A Recessive Major Gene Controls the Mitsuda Reaction in a Region Endemic for Leprosy

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Background. Leprosy is a chronic infectious disease caused by Mycobacterium leprae. The Mitsuda reaction is a delayed granulomatous skin reaction elicited by intradermal injection of heat-killed M. leprae. Interestingly, results of the Mitsuda test are positive in the majority of individuals, even in areas not endemic for M. leprae. Like leprosy, the Mitsuda reaction is thought to be genetically controlled, but its mode of inheritance is unknown, although the role of the NRAMP1 gene has previously been reported.

Methods. We conducted a segregation analysis of quantitative Mitsuda reactivity in 168 Vietnamese nuclear families ascertained through patients with leprosy.

Results. We found strong evidence (P < 10−7) for a major gene controlling the Mitsuda reaction independently of leprosy clinical status. Subsequent linkage analysis showed that this major gene was distinct from NRAMP1. Under the major-gene model, ~12% of individuals are homozygous for the recessive predisposing allele and are predicted to display high levels of Mitsuda reactivity (mean, ~10 mm, versus 5 mm in other individuals).

Conclusion. We provide evidence that the Mitsuda reaction is controlled by a major gene. Our study paves the way for the identification of this gene and should provide novel insight into the mechanisms involved in granuloma formation, especially in M. leprae infection.

Leprosy is a chronic infectious disease, caused by Mycobacterium leprae, that mainly affects skin and peripheral nerves [1]. Despite a dramatic decrease in prevalence during the past 15 years, partly due to the efficacy of multidrug therapy [2], the worldwide leprosy incidence is still approaching 500,000 novel cases/year. Whereas most infected individuals do not develop clinical disease, even after sustained exposure to M. leprae, others present a broad spectrum of clinical symptoms. Leprosy ranges from the tuberculosis form, characterized by mature, well-delineated, and well-differentiated paucibacillary granulomas, to the lepromatous form, characterized by ill-delineated, ill-differentiated multibacillary lesions, via several intermediate forms, designated as “borderline” [3]. Recently, the World Health Organization proposed to classify leprosy-affected people into 2 groups, paucibacillary and multibacillary.

The clinical expression of infection with M. leprae results from a complex interplay between genetic and nongenetic human factors and mycobacterial and nonmycobacterial environmental factors [4]. In particular, the existence of genetic control of susceptibility to leprosy has long been inferred from epidemiological studies and segregation analyses (reviewed in [5, 6]). Studies with genetic markers, mainly based on a candidate-gene approach, have provided other sources of evidence for a genetic component of leprosy (reviewed in [7, 8]). More recently, 2 genome scans have shown significant linkage of paucibacillary leprosy to chromosome 10p13 in an Indian population [9] and of leprosy per se to chromosome region 6q25 in a Vietnamese population [10]. This latter locus was subsequently identified as the regulatory region shared by PARK2 (juvenile Parkinson disease susceptibility gene) and PACRG (PARK2...
coregulated gene) [11]. This was the first identification by positional cloning of a major gene controlling susceptibility to a common infectious disease in humans.

The Mitsuda reaction is a skin granulomatous reaction that is measured 30 days after an intradermal injection of 0.1 mL of lepromin (heat-killed *M. leprae*). Results of the Mitsuda test are classically coded as a binary outcome—that is, as positive (≥3 mm) or negative (<3 mm). Positive Mitsuda test results reflect the formation of a nodular granuloma composed of epithelioid cells with giant multinucleated cells, associated with a lymphocytic infiltrate [4, 12]. The positivity of the test results usually coincides with the clearance of *M. leprae* by macrophages—that is, no more acid-fast bacilli are visible [13–15]. Whether they are asymptomatic or exhibit leprosy, not all infected individuals will have a positive Mitsuda test result; in particular, most lepromatous patients have negative test results [16, 17]. In endemic areas, 60%–96% of healthy adults have positive test results [4, 12, 18–20]. Mitsuda test results have rarely been studied as quantitative values, but 1 cross-sectional study of 3 different healthy populations in India showed that the distribution of results is Gaussian-like, with mean values ranging from 4.5 to 6 mm [20].

