The purpose of our study was to investigate the association between a functional single nucleotide polymorphism (SNP) in the interleukin-23 receptor gene (IL23R; rs11209026, 1142 G<sup>野生型</sup> → A<sup>功能减退</sup>, Arg381Gln) and disease severity outcome in pulmonary tuberculosis (TB) in the Tunisian population. SNP was investigated in a population of 168 patients with active pulmonary TB (cases were stratified into patients with minimal/moderate lung involvement, i.e., patients with minimal/moderate disease [Pmd], and patients with extensive lung involvement, i.e., patients with active disease [Pad]) and 150 healthy subjects. Genotype analyses were carried out using the PCR-restriction fragment length polymorphism method. We have found that the IL23R reduced-function allele 1142A and genotypes AA and AG were overrepresented, especially in the Pad subgroup compared with the control group (51% versus 18% [P = 10<sup>−8</sup>], 33% versus 5% [P = 10<sup>−8</sup>], and 36% versus 26% [P = 5 × 10<sup>−3</sup>], respectively). Additionally, comparison of the Pad and the Pmd groups showed that the A allele and AA genotype seemed to be associated with 2.79-fold (P = 4 × 10<sup>−5</sup>) and 7.74-fold (P = 10<sup>−5</sup>) increased risks of TB with minimal/moderate lung involvement, respectively. Our results demonstrate that the reduced-function polymorphism 1142G → A encoded by IL23R influences the outcome of disease severity of active pulmonary TB in Tunisian patients.
Susceptibility to Tuberculosis

IL-23-deficient mice have shown that the absence of IL-23 has little or no effect on host resistance to *Toxoplasma gondii*, *Cryptoccus neoformans*, and *M. tuberculosis* infection, unless IL-12 is also absent (27, 29, 31). These studies suggest that, compared to the dominant role of IL-12, the role of IL-23 in chronic infections is more subtle. Recently, Khader and collaborators have reported that IL-23 plays an essential role in chronic infection. They showed that this cytokine is required for long-term control of tuberculosis and its severity in TB patients in Sousse, Tunisia, a region with a high incidence of tuberculosis (26).

In our study, we have investigated the association between a polymorphism encoded by *IL23R* ([IL23R] 1142G→A), a Th1-promoting cytokine (29, 40) which has also been considered a candidate susceptibility gene in many autoimmune diseases. The IL-23 receptor also shares a subunit, IL-12 receptor β1 ([IL-12Rβ1], with the IL-12 receptor, but it is the specific subunit of the IL-23 receptor, named IL-23R. IL-23 stimulates the proliferation of Th17 cells, a T-cell population which produces inflammatory cytokines such as IL-17, tumor necrosis factor, and IL-6 (28).

Several polymorphisms within the IL-23R gene ([IL23R]; such as the 1142G→A polymorphism encoded by [IL23R] [IL23R] 1142G→A); rs11209026, 1142 G°/° type → A°/A° reduced function, Arg381Gln) have been associated with immune-related diseases, including inflammatory bowel disease, psoriasis, and ankylosing spondylitis (9, 17, 36, 42). However, to date, there have been no studies evaluating the association between this polymorphism and the risk of development of active TB in the world or in Tunisian patients.

In our study, we have investigated the association between [IL23R] (1142G→A) and the risk of development of active pulmonary TB and its severity in TB patients in Sousse, Tunisia, a region characterized by a moderate TB prevalence (31 new cases per 100,000 population) and incidence (25 cases/100,000 population/year) and a predominating *M. tuberculosis* strain (46).

### MATERIALS AND METHODS

#### Study populations

One hundred sixty-eight patients with active pulmonary TB from Sousse, Tunisia, which is in the central region of the country, were enrolled in this study (Table 1). One hundred fifty healthy blood donors (135 males and 15 females) were studied as controls.

Patients and healthy blood donors were selected over the period from January 2009 to June 2010. Individuals with a history of severe pathologies, including HIV infection, cardiovascular disease, asthma, or atopy autoimmune diseases, and cancer were excluded from the study. An informed written consent was obtained from all individuals prior to blood sampling. Moreover, our study was approved by the ethics committee of the Farhat Hached University Hospital.

Patients were recruited from the Pneumology Unit, CHU Farhat Hached, and the health care service, Sousse, Tunisia. Inclusion criteria for the patients in this group were determined according to the criteria defined by the American Thoracic Society (1).

Diagnosis of active pulmonary TB was based on clinical symptoms, the presence of acid-fast bacilli in sputum smears, and culture on Lowenstein-Jensen and Coletos medium in all cases.

Pulmonary TB patients whose radiological lung tissue involvement (n = 168) was available were further stratified into pulmonary patients with minimal (n = 80)/moderate (n = 43) disease or lung involvement (Pmd) and pulmonary patients with advanced disease and extensive lung involvement (Pad; n = 45), according to non-HIV-related TB guidelines for disease classification (14, 32).

All controls had the same ethnic and geographic origins and lived in the same city as the TB patients. The inclusion criteria for the control group were the absence of acute or chronic pulmonary disease, a negative history for TB, and proof of being healthy.

#### DNA extraction and genotyping

Genomic DNA was isolated from fresh whole blood-EDTA and buffy-coat lymphocytes of TB patients and controls using a Wizard Genomic DNA purification kit (Promega Corporation, Madison, WI), according to the manufacturer’s instructions.

To genotype Arg381Gln (1142G→A), a PCR-restriction fragment length polymorphism (RFLP) method was used as previously reported (42). Briefly, 100 ng of genomic DNA was added to 25 μl of a reaction mixture containing 1 mM each primer. The forward and reverse primers were 5'-CTTTTTCGCAACGCTATTGTTG-3' and 5'-AAATTTGCTTCC TGGGGTATGTGTTG-3', respectively. The remainder of the mixture consisted of 1X PCR GoTaq buffer (Promega), 1.5 mM MgCl₂, 0.2 mM deoxynucleoside triphosphates, and 1 U GoTaq Hot Start polymerase (Promega). The mixture was then initially subjected to 95°C for 10 min, followed by 40 cycles of denaturation for 30 s at 95°C, annealing for 1 min at 53°C, and extension for 1 min and 30 s at 72°C; final extension was for 7 min at 72°C. Amplifications were performed in a MyCycler thermal cycler (Bio-Rad). RFLP of the PCR product was used for the detection of

### Table 1 Demographic and clinical data for tuberculosis patients and controls

<table>
<thead>
<tr>
<th>Study group</th>
<th>No. of cases</th>
<th>Gender (no. M:no. F)</th>
<th>Mean (range) age (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pTB</td>
<td>168</td>
<td>127:41</td>
<td>44 (14–78)</td>
</tr>
<tr>
<td>Pmd</td>
<td>123</td>
<td>100:23</td>
<td>39 (14–65)</td>
</tr>
<tr>
<td>Pad</td>
<td>45</td>
<td>27:18</td>
<td>45 (37–78)</td>
</tr>
<tr>
<td>Healthy</td>
<td>150</td>
<td>135:15</td>
<td>35 (24–55)</td>
</tr>
</tbody>
</table>

*pTB, pulmonary tuberculosis; Pmd, pulmonary patients with minimal/moderate lung involvement; Pad, pulmonary patients with extensive lung involvement; M, male; F, female.

### Table 2 IL23R 1142 G→A allele and genotype frequencies in pulmonary tuberculosis cases and controls

<table>
<thead>
<tr>
<th>Allele or genotype</th>
<th>pTB cases vs controls</th>
<th>Pmd vs controls</th>
<th>Pad vs controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>113 (34)</td>
<td>67 (27)</td>
<td>46 (51)</td>
</tr>
<tr>
<td></td>
<td>55 (18)</td>
<td>245 (82)</td>
<td>10⁻⁵</td>
</tr>
<tr>
<td>G</td>
<td>223 (66)</td>
<td>179 (73)</td>
<td>44 (49)</td>
</tr>
<tr>
<td></td>
<td>245 (82)</td>
<td>10⁻⁵</td>
<td></td>
</tr>
</tbody>
</table>

*pTB, pulmonary tuberculosis; Pmd, pulmonary patients with minimal/moderate lung involvement; Pad, pulmonary patients with extensive lung involvement.

To determine the susceptibility of this polymorphism to TB, we studied the association between [IL23R] (1142G→A) and the risk of development of active pulmonary TB and its severity in TB patients in Sousse, Tunisia, a region characterized by a moderate TB prevalence (31 new cases per 100,000 population) and incidence (25 cases/100,000 population/year) and a predominating *M. tuberculosis* strain (46).
Allele or genotype | No. (%) of patients | Allele | Genotype | Allele | Genotype
--- | --- | --- | --- | --- | ---
Pad (n = 45) | Pmd (n = 123) | P | OR (95% CI)
Allele | | | | | |
A | 46 (51) | 67 (27) | 4 × 10⁻³ | 2.79 (1.64–4.75)
G | 44 (49) | 179 (73) | | |
Genotype | | | AA | AG | GG
AA | 15 (33) | 9 (7) | 10⁻⁵ | 7.74 (2.54–24.2)
AG | 16 (36) | 49 (40) | 0.31 | 1.52 (0.63–3.67)
GG | 14 (31) | 65 (53) | | |

