Association of IL12RB1 Polymorphisms with Pulmonary Tuberculosis in Adults in Morocco

Natascha Remus,1,a Jamila El Baghdadi,2,a Claire Fieschi,3 Jacqueline Feinberg,1 Thibaut Quintin,1 Mohamed Chentoufi,2 Erwin Schurr,4 Abdellah Benslimane,4 Jean-Laurent Casanova,1 and Laurent Abel1

1Laboratory of Human Genetics of Infectious Diseases, University of Paris René Descartes, INSERM U550, Paris, France; 2Laboratory of Immunology, Military Hospital Mohamed V, Rabat, and 3Centre for Tuberculosis Diagnosis, Hay Mohammadi, and 4Immunology Center, Medical School of Casablanca, Casablanca, Morocco; Departments of Human Genetics and Experimental Medicine, McGill University, Montreal General Hospital, Montreal, Canada

Five disease-causing genes, including the IL12RB1 gene that encodes the β1 chain of the receptor for interleukin (IL)–12 (IL-12Rβ1), are known to be associated with the syndrome of Mendelian susceptibility to mycobacterial diseases. Some IL-12Rβ1–deficient patients present with tuberculosis as the only clinical phenotype. A comprehensive genetic study of IL12RB1 was conducted among 101 Moroccan families, including 157 offspring (age, >15 years) who had culture-positive pulmonary tuberculosis (PTB). The promoter, exons, and flanking intron regions of IL12RB1 in 40 randomly selected patients with PTB were entirely sequenced, leading to the detection of 19 variants (including 10 novel mutations). Blood cells obtained from individuals who were homozygous for any of the 13 most common variants responded to IL-12, indicating that these polymorphisms were not loss-of-function mutations. By use of a family-based study, 2 promoter polymorphisms that were in strong linkage disequilibrium were found to be associated with PTB, especially –2C→T (odds ratio for CT or TT vs. CC, 2.69 [95% confidence interval, 1.19–6.09]). This result suggests that IL12RB1 polymorphisms might influence the risk of development of PTB in adults.

Tuberculosis (TB), which is caused by Mycobacterium tuberculosis and related Mycobacterium species (e.g., M. bovis), is a major cause of morbidity and mortality worldwide, and a recent World Health Organization report estimated that there were 8–9 million new cases of clinical TB in 2000, with 1.9 million deaths resulting from the disease [1]. Progression to clinical disease is far from being an inevitable consequence of infection, and it is generally estimated that 90% of M. tuberculosis–infected subjects will never develop TB, whereas one-half of the remaining 10% of these subjects will have progression to disease occur during the first 2 years after infection [2]. Substantial epidemiological evidence supports the role of host genetic factors in susceptibility to TB. Several candidate genes have been tested using association studies (recently reviewed in [3, 4]), but conclusive genetic studies remain scant. In studies of pulmonary TB (PTB), convincing results were observed for HLA-DR2 and some alleles of the natural resistance–associated macrophage protein 1 gene (NRAMP1, also known as “SLC11A1”) [see [3, 4] and references therein]. The pathophysiology of the associated alleles, however, remains to be established at the cellular and molecular levels.

The recent identification of mutations in the interleukin (IL)–12/IL-23 (IL-12/IL-23)–interferon-γ (IFN-γ) axis in patients with Mendelian susceptibility to mycobacterial disease (MSMD; MIM 209950) was the first demonstration that genetic defects could cause severe mycobacterial infections in humans. MSMD is characterized by vulnerability to poorly virulent mycobac-
The present study was conducted in the area of Casablanca, Morocco, where the annual incidence of TB is estimated to be 108 cases/100,000 inhabitants [1]. Enrollment of patients with PTB and their relatives, as well as collection of clinical, bacteriological, and biological data, was done at the Pasteur Institute of Casablanca, in collaboration with the Immunology Center of the Casablanca Medical School and the Centre for Tuberculosis Diagnosis of Hay Mohammadi (Casablanca, Morocco). Of the patients who had been given a diagnosis of PTB on the basis of clinical symptoms (persistent cough was noted in all case patients) and pathologic findings on chest radiographs (i.e., upper lobe infiltrates, cavitory infiltrates, and/or hilar or paratracheal adenopathy), we included in the present study only the patients who had positive results of sputum culture examination (performed with the use of Lowenstein-Jensen medium).