Several studies have provided evidence of a genetic component influencing the Mitsuda reaction as a binary trait (i.e., positive/negative). Familial correlation studies were first conducted in Brazil and demonstrated significant intrafamilial aggregations for Mitsuda test results [21]. Only 1 segregation analysis of the Mitsuda reaction has yet been published, conducted in 544 Brazilian nuclear families, with the Mitsuda reaction coded as a binary trait [22]. The authors found evidence of a codominant major gene, but its effect on the Mitsuda reaction was not clearly specified. More recently, we conducted a candidate-gene linkage study that considered the Mitsuda reaction as a quantitative trait in 20 Vietnamese families and found significant linkage to the *NRAMP1* region [23]. *NRAMP1* (or *SLC11A1*) is the human ortholog of a murine gene controlling susceptibility/resistance to different intracellular pathogens, including *M. lepraemurium* and *M. bovis* [24]. However, this linkage analysis based on a genetic model–free method could not specify the effect of *NRAMP1* on Mitsuda reactivity. Furthermore, it focused on a single chromosomal region and did not address the question of the global genetic control of the Mitsuda reaction.

To further investigate the genetic component of the Mitsuda reaction, we conducted an epidemiologic study in a larger Vietnamese population. Our objectives were to assess the influence of several measured factors on the Mitsuda reaction, to investigate the presence of familial correlations, and to perform a complex segregation analysis. The aim of segregation analysis is to discriminate between the different factors causing familial resemblance, with the main goal being to test for the existence of a major gene. The term “major gene” does not mean that it is the only gene involved in the control of the Mitsuda reaction but, rather, that there is at least 1 gene with an effect on the extent of Mitsuda reactivity sufficient to make it distinguishable from other genes with effects on the trait. In the present study, we show that the Mitsuda reaction is under the control of a major gene distinct from *NRAMP1*.

**SUBJECTS, MATERIALS, AND METHODS**

*Family study and subjects.* Patients with leprosy were identified from the records of the Dermatology Hospital in Ho Chi Minh City, Vietnam. At the time of enrollment (1990–1992), the annual incidence of leprosy in this area was ∼6/100,000 individuals. Patients with leprosy were randomly selected from the registry of this hospital. Nuclear families were ascertained through each of these index case patients (probands) and consisted of either the spouse and children or the parents and siblings of the proband, according to the proband parental status, as detailed elsewhere [5]. Access to the registry was approved by the Institutional Review Board of the Dermatology Hospital in Ho Chi Minh City.

The family members were visited by experienced leprologists.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Distribution of Mitsuda values in the whole population (*n* = 1118) (A) and in the healthy population (*n* = 870) (B).
Table 1. Factors influencing the quantitative Mitsuda reaction: results from univariate and multivariate linear regression.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Sample size</th>
<th>Mitsuda value, mm</th>
<th>P</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (SD) Median</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>537</td>
<td>5.89 (3.15) 5.5</td>
<td>.077</td>
<td>.004</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>581</td>
<td>6.24 (3.29) 6.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethnic group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vietnamese</td>
<td>738</td>
<td>5.72 (3.13) 5.0</td>
<td>&lt;10^-4</td>
<td></td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>Chinese</td>
<td>380</td>
<td>6.80 (3.31) 7.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leprosy status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>870</td>
<td>6.38 (2.93) 6.0</td>
<td>&lt;10^-5a</td>
<td></td>
<td>&lt;10^-5a</td>
</tr>
<tr>
<td>Nonlepromatous</td>
<td>159</td>
<td>6.47 (3.50) 6.0</td>
<td>.746b</td>
<td></td>
<td>.841b</td>
</tr>
<tr>
<td>Lepromatous</td>
<td>89</td>
<td>2.30 (3.06) 2.0</td>
<td>&lt;10^-5c</td>
<td></td>
<td>&lt;10^-5c</td>
</tr>
</tbody>
</table>

* Leprosy status as a whole.
* Nonlepromatous vs. healthy.
* Lepromatous vs. healthy.

Disease evaluation was based on clinical, bacteriological, and histological data. The clinical forms of leprosy were classified in accordance with the Ridley and Jopling classification [3] and then were grouped into 2 classes, as proposed elsewhere [5]: lepromatous (gathering polar lepromatous and borderline-lepromatous forms) and nonlepromatous (polar tuberculoid, borderline-tuberculoid, borderline-borderline, and undetermined forms). The Mitsuda test was performed on each individual by intradermal injection of 0.1 mL of lepromin A (armadillo-derived; provided by The National Hansen’s Disease Center, Carville, LA) into the volar surface of the forearm. The reaction was read 28–30 days later, and the diameter of induration in millimeters was measured by 2 independent leprologists.

**Statistical methods.** The phenotype of interest was quantitative Mitsuda reaction diameter (Mitsuda value), measured in millimeters. First, we investigated the effects of 4 covariates: sex, age, ethnic group (Chinese or Vietnamese), and leprosy status. Leprosy status was coded as a categorical variable with 3 classes: healthy (the reference class), lepromatous, and nonlepromatous. The analyses were performed with SAS software (version 8.2; SAS Institute), using parametric linear regression (GLM procedure) and nonparametric rank-sum tests (RANK procedure).

We then performed a segregation analysis, using the regressive model developed by Bonney [25], which specifies a regression relationship between each individual phenotype and 3 sets of explanatory variables, as detailed elsewhere [26, 27]: (1) the effect of measured covariates (e.g., sex), which is specified through regression coefficients denoted as $\beta$ (e.g., $\beta_x$); (2) the dependence on phenotypes of preceding relatives, which is parameterized in terms of familial correlations—that is, in the present study, father-mother ($\rho_{FM}$), father-offspring ($\rho_{FO}$), mother-offspring ($\rho_{MO}$), and sibling-sibling ($\rho_{SS}$) correlations; and (3) the effect of a major biallelic gene, defined by 5 parameters: $q$ (the frequency of allele A, assumed to predispose to high Mitsuda reactivity); $\mu_{AA}$, $\mu_{Aa}$, and $\mu_{aa}$ (the genotypic means of individuals with genotypes AA, Aa, and aa, respectively); and $\sigma^2$ (the common residual variance of the phenotype). Additional parameters (defined in table 2) were used to test for gene-covariate interactions and to ensure that the parent-offspring transmission of the major gene is Mendelian. Parameter estimation and hypothesis testing were performed using classic likelihood theory.

Families in the sample were ascertained through leprosy-affected probands, so the phenotype leading to ascertainment differed from our phenotype of interest. However, lepromatous forms are related to low Mitsuda values, which may introduce a selection bias. As a first approach, a “naive” analysis was conducted on the whole sample, without any corrections. Then, several strategies of correction were used to assess the robustness of the results to the mode of ascertainment. First, we performed an analysis in the healthy population only. Second, we introduced leprosy status among the explanatory covariates in the whole population. Third, we performed a stratified analysis separating families into 2 subsamples according to proband familial status—parent or child—as proposed elsewhere in a sim-
Family sample and phenotype distribution. A total of 168 nuclear families, consisting of either the spouse and children (80 families) or the parents and siblings (88 families) of the proband, were ascertained. Sibships were large (range, 1–12 children), with a median size of 5 children, leading to an overall sample size of 1118 individuals. One hundred four families were of Vietnamese origin (738 individuals; 66%) and 64 were of Chinese origin (380 individuals; 34%), which roughly reflects the proportion of these 2 ethnic groups in the registry of the Dermatology Hospital. The sex ratio (male/female) was 1.08, and the mean age was 32.7 years (range, 2–85 years). There were 870 healthy individuals (78%) and 248 individuals with leprosy (22%). Among affected individuals, 159 (64.1%) were nonlepromatous, and 89 (35.9%) were lepromatous.

The mean (median) value of the Mitsuda reaction was 6.06 (6.00) mm, with an SD of 3.23 mm. Compared with a normal distribution, the distribution of observed Mitsuda values in the whole population (figure 1A) presented 2 characteristics: (1) there was an excess of null values, largely imputable to the high proportion of lepromatous patients in this population, as a result of the type of ascertainment; and (2) the distribution was skewed to the right by an excess of high values (skewness, +0.21). When we focused on the healthy population (figure 1B), the excess of null values markedly decreased, as expected, and skewness increased (to +0.47).

Analysis of covariates. Results of parametric and non-parametric tests were very similar, and only the results of parametric analyses will be presented here (table 1). In the univariate analysis, there was a strong effect of leprosy status ($P < 10^{-3}$), mainly reflecting the lower Mitsuda reactivity among lepromatous patients (figure 2A). The mean Mitsuda value was 2.30 mm in lepromatous patients, versus 6.47 mm in nonlepromatous patients and 6.38 mm in healthy subjects. The difference between nonlepromatous patients and healthy subjects was not significant ($P > 0.746$). There was no significant effect of age, whatever the coding scheme (continuous variable or ordinal variable with 8 classes, as shown in figure 2B). The mean Mitsuda value was higher in male subjects (6.24 mm) than in female subjects (5.89 mm), although this difference was not significant ($P = 0.746$). A significant effect of ethnic group was observed ($P < 10^{-4}$), with the mean Mitsuda values being higher in the Chinese population (6.80 mm) than in the Vietnamese population (5.72 mm). Multivariate analysis confirmed the strong effects of leprosy status ($P < 10^{-3}$) and ethnic group ($P < 10^{-4}$). Moreover, the effect of sex became significant after adjustment for leprosy status ($P = 0.004$); this may be explained by there being a higher proportion of lepromatous patients among males (0.11) than among females (0.05).

Segregation analysis. As detailed above, we performed a “naive” analysis and different analyses to test the robustness to the mode of ascertainment. In all tested models, there were neither significant correlations between spouses ($\rho_{sp}$) nor significant differences between father-offspring ($\rho_{fo}$) and mother-offspring ($\rho_{mo}$) correlations. Thus, all results are presented fixing $\rho_{sp} = 0$ and $\rho_{fo} = \rho_{mo} = \rho_{po}$, where $\rho_{po}$ is the common parent-offspring correlation.

Results of the “naive” analysis are presented in table 2. There was evidence of strong familial correlations, since the sporadic model without familial correlations was highly rejected against the model that included parent-offspring correlation and sibling-sibling correlation (model I vs. IIc; $\chi^2 = 141$; 2 df; $P < 10^{-20}$).
Table 2. Segregation analysis of quantitative Mitsuda values in the whole population, accounting for sex and ethnic group.

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter</th>
<th>(\mu_{AA}^a)</th>
<th>(\mu_{Aa}^b)</th>
<th>(\mu_{aa}^a)</th>
<th>(Q_0^c)</th>
<th>(\tau_{AAA}^c)</th>
<th>(\tau_{Aa}^c)</th>
<th>(\tau_{aa}^c)</th>
<th>(\sigma^2d)</th>
<th>(\rho_{PO}^e)</th>
<th>(\rho_{SS}^e)</th>
<th>(\beta_{PO}^f)</th>
<th>(\beta_{SS}^f)</th>
<th>(\beta_{PO\times eth}^f)</th>
<th>(-2\ln L + C^g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Sporadic</td>
<td></td>
<td>6.59(p)</td>
<td>[6.59]</td>
<td>[6.59]</td>
<td>(0)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>10.38</td>
<td>(0)</td>
<td>(0)</td>
<td>-0.27</td>
<td>1.06</td>
<td>214.5</td>
<td></td>
</tr>
<tr>
<td>II. FC</td>
<td>a. PO</td>
<td>5.05</td>
<td>[5.05]</td>
<td>[5.05]</td>
<td>(0)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>9.91</td>
<td>0.11</td>
<td>(0)</td>
<td>-0.29</td>
<td>1.10</td>
<td>174.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b. SS</td>
<td>5.15</td>
<td>[5.15]</td>
<td>[5.15]</td>
<td>(0)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>10.58</td>
<td>[0]</td>
<td>0.37</td>
<td>-0.31</td>
<td>1.01</td>
<td>111.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c. PO and SS</td>
<td>5.15</td>
<td>[5.15]</td>
<td>[5.15]</td>
<td>(0)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>10.36</td>
<td>0.23</td>
<td>0.34</td>
<td>-0.32</td>
<td>1.01</td>
<td>73.5</td>
<td></td>
</tr>
<tr>
<td>III. MG without FC</td>
<td>Recessive</td>
<td>8.04</td>
<td>3.68</td>
<td>[3.68]</td>
<td>0.55</td>
<td>(1)</td>
<td>(0.5)</td>
<td>(0)</td>
<td>5.92</td>
<td>(0)</td>
<td>(0)</td>
<td>-0.31</td>
<td>1.06</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>IV. MG and FC</td>
<td>a. Dominant</td>
<td>9.49</td>
<td>[9.49]</td>
<td>4.42</td>
<td>0.11</td>
<td>(1)</td>
<td>(0.5)</td>
<td>(0)</td>
<td>6.91</td>
<td>0.16</td>
<td>0.37</td>
<td>-0.35</td>
<td>0.80</td>
<td>10.7</td>
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<tr>
<td></td>
<td>b. Codominant</td>
<td>10.59</td>
<td>5.52</td>
<td>5.52</td>
<td>0.34</td>
<td>(1)</td>
<td>(0.5)</td>
<td>(0)</td>
<td>7.33</td>
<td>0.29</td>
<td>0.43</td>
<td>-0.29</td>
<td>0.85</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c. Recessive</td>
<td>10.59</td>
<td>5.52</td>
<td>5.52</td>
<td>0.34</td>
<td>(1)</td>
<td>(0.5)</td>
<td>(0)</td>
<td>7.33</td>
<td>0.29</td>
<td>0.43</td>
<td>-0.29</td>
<td>0.85</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>d. Recessive + MG–ethnic group interaction</td>
<td>10.95</td>
<td>4.50</td>
<td>[4.50]</td>
<td>0.34</td>
<td>(1)</td>
<td>(0.5)</td>
<td>(0)</td>
<td>7.33</td>
<td>0.29</td>
<td>0.43</td>
<td>-0.29</td>
<td>0.85</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>V. Absence of transmission</td>
<td>10.72</td>
<td>4.82</td>
<td>[4.82]</td>
<td>0.27</td>
<td>0.77</td>
<td>[0.77]</td>
<td>[0.77]</td>
<td>8.85</td>
<td>0.31</td>
<td>0.43</td>
<td>-0.33</td>
<td>0.99</td>
<td>54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI. General transmission</td>
<td>10.64</td>
<td>4.59</td>
<td>[4.59]</td>
<td>0.34</td>
<td>1.00</td>
<td>0.45</td>
<td>0.07</td>
<td>7.27</td>
<td>0.30</td>
<td>0.44</td>
<td>-0.31</td>
<td>0.81</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Brackets indicate a parameter fixed to the same value as the preceding estimated parameter; parentheses indicate a parameter fixed before the analysis; ellipