 16 h, and IL-12p70 concentration was assayed in duplicate by ELISA, according to the manufacturer's instructions (DuoSet ELISA Development Kit; R&D Systems). Correlation of IL-12p70 protein levels and genetic polymorphisms was analyzed by the Mann-Whitney U test.

## RESULTS

*Association between 1/1 genotype of* **IL12B** *intron 2 and TB.* Significant differences between the genotype frequencies of promoter, intron 2, intron 4, and 3′ UTR polymorphisms of *IL12B* in patients with TB and those in control subjects were detected ($P < .05$) (table 2). Intron 2 polymorphism showed the most significant result. Individuals homozygous for the 1/1 genotype of intron 2 had a 2.14-fold increased risk of developing TB (95% confidence interval [CI], 1.45–3.19 [$P < .001$]). Importantly, the genotype analyses of the whole cohort of 516 patients with TB and of the subset of 306 culture- or smear-positive patients with TB yielded very similar genotype frequencies, ORs, and P values for all 4 informative *IL12B* polymorphisms (see the Appendix). In the latter analysis, the OR for the 1/1 genotype of intron 2 was 1.98, with a 95% CI of 1.25–3.13 and P value of .009. Therefore, we used the whole cohort of 516 patients with TB for subsequent analysis. The genotype frequencies of all 4 informative *IL12B* polymorphisms in the patients with TB and in the control subjects were in HWE, except for (1) excess homozygosity at promoter genotypes in the control subjects and (2) excess homozygosity and excess hetero-

**Table 3.** Estimated *IL12B* haplotype frequencies in patients with tuberculosis (TB) and in control subjects.

| *IL12B* haplotype[a] | Promoter | Intron 2 | Intron 4 | 3′ UTR | Percentage (95% CI) | |
|---|---|---|---|---|---|---|
| | | | | | Patients with TB (*n* = 516) | Control subjects (*n* = 514) |
| A | 1 | 1 | 1 | 1 | 42.2 (39.2–45.3) | 35.4 (32.5–38.4) |
| B | 2 | 2 | 2 | 2 | 26.9 (24.2–29.6) | 34.8 (31.9–37.7) |
| C | 2 | 1 | 1 | 1 | 6.6 (5.1–8.2) | 7.0 (5.5–8.6) |
| D | 2 | 2 | 2 | 1 | 3.0 (1.9–4.0) | 3.7 (2.5–4.8) |
| E | 1 | 2 | 2 | 2 | 3.7 (2.6–4.9) | 3.3 (2.2–4.4) |
| F | 1 | 1 | 1 | 2 | 1.3 (0.6–2.0) | 2.6 (1.7–3.6) |
| G | 1 | 1 | 2 | 1 | 0.6 (0.1–1.1) | 2.5 (1.5–3.4) |
| H | 2 | 2 | 1 | 2 | 0.7 (0.2–1.1) | 2.1 (1.2–3.0) |
| I | 1 | 2 | 1 | 1 | 1.2 (0.5–1.9) | 1.1 (0.5–1.7) |
| J | 2 | 2 | 1 | 1 | 1.3 (0.6–2.0) | 1.1 (0.5–1.7) |
| K | 2 | 1 | 2 | 2 | 4.5 (3.3–5.8) | 0.6 (0.1–1.1) |
| L | 2 | 1 | 1 | 2 | 1.1 (0.5–1.8) | 0.7 (0.2–1.2) |

**NOTE.** The frequencies of multiple-locus haplotypes composed of *IL12B* promoter, intron 2, intron 4, and 3′ UTR alleles were estimated by use of the expectation-maximization algorithm. Frequencies are given in percentages. An overall difference in haplotype frequency between the patients with TB and the control subjects was found ($P < .001$). The test statistic was defined as $G = -2(\ln L_{PC} - \ln L_P - \ln L_C)$, where $\ln L_{PC}$ is the natural logarithm of the maximum-likelihood estimates for patients plus control subjects, $\ln L_P$ is the natural logarithm of the maximum-likelihood estimates for the patients with TB, and $\ln L_C$ is the natural logarithm of the maximum-likelihood estimates for the control subjects. CI, confidence interval; UTR, untranslated region.

[a] Twelve haplotypes (designated "A–L") with frequencies >1% are shown.

zygosity at intron 2 and 3′ UTR genotypes, respectively, in the patients with TB. Our sample of patients with TB consisted mainly of individuals who were newly diagnosed at territory-wide chest clinics, which treat >90% of newly diagnosed patients with TB in Hong Kong. Furthermore, the cohort of 516 patients with TB had characteristics (male, 69.1%; mean age, 48.4 years [SD, 19.8 years]; pulmonary TB, 86.3%) that were similar to those in a large survey of 5757 patients with TB who registered for treatment in Hong Kong during 1996 [36]. Departure from HWE in the patients with TB probably represented the association between TB and the intron 2 allele and a particular haplotype in our population, rather than sampling bias.