From patients with PTB who were included in the study, we enrolled affected siblings when they fulfilled the PTB inclusion criteria and, also, both parents. When both parents were not available (as occurred for 10 of the 101 families in the study sample), we also enrolled siblings who were considered to be unaffected on the basis of normal findings of clinical examination, normal findings on chest radiographs, and a tuberculin skin test response of <12 mm of induration. BCG vaccination was ascertained through the presence of a characteristic scar. The families included in the study belonged to 2 ethnic groups (Arabs and Berbers), and the distinction between the 2 ethnic groups was based on the language spoken by the parents and grandparents of the patients with PTB. Blood samples for all functional assays were obtained from a total of 78 healthy white individuals from the French Blood Center (Etablissement Français du Sang, Hôpital Saint Vincent de Paul, Paris, France).

The study received ethical approval from the Moroccan Ministry of Public Health (Délégation du Ministère de Santé publique à Casablanca et Direction d’Épidémiologie et de Lutte contre les Maladies Infectieuses). Families of patients with PTB were contacted by a primary health care worker, and informed consent was obtained from adults or from the parents of minors. Informed consent was obtained from all healthy blood donors of the Etablissement Français du Sang, Hôpital Saint Vincent de Paul (Paris, France).

**Genotyping.** Genomic DNA in the Moroccan samples was extracted from whole blood, as described elsewhere [7]. In brief, red blood cells were lysed and separated from white blood cells by repeated washings with Tris-EDTA buffer (TE20/5). DNA was extracted with protease K, SDS, and a series of phenol-chloroform washings, and it then was precipitated with ethanol. The 17 IL12RB1 coding exons with flanking intron regions were amplified using pairs of primers and PCR conditions that have been used elsewhere [11] and that are available from the authors on request. For amplification of the promoter, several new primer pairs were designed (also available on request) that spanned a 1059-bp segment. Amplified PCR products were analyzed by gel electrophoresis on a 2% agarose gel and were purified by centrifugation through Sephadex G-50 Superfine resin (Amersham) on filter plates (Multiscreen MAHV-N45; Millipore). PCR products were sequenced using dideoxynucleotide termination, with nested primers used for the pro-
Functional assays. Functional assays were conducted to test whether some of the detected mutations could be loss-of-function mutations in subjects homozygous for the minor allele of these mutations. Peripheral blood mononuclear cells (PBMCs) were separated through Ficoll-Paque Plus (Amer- sham) gradient centrifugation. Genomic DNA was extracted from the sedimented polymorphonuclear cells by use of the method described above. PBMCs from selected individuals were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated pooled human AB serum and were activated by incubation with phytohemagglutinin-P 1/700 (Bacto PHA-P; Becton Dickinson) for 72 h. T cell blasts were restimulated with IL-2 (40 IU/mL; Chiron) every 48 h. T cell blasts were incubated with an IL-12Rβ1–specific mouse IgG1 monoclonal antibody (24E6) or an isotypic control monoclonal antibody (BD Biosciences/Becton Dickinson). Signals were analyzed with a FACScan machine, by use of CellQuest software (Becton Dickinson). Alternatively, T cell blasts were stimulated with incremental doses (from 0.001 ng/mL to 100 ng/mL) of human recombinant IL-12. Supernatants were harvested after 48 h and were assayed for IFN-γ by use of ELISA, according to the manufacturer’s recommendations (Pelikin Compact; CLB). IFN-γ concentrations per 1 million cells were calculated.

Statistical analysis. Pairwise linkage disequilibrium (LD) between common polymorphisms was assessed using Lewontin’s coefficient $D'$, as computed by use of GOLD software [16]. A $D'$ value of 0 corresponds to no LD, whereas an absolute $D'$ value of 1 corresponds to maximum LD. Haplotype frequencies were estimated using haplotype FBAT software [17]. The role of common IL12RB1 polymorphisms in susceptibility to PTB was investigated using a family-based association study, which avoids possible confounding of gene-phenotype associations resulting from inappropriately chosen control subjects or population substructures.

The general principle of the present study was to search for a distortion of the transmission of alleles from parents to affected offspring, a strategy that has been designated as a “transmission disequilibrium test” (TDT) [18]. Families with missing parental data (10 of 101 families in the study sample here) can be analyzed either by reconstructing parental genotypes from children (RC-TDT) [19] or by using unaffected siblings as control subjects (Sib-TDT) [20]. Data were analyzed using the family-based method implemented in the haplotype FBAT program [17,21], which combined the 3 different methods (i.e., TDT, RC-TDT, and Sib-TDT). Such analysis allows the use of an empirical variance-covariance estimator for the statistic, which is consistent when sibling marker genotypes are correlated (e.g., when the analysis includes multiplex families).

In addition to using the nominal $P$ values provided by the haplotype FBAT software, we performed a simulation study to assess levels of significance in our specific context and to account for the problem of multiple testing. In the simulation study, haplotypes that consisted of the 9 polymorphisms tested for association were randomly assigned to parents, according to their frequencies estimated in the sample, and the haplotypes then were transmitted to offspring under the null hypothesis of no association. Ten thousand replicates were generated under this null hypothesis and were analyzed by haplotype FBAT software, allowing for estimation of the probability that a given result observed in the real data occurred just by chance.

Alles that showed some evidence for association were also analyzed using conditional logistic regression, as described elsewhere [22]. This analysis allowed odds ratio (OR) estimates to be provided and also tested for different modes of inheritance (e.g., additive, dominant, and recessive modes). Finally, because the sample in the present study consisted of 2 main ethnic groups, Arabs and Berbers, the analysis was also conducted separately in these 2 subsamples.

RESULTS

A total of 101 nuclear families (91 families with 2 genotyped parents, 5 families with 1 genotyped parent, and 5 families with 0 genotyped parents) with $\geq 1$ adult offspring (age, $\geq 15$ years) with PTB were identified. A substantial proportion (32%) of the 187 available parents from the 101 families was also affected by PTB (40% of affected parents had concurrently active PTB, whereas the remaining 60% had been previously affected). The majority of the 101 families included in the study (77 families) were of Arabic origin, whereas the 24 remaining families were of Berber origin. Fifty-nine, 29, 12, and 1 families had 1, 2, 3, and 4 affected adult siblings, respectively. Overall, there were 157 affected adult offspring, including 97 men and 60 women (mean age at diagnosis, 24.1 years; range, 16–46 years). From the 10 families with missing parents, a total of 14 unaffected siblings were recruited for the study. All persons had scars that were characteristic of BCG vaccination.

We first investigated 40 randomly chosen unrelated patients with PTB (29 patients of Arabic origin and 11 patients of Berber origin), using sequence analysis of all coding IL12RB1 exons and their flanking intron regions, as well as a 1059-bp promoter segment (for an overall total of 5944 bp). Nineteen point mutations (9 known and 10 unknown) were detected (figure 1); none of these point mutations corresponded to previously reported IL12RB1 null mutations. Four common exonic mutations (467G→A, 641A→G, 1094T→C, and 1132G→C) that cause 4 aa changes (R156H, Q214R, M365T, and G378R), as well as another silent exonic mutation (684C→T), had already been reported elsewhere [14,23–25]. One 5’ untranslated region (UTR; −2C→T) and 3 intronic mutations
Figure 1. Schematic representation of the structural organization of the IL12RB1 gene with the variants found in the Moroccan population. Variants with a minor allele frequency \( q \) <5% are shown in italics, and variants for which \( q \) >5% (denoted as “single-nucleotide polymorphisms” [SNPs]) are shown in bold. Exons 1–17 are depicted as boxes (shown with a roman numeral), with lengths proportional to exon sizes; the promoter, introns, and the 3′ untranslated region are shown as lines of arbitrary size. Shaded boxes denote exons that contain SNPs for which the entire Moroccan population has been genotyped. Missense SNPs are indicated above the gene, with the nucleotide changes shown in parentheses; synonymous and intronic variants are indicated below the gene.
Figure 2. Interferon (IFN)-γ production of T cell blasts in response to increasing interleukin (IL)-12 stimulation, according to individual genotypes. The continuous bold line denoted as “wt-ct” (i.e., wild-type–control) is the mean of the values for 3 individuals who were homozygous for the common alleles of all 13 common single-nucleotide polymorphisms (SNPs) detected in IL12RB1, with bars denoting maximum and minimum observed values. Because of strong linkage disequilibrium between several of the 13 SNPs, subjects 1–5 were homozygous for the minor alleles of several SNPs at a time. Subject 1 was homozygous for the minor alleles of −111A→T, −2C→T, 467G→A, the cluster 641A→G-684C→T-1094T→C-1132G→C (known as “cluster A”), 1791+46T→C, and the cluster 1983+24C→T-1983+47G→T-1989+34C→T (known as “cluster B”). Subjects 2 and 3 were homozygous for the minor alleles of the cluster A SNPs and 1791+46T→C, and subject 3 was also homozygous for the minor alleles of 387G→C. Subjects 4 and 5 were homozygous for the minor alleles of 1791+46T→C and the cluster B SNPs, and subject 5 was also homozygous for the minor alleles of 409+21C→A. Subject 6 served as a negative control; this subject had abolished surface expression of the β1 chain of the receptor for IL-12 (IL-12R β1) on T cell blasts resulting from a homozygous premature stop codon, Q32X (patient 20.II.1 in [11]).