**TABLE 3** IL23R 1142 G → A allele and genotype frequencies in pulmonary patients with extensive lung involvement and pulmonary patients with minimal/moderate lung involvement

The frequency distributions of different IL23R 1142 G → A genotypes are summarized in Table 2. We observed that the IL23R 1142A (reduced-function) allele was significantly overrepresented in the pulmonary TB group in comparison to the control group (34% versus 18%; odds ratio [OR] = 2.26, 95% confidence interval [CI] = 1.53 to 3.32) (Table 2). Moreover, when this group was stratified into pulmonary patients with minimal/moderate lung involvement (Pmd) and pulmonary patients with extensive lung involvement (Pad), we found that the 1142A allele was significantly more frequent in these two groups (27% versus 18% [P = 12 × 10⁻³]) and 51% versus 18% [P = 10⁻⁸], respectively) (Table 2).

Additionally, the 1142A allele seemed to be associated with the increased risk of development of TB with minimal/moderate lung involvement (OR = 1.67, 95% CI = 1.09 to 2.55) and TB with extensive lung involvement (OR = 4.66, 95% CI = 2.72 to 7.98).

Three genotypes, AA, AG, and GG, were observed in the different TB and control groups (Table 2). The AA and AG genotypes were significantly more frequent in pulmonary TB patients and pulmonary patients with extensive lung involvement (Pad) than in the control group (14% versus 5% [P = 9 × 10⁻⁴] and 33% versus 5% [P = 10⁻⁸], respectively, for the AA genotype and 39% versus 26% [P = 10⁻³] and 36% versus 26% [P = 5 × 10⁻³], respectively, for the AG genotype). Additionally, these genotypes seemed to be associated with an increased risk for development of active pulmonary TB with extensive lung involvement (Table 3).

When the frequency distribution of different allele and genotypes of the IL23R 1142 G → A single nucleotide polymorphism (SNP) was adjusted by gender in the Pmd, Pad, and control groups, we found that (i) the A allele seemed to be associated with an increased risk of development of TB with minimal/moderate lung involvement (OR = 1.67, 95% CI = 1.02 to 2.74, P = 0.031) and extensive lung involvement (OR = 3.37, 95% CI = 1.89 to 7.38, P = 2 × 10⁻⁵) only in men (Tables 4 and 5) and (ii) men harboring the AA genotype seemed to be at greater risk of development of active TB with extensive lung involvement than women (OR = 12.06, 95% CI = 2.97 to 51.4, P = 10⁻⁴) (Table 5).

**RESULTS**

**Hardy-Weinberg equilibrium.** In this study, evaluation of Hardy-Weinberg equilibrium showed that the genotype frequencies of the IL23R 1142 G → A polymorphism were in Hardy-Weinberg equilibrium in the pulmonary TB, Pmd TB, and Pad TB groups and healthy blood donors (P = 0.05).

**Association of the IL23R 1142 G → A reduced-function polymorphism with pulmonary tuberculosis.** We have used PCR-RFLP to examine the status of the IL23R gene polymorphism linked to Gwild-type → A-reduced-function phenotypes.

**TABLE 4** IL23R 1142 G → A allele and genotype frequencies in Pmd and control groups, by gender

<table>
<thead>
<tr>
<th>Allele or genotype</th>
<th>No. (%) of patients</th>
<th>Male and female Pmd vs male and female control cases</th>
<th>Male Pmd vs male control cases</th>
<th>Female Pmd vs female control cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pmd (n = 123)</td>
<td>Controls (n = 150)</td>
<td>M + F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>67 (27)</td>
<td>46 (23)</td>
<td>21 (46)</td>
<td>55 (18)</td>
</tr>
<tr>
<td>G</td>
<td>179 (73)</td>
<td>154 (77)</td>
<td>25 (54)</td>
<td>245 (82)</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td>AA</td>
<td>AG</td>
</tr>
<tr>
<td>AA</td>
<td>9 (7)</td>
<td>7 (7)</td>
<td>2 (9)</td>
<td>8 (5)</td>
</tr>
<tr>
<td>AG</td>
<td>49 (40)</td>
<td>32 (32)</td>
<td>17 (74)</td>
<td>39 (26)</td>
</tr>
<tr>
<td>GG</td>
<td>65 (53)</td>
<td>61 (61)</td>
<td>4 (17)</td>
<td>103 (69)</td>
</tr>
</tbody>
</table>

* Pad, pulmonary patients with extensive lung involvement; M, male; F, female.

{Fish’s exact test}
of cytokine receptor genes, such as the gamma interferon receptor (20) and interleukin-10 receptor, as we have recently reported (7), to be host factors influencing the development of active TB. This is the first study demonstrating that the IL23R 1142G → A functional polymorphism is associated with increased susceptibility to active pulmonary TB and its severity in Tunisian patients. In fact, our result showed that patients carrying the IL23R 1142A allele or AA genotype had 2.79- and 7.74-fold increased risks of developing active TB with extensive lung involvement, respectively.

Recently, Khader and collaborators have reported that IL-23 plays a crucial role in the long-term immune response against M. tuberculosis (26). They showed that IL-23 is required for long-term containment of M. tuberculosis, as well as the expression of CXCL13 within and the maintenance of B-cell follicles within the lung lesions. Additionally, they demonstrated that IL-17RA and IL-22 were involved in B-cell-follicle development at distinct times during infection and that IL-23 is necessary for the expression of both of these cytokines in the lung.

Many studies have revealed that several single nucleotide polymorphisms in the IL23R gene are associated with immune-related diseases, including inflammatory bowel disease, psoriasis, and ankylosing spondylitis (9, 17, 36, 42). The most studied SNP was IL23R R381Q. Sarin et al. indeed showed that the 381Q variant has a reduced percentage of cells that secrete IL-17 and IL-22 in response to IL-23 stimulation and reduced levels of STAT3 phosphorylation and IL-17 and IL-22 production in T-cell subsets (38). The group of Pidasheva et al. also showed reduced STAT3 phosphorylation in response to IL-23 in T cells with the 381Q variant, although they did not find reduced numbers of IL-17- or IL-22-producing cells (36). Sarin et al. showed that IL-23-induced IFN-γ production is not affected in the 381Q variant (38), and Pidasheva et al. showed that IFN-γ-producing cells are not reduced (36). Another group has also shown that the 381Q variant of IL-23R is comparable to the wild-type variant in an overexpression system, when analyzing the ability to induce IFN-γ and IL-10 production (16). The heterogeneity of these results could be related to the cytokines investigated or the use of different cell types, such as retrovirally transduced T-cell blasts (16) rather than isolated primary CD8+ T cells expressing the endogenous wild type or R381Q IL23R variant (38).

To our knowledge, no study in the world has investigated the association between the IL23R R381Q reduced-function polymorphism and the risk of development of active pulmonary TB. This is the first study demonstrating an association between the IL23R R381Q reduced-function polymorphism and pulmonary active TB and its severity in the Tunisian population. Our result has shown that patients carrying the A allele of the IL23R R381Q reduced-function polymorphism had 2.26-, 1.67-, and 4.66-fold increased risks of developing pulmonary TB, pulmonary TB with minimal/moderate lung involvement, and pulmonary TB with extensive lung involvement, respectively. Additionally, comparison of the Pad (advanced TB) and Pmd (mild to moderate TB) groups showed that the A allele and AA genotype of the 1142G → A polymorphism seemed to be associated with 2.79- and 7.74-fold increased risks of advanced disease and disease of minimal/moderate severity, respectively. The A allele of the IL23R 381Q polymorphism corresponds to decreased IL-23-dependent IL-17 and IL-22 production, which may impair the immune response against M. tuberculosis infection, resulting in pulmonary TB development.

Interestingly, in our study, the IL23R 1142A allele appeared to be associated with 1.67- and 3.37-fold increased risks of development of active TB with minimal/moderate lung involvement and extensive lung involvement, respectively, in males. Additionally, men with the AA genotype appeared to be at a 12.06-fold increased risk of development of the active form of pulmonary TB with extensive lung involvement.

In conclusion, our study shows for the first time that the IL23R 1142G → A reduced-function polymorphism seems to be associated with an increased risk of development of a severe form of active pulmonary TB. Further studies with both adult and pediatric populations in ethnically diverse settings are needed to confirm our findings.

ACKNOWLEDGMENTS

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W.B.-S. performed the experiments and wrote the manuscript. J.B. conceived the project, supervised experiments, and revised the manuscript. Both W.B.-S. and J.B. read and approved relevant portions of the manuscript.

We state that we have no conflicts of interest.
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