*Associations between risk haplotypes (designated "A" and "K") of* IL12B *and TB and between a protective haplotype (designated "B") of* IL12B *and TB.* Estimation of the haplotype frequencies from the diploid genotype data was performed by use of the EM algorithm [35]. In the total sample of patients with TB and control subjects, 12 of the 38 possible haplotypes composed of the promoter, intron 2, intron 4, and 3′ UTR alleles of *IL12B* had frequencies >1% (table 3). Most of the remaining haplotypes had frequencies of zero or of close to zero. We tested the genotype frequencies for HWE separately in the patients with TB and the control subjects and noted deviations from HWE in 1 locus in the control subjects and in 2 loci in the patients with TB, as described above. Estimation error might result from these HWE deviations. However, the HWE deviations in our sample were mainly due to excess homozygosity (2 of 3 loci), which caused little increase in error

between the EM estimates and the sample values [37]. Haplotypes A and B account for ~70% of the total haplotypes of *IL12B* in the Hong Kong Chinese population. An overall difference in haplotype frequency between the patients with TB and the control subjects was found ($P < .001$). Among the different *IL12B* haplotypes, haplotypes A and K occurred at higher frequencies in the patients with TB than in the control subjects, whereas the converse was found for haplotype B, with no overlapping of the 95% CIs for the haplotype frequencies in the patients with TB and the control subjects (table 3).

*Associations between a risk diplotype (designated "I") of* IL12B *and TB and between a protective diplotype (designated "II") of* IL12B *and TB.* We combined the genotypes at each of the 4 informative *IL12B* polymorphic loci (promoter, intron 2, intron 4, and 3′ UTR) and observed 61 diplotypes in the patients with TB and the control subjects. In the total sample of patients and control subjects, one-quarter of the diplotypes had frequencies >1%. The remaining diplotypes were grouped together as "others" (table 4). An overall difference in diplotype frequency between patients with TB and control subjects was found ($P < .001$). Furthermore, diplotype I (composed of A and K haplotypes) was found to be strongly associated with TB, with 25 patients with TB and none of the control subjects showing the genotype pattern. In contrast, diplotype II (homozygous BB haplotypes) was underrepresented in patients with TB, compared with its frequency in control subjects (table 4).

*IL-12p70 expression levels and* IL12B *genetic polymorphisms.* To investigate whether *IL12B* genetic polymorphisms

**Table 4. *IL12B* diplotype frequencies in patients with tuberculosis (TB) and in control subjects.**

| *IL12B* diplotype[a] | Promoter | Intron 2 | Intron 4 | 3′ UTR | No. (%) Patients with TB (*n* = 516) | No. (%) Control subjects (*n* = 514) | Possible haplotype combinations |
|---|---|---|---|---|---|---|---|
| I | 1/2 | 1/1 | 1/2 | 1/2 | 25 (4.8) | 0 (0) | AK >> GL |
| II | 2/2 | 2/2 | 2/2 | 2/2 | 32 (6.4) | 60 (11.7) | BB only |
| III | 1/2 | 1/2 | 2/2 | 1/2 | 0 (0) | 10 (1.9) | BG only |
| IV | 1/1 | 1/1 | 1/1 | 1/1 | 89 (17.2) | 79 (15.4) | AA only |
| V | 1/2 | 1/2 | 1/2 | 1/2 | 136 (26.4) | 132 (25.7) | AB, CE, DF, GH, IK |
| VI | 1/1 | 1/1 | 1/1 | 1/2 | 8 (1.5) | 8 (1.6) | AF only |
| VII | 1/1 | 1/2 | 1/2 | 1/2 | 17 (3.3) | 12 (2.3) | AE only |
| VIII | 1/2 | 1/1 | 1/1 | 1/2 | 7 (1.4) | 3 (0.6) | AL, CF |
| IX | 1/2 | 1/2 | 1/2 | 1/1 | 7 (1.4) | 7 (1.4) | AD, GJ |
| X | 1/2 | 2/2 | 2/2 | 2/2 | 9 (1.7) | 12 (2.3) | BE only |
| XI | 2/2 | 1/1 | 1/1 | 1/1 | 4 (0.8) | 7 (1.4) | CC only |
| XII | 2/2 | 1/2 | 1/2 | 1/2 | 22 (4.3) | 35 (6.8) | BC, DL, JK |
| XIII | 2/2 | 2/2 | 2/2 | 1/2 | 15 (2.9) | 19 (3.7) | BD only |
| Others | … | … | … | … | 144 (27.9) | 130 (25.3) | … |

**NOTE.** The frequencies of the multiple-locus genotypes (termed "diplotypes") in patients with TB and control subjects are cross-tabulated here. An overall difference in diplotype frequency between the patients with TB and the control subjects was found ($P < .001$, $\chi^2$ test). UTR, untranslated region.

[a] Thirteen diplotypes (designated "I"–"XIII") with frequencies >1% are shown.

are associated with gene expression, we stimulated PBMCs from 98 randomly selected control subjects for IL-12p70 production. A stronger trend for IL-12p70 production was observed in haplotype B carriers ($n = 57$; median, 352 pg/mL), compared with non–haplotype B carriers ($n = 41$; median, 283 pg/mL) ($P = .154$), whereas a weaker trend for IL-12p70 production was observed in *IL12B* promoter heterozygotes ($n = 45$; median, 287 pg/mL), compared with *IL12B* promoter homozygotes ($n = 53$, median, 357 pg/mL) ($P = .423$). Large variations in levels of IL-12p70 expression among individuals with the same *IL12B* genotypes were observed, and no statistical correlation between levels of IL-12p70 expression and specific *IL12B* genetic polymorphisms was found.

## DISCUSSION

To test whether there is an association between *IL12B* polymorphisms and TB susceptibility, we performed a large-scale population-based case-control study involving >1000 individuals from the Hong Kong Chinese population. Whether the whole cohort of 516 patients with TB or just the 306 culture- or smear-positive patients were considered, the genotype analyses showed that individuals homozygous for the intron 2 (ATT)$_8$ allele of *IL12B* had a 2-fold increased risk for TB. Only one study has reported an association between intron 2 polymorphism of *IL12B* and clinical disease; in white American families, the intron 2 (ATT)$_9$ allele has been found to be preferentially transmitted to individuals with type 1 diabetes [27]. Our findings for patients with TB could reconcile with those for individuals with type 1 diabetes, because we found the intron 2 (ATT)$_9$ allele to be protective against an infectious disease such as TB, whereas the same allele appeared to be a risk factor for an autoimmune disease

such as diabetes. This finding is consistent with the observation that IL-12 is a costimulator of protective immune responses as well as a mediator of immunopathological diseases.

We next estimated the frequencies of *IL12B* haplotypes composed of 4 informative polymorphic alleles at promoter, intron 2, intron 4, and 3′ UTR and identified risk (A and K) and protective (B) haplotypes. In agreement with the genotype findings, the intron 2 (ATT)$_8$ allele was contained in the risk haplotypes A and K, whereas the alternative (ATT)$_9$ allele was contained in the protective haplotype B. Correlation of the haplotypes and the diplotypes revealed interesting results. Diplotype I (heterozygous A and K haplotypes) was found in only 25 patients with TB and in none of the control subjects. The other probable pairings of haplotypes for diplotype I were unlikely, because they involved haplotypes with low frequencies and at least 1 haplotype with a frequency <1% (tables 3 and 4). Because diplotype IV (homozygous AA haplotypes) occurred at similar frequencies in patients with TB and control subjects, it was inferred that haplotype K might be strongly linked to a TB-susceptibility locus.

In contrast to haplotypes A and K, haplotype B was found to confer resistance to TB. Both diplotypes II (homozygous BB haplotypes) and III (heterozygous B and G haplotypes) occurred at higher frequencies in control subjects, compared with those in patients with TB. Diplotype II was found in twice the number of control subjects than in patients with TB, and diplotype III was found in 10 of the control subjects and in none of the patients with TB. The effect that haplotype G has on resistance to TB is probably small, because it was found to be rare in both the patients with TB and the control subjects. A particular *IL12B* haplotype (allele 2 at the promoter and allele 2 at 3′ UTR) was found to be associated with fatal cerebral

malaria in Tanzanians [38]. In our study population, 9% of patients with TB and 14% of control subjects were homozygous for this haplotype, which indicates that it plays a protective role against TB (OR, 0.60 [95% CI, 0.41–0.88]) ($P = .012$). The discordant effects that the *IL12B* haplotype has on malaria and TB might reflect the complexity of the influence of IL-12 and its genetic polymorphisms on host immune responses against different infectious diseases.

We attempted to correlate the *IL12B* haplotypes and levels of IL-12p70 expression. A higher median level of IL-12p70 was observed in haplotype B carriers, compared with non–haplotype B carriers; however, the result did not reach statistical significance. We could not investigate the level of IL-12p70 expression in individuals with diplotype I, because of the low frequency of such individuals among the control subjects. To date, no functional studies have reported on *IL12B* intron 2 and 4 polymorphisms; conflicting results have been reported on the functional effects of 3′ UTR polymorphisms. Morahan et al. [31] indicated that *IL12B* mRNA expression was significantly reduced in Epstein-Barr virus–transformed cell lines of the 3′ UTR 2/2 genotype, compared with those of the 3′ UTR 1/1 genotype. Dahlman et al. [39] examined 19 cell lines of 3 different 3′ UTR genotypes but did not find any correlation between genotype and expression; wide variation in levels of expression between different B-lymphoblastoid cell lines, independent of 3′ UTR genotype, was observed. Contrary to the findings of Morahan et al., Seegers et al. [32] demonstrated an association between significantly higher IL-12p70 protein levels and the 3′ UTR 2/2 genotype, after stimulation of monocytes with *Staphylococcus aureus* strain Cowan and IFN-γ. Such inconsistent results might be due in part to different experiment protocols, but they could also be due to factors other than *IL12B* genotypes regulating the expression of IL-12. We also found great variation in levels of IL-12p70 expression among individuals with the same *IL12B* genotypes. Furthermore, after multiple logistic regression, we did not observe any strong independent effect of 3′ UTR polymorphism on TB susceptibility, which concurs with the observed lack of association between 3′ UTR polymorphism of *IL12B* and TB susceptibility in a case-control study of African American and white populations [40]. However, the latter study did not evaluate the effects of other *IL12B* polymorphisms.

Morahan et al. [26] found reduced *IL12B* gene transcription and decreased secretion of IL-12p70 in asthmatic children with *IL12B* promoter heterozygosity. We found a weaker trend for levels of IL-12p70 expression in *IL12B* promoter heterozygotes than in homozygotes among the control subjects in our population, but there was substantial overlap of levels between the 2 groups, as well as great variation in the levels of IL-12 among individuals. Taken together, the current functional assays of levels of IL-12 expression are confounded by the large natural variation in cytokine production among different individuals. A better standardized biologic assay system is needed to dissect genotype-phenotype correlation.

In summary, the present study supports the association between *IL12B* intron 2 polymorphism and TB and between specific haplotypes and TB. Novel risk and protective haplotypes of *IL12B* have been identified, of which haplotype K was found to be strongly linked to TB susceptibility and haplotype B was found to confer a protective effect. Further genetic and biologic studies of these haplotypes should render valuable insight into TB susceptibility.

## APPENDIX

***IL12B* genotype frequencies in culture- or smear-positive patients with tuberculosis (TB) and in control subjects.**

| *IL12B* genotype | Patients with TB ($n = 306$) | Control subjects ($n = 514$) | Multiple logistic regression[a] | |
|---|---|---|---|---|
| | | | OR (95% CI) | P |
| Promoter | | | | .126 |
| 1/1 | 92 (30) | 134 (26) | 1.40 (0.90–2.19) | … |
| 1/2 | 144 (47) | 225 (44) | 1.50 (1.00–2.25) | … |
| 2/2 | 70 (23) | 155 (30) | Reference | … |
| Intron 2 | | | | .009 |
| 1/1 | 124 (41) | 149 (29) | 1.98 (1.25–3.13) | … |
| 1/2 | 129 (42) | 239 (46) | 1.34 (0.86–2.09) | … |
| 2/2 | 53 (17) | 126 (25) | Reference | … |
| Intron 4 | | | | .014 |
| 1/1 | 87 (28) | 139 (27) | 1.59 (0.97–2.56) | … |
| 1/2 | 136 (44) | 220 (43) | 1.08 (0.72–1.61) | … |
| 2/2 | 49 (16) | 121 (24) | Reference | … |
| Others[b] | 34 (11) | 34 (7) | 0.53 (0.28–0.99) | … |
| 3′ UTR | | | | .100 |
| 1/1 | 104 (34) | 143 (28) | 1.70 (1.03–2.80) | … |
| 1/2 | 158 (52) | 273 (53) | 1.52 (0.95–2.42) | … |
| 2/2 | 44 (14) | 98 (19) | Reference | … |

**NOTE.** Data are no. (%) of patients with TB or control subjects, unless otherwise noted. CI, confidence interval; OR, odds ratio; UTR, untranslated region.

[a] Genotype frequencies at each polymorphic locus were compared between the patients with TB and the control subjects, and ORs were calculated by assigning the reference value (1.0) to the homozygous genotype that was more frequent in the control subjects than in the patients with TB. The OR and P values were calculated by multiple logistic regression analysis, adjusted for the age and sex of the patients with TB and the control subjects. $P < .05$ was considered to be significant.

[b] Repeats other than (TA)$_{10}$(TG)$_{10}$ and (TA)$_9$(TG)$_9$ were grouped together.

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