(1327+84G→A, 1791+46T→C, and 1989+34C→T) are published in the National Center for Biotechnology Information single-nucleotide polymorphism (SNP) database (GenBank accession number, NT_011295). Eight novel mutations, including 2 silent exonic mutations (387G→C and 1098G→A, with minor allele frequencies [q] of 0.13 and 0.02, respectively), 4 intronic mutations (240+20C→T, 409+21C→A, 1983+24C→T, and 1983+47G→T, with q values of 0.02, 0.09, 0.25, and 0.25, respectively), and 2 mutations in the promoter region (−430C→T and −111A→T, with q values of 0.05 and 0.17, respectively) were found in several patients. Finally, a single copy of 2 novel intronic mutations (700+22G→A and 1021+24C→T) was observed among the 80 tested chromosomes.

We then studied whether the detected mutations were loss-of-function mutations, by testing IL-12Rβ1 expression on, and the IL-12 responsiveness of, T cell blasts in healthy white control subjects who were homozygous for common alleles of all 13 SNPs or homozygous for minor alleles of different SNPs (figure 2, subjects 1–5) (data not shown). T cell blasts subsequently were stimulated with incremental doses of IL-12, and IFN-γ production was measured by ELISA, as shown in figure 2. All subjects who were homozygous for various combinations of the minor alleles of the 13 SNPs responded to increasing doses of IL-12 with a pattern of IFN-γ production close to that of control subjects with the wild type. This finding excluded the possibility that any of the 13 IL12RB1 SNPs identified—of note, the 5 novel SNPs found in our Moroccan population—were loss-of-function mutations.

We then genotyped the entire family sample for the 13 detected SNPs, to test for association with PTB. All IL12RB1 SNPs were in Hardy-Weinberg equilibrium, and segregation patterns were consistent with Mendelian transmission. The pattern of LD between the 13 SNPs is shown in figure 3. Complete LD (with equal allele frequencies) was found within the SNP couple 641A→G-684C→T, the couple 1094T→C-1132G→C, and the trio 1983+24C→T-1983+47T→C-1989+34C→T, and only 1 SNP (the
Figure 3. Linkage disequilibrium pattern between the 13 common IL12RB1 single-nucleotide polymorphisms. Pairwise Lewontin’s coefficients $D$ (absolute values) are plotted using GOLD software.

first one) per couple or trio was retained for further analysis, resulting in a total of 9 SNPs for the association study. The most common haplotypes observed with these 9 SNPs are shown in table 1. Almost complete LD (with slightly different allele frequencies) was found between the 2 couples 641A→G-684C→T and 1094T→C-1132G→C, so that these 4 SNPs only defined 2 common alleles, as previously reported in a Japanese population [23]. Strong LD was also observed between the promoter SNPs /H11002 111A→T and /H11002 2C→T with 2 common haplotypes (A-C and T-T), a rare haplotype T-C, and a missing haplotype A-T.

Results of the family-based association study between PTB and the 9 retained IL12RB1 SNPs are shown, in table 2, for a dominant mode of inheritance (similar results were obtained with an additive model; data not shown). Only 2 SNPs in strong LD (∼2C→T and −111A→T) provided evidence ($P = .013$ and $P = .019$, respectively) for an association with PTB. The simulation study indicated that the probability of observing ≥1 test for which $P = .013$ was 0.082, when 9 SNPs in this specific sample were tested. However, the probability of obtaining ≥1 test for which $P = .013$ and another test for which $P = .019$, as actually observed, was 0.012; this finding indicates that our results are unlikely to occur just by chance. Under a dominant model, for −2CT or −2TT vs. −2CC, the OR of developing PTB was 2.69 (95% confidence interval [CI], 1.19–6.09), and, for −111AT/TT vs. −111AA, it was 2.03 (95% CI, 1.03–4.03). The previous finding that the A-T haplotype (which contains allele A of −111A→T and allele T of −2C→T) is missing indicates that (1) the differences in the results obtained with those 2 SNPs are a result of the few subjects carrying the T-C haplotype and (2) the analyses performed with the T allele of −2C→T exactly correspond to analyses performed with the T-T haplotype. When the analysis was performed with stratification done according to ethnic group, the association was stronger ($P = .004$ and $P = .008$ for −2C→T and −111A→T, respectively) in families of Arabic origin, with an estimated OR of 3.80 (95% CI, 1.62–10.19) for −2CT/TT vs. −2CC. In this specific sample, the probability of observing ≥1 test for which $P = .004$ was estimated, by simulation, to be 0.021. Of interest, we also observed, in Arabic families, a greater proportion of subjects with −2CT/TT (27.9%) among the 43 affected parents with PTB, compared with the unaffected parents (16.7% of 96 unaffected subjects), although this difference was not statistically significant ($P = .10$).

DISCUSSION

The present study is the first comprehensive study to investigate the Mendelian and complex contribution to adult PTB of 1 of the 5 known genes associated with MSMD. We did not find any previously described null IL12RB1 mutations, and the 13 common SNPs that were detected were shown not to be loss-of-function mutations. This result does not exclude the possibility that these SNPs may exert more subtle effects that could be detected by more sensitive assays. In addition, we could not exclude the possible existence of rare loss-of-function IL12RB1 mutations causing PTB in other patients. Because we did not
observe any null recessive mutations in a sample of 40 patients, the 95% CI of the proportion of patients who carry such mutations could be estimated to be 0%–7.2%. Furthermore, null IL12RB1 mutations are more likely to be found in children or young adults with severe TB (disseminated, extrapulmonary TB). Indeed, complete IL-12Rβ1 deficiency has been diagnosed, to date, in 2 Spanish children, aged 5 and 13 years [12], and in an 18-year-old patient from Morocco [10]; these 3 individuals developed disseminated multicabulary TB, PTB (despite receipt of chemoprophylaxis), and abdominal TB, respectively. Recently, an additional 12-year-old Turkish girl with the same mutations that alter the sequence flanking the start codon may be involved in Mendelian hereditary diseases, such as α-thalassemia, androgen insensitivity syndrome, and ataxia with vitama.

In studying the common polymorphisms, we found a significant association of 2 SNPs of the IL12RB1 gene (−2C→T and −111A→T) with PTB in our study population. Both SNPs are in almost complete LD, but the effect of −2T→C was slightly stronger, notably in the Arabic population. Because of the low number of informative Berber families, it is difficult to come to definitive conclusions about the existence of genetic heterogeneity in the phenotype control. We did not find any association with the cluster of 4 SNPs that were in complete LD (641A→G-684C→T and 1094T→C-1132G→C), although the less common haplotype of this cluster was reported to predispose an adult Japanese population to PTB [23]. We also did not find a strong influence of NRAMP1 variants on PTB in a previous study performed in the same Moroccan population [26], although polymorphisms of this gene were found to be associated with PTB in other populations [3, 4]. Because of the large number of informative families, these negative results could not be explained by a lack of power, and they are consistent with the hypothesis that the genes involved in the control of phenotypes such as PTB depend on the populations studied, as discussed in previously published reviews [3, 4].

Whether the SNPs −2C→T and −111A→T are in LD with other causative polymorphisms elsewhere and/or are themselves hypomorphic and involved functionally in the risk for TB remains to be established. Both SNPs were found to be in rather strong LD with the 467G→A SNP that causes an amino acid substitution (R156H), but haplotype analysis did not support a strong contribution of the 467G→A SNP to the association of PTB with −2C→T and −111A→T. However, we could not exclude the presence of variants in LD with the −2T and −111T alleles located in intronic regions, further upstream in the promoter, or in the 3′ UTR, which might influence gene transcription or mRNA translation. No obvious functional role can be predicted for the region surrounding the SNP −111A→T. Regarding the SNP −2C→T, it is interesting to note that ribosomes do commence translation from mRNA preferentially at AUG start codons surrounded by consensus sequences [27]. Mutations that alter the sequence flanking the start codon may be involved in Mendelian hereditary diseases, such as α-thalassemia, androgen insensitivity syndrome, and ataxia with vit-

### Table 1. Most frequent (frequency, ≥0.01) haplotypes observed with the 9 common IL12RB1 single-nucleotide polymorphisms, as estimated using haplotype FBAT software.

<table>
<thead>
<tr>
<th>Position</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>−111T</td>
<td>0.12</td>
</tr>
<tr>
<td>−2C→T</td>
<td>0.10</td>
</tr>
<tr>
<td>387G→C</td>
<td>0.13</td>
</tr>
<tr>
<td>409+21C→A</td>
<td>0.06</td>
</tr>
<tr>
<td>467G→A</td>
<td>0.10</td>
</tr>
<tr>
<td>641A→G</td>
<td>0.24</td>
</tr>
<tr>
<td>1094T→C</td>
<td>0.26</td>
</tr>
<tr>
<td>1791+46T→C</td>
<td>0.46</td>
</tr>
<tr>
<td>1983+24T→C</td>
<td>0.24</td>
</tr>
</tbody>
</table>

### Table 2. Results of an association study of common IL12RB1 polymorphisms and pulmonary tuberculosis in adults, assuming a dominant effect for the marker minor allele.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Minor allele frequency</th>
<th>P&lt;sub&gt;0&lt;/sub&gt; (no. of informative families)</th>
<th>OR&lt;sup&gt;b&lt;/sup&gt; (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−111A→T</td>
<td>0.12</td>
<td>0.01 (28)</td>
<td>2.03 (1.04–4.04)</td>
</tr>
<tr>
<td>−2C→T</td>
<td>0.10</td>
<td>0.013 (25)</td>
<td>2.69 (1.19–6.09)</td>
</tr>
<tr>
<td>387G→C</td>
<td>0.13</td>
<td>.83 (21)</td>
<td>1.03 (0.55–1.94)</td>
</tr>
<tr>
<td>409+21C→A</td>
<td>0.06</td>
<td>.80 (13)</td>
<td>0.69 (0.24–2.02)</td>
</tr>
<tr>
<td>467G→A</td>
<td>0.10</td>
<td>.39 (27)</td>
<td>1.35 (0.68–2.68)</td>
</tr>
<tr>
<td>641A→G</td>
<td>0.24</td>
<td>.40 (36)</td>
<td>0.83 (0.52–1.32)</td>
</tr>
<tr>
<td>1094T→C</td>
<td>0.26</td>
<td>.90 (36)</td>
<td>1.18 (0.51–2.72)</td>
</tr>
<tr>
<td>1791+46T→C</td>
<td>0.46</td>
<td>.98 (34)</td>
<td>0.97 (0.53–1.77)</td>
</tr>
<tr>
<td>1983+24T→C</td>
<td>0.24</td>
<td>.96 (45)</td>
<td>1.02 (0.61–1.69)</td>
</tr>
</tbody>
</table>

**NOTE.** CI, confidence interval; OR, odds ratio.

<sup>a</sup> Haplotype FBAT software.

<sup>b</sup> OR of developing pulmonary tuberculosis, for carriers of ≥1 copy of the minor allele vs. individuals homozygous for the common allele, as estimated by conditional logistic regression.
IL12RB1 Polymorphisms in Adults with PTB • JID 2004:190 (1 August) • 587

min E deficiency resulting from point mutations at positions –4 of the α-globin gene, +4 of the androgen receptor gene, and –1 of the α-tocopherol transfer protein gene, respectively (see [27] and references therein). A purine base (A or G) at position –3 and a G at position +4 are the most conserved nucleotides in genes of vertebrates [28]. The base at position –2 is less conserved, but C (40%) and A (31%) are the most favored nucleotides, whereas T is the least common (10%) [28, 29]. Despite a grossly normal surface expression of IL-12Rβ1, as shown by flow cytometric analysis of T cell blasts from a healthy white control subject, additional in-depth immunological studies of Moroccan patients are required to investigate whether a C→T change at position –2 and/or an A→T change at position –111 may be hypomorphic by influencing IL12RB1 mRNA processing, stability, and/or translation.

Acknowledgments

We thank Alexandre Alcaïs for critical reading of the manuscript and members of the Laboratory of Human Genetics of Infectious Diseases (Paris, France) for fruitful discussions. We are grateful to the Établissement Français du Sang–Île de France, Hôpital Saint Vincent de Paul (Paris, France) for helpful collaboration.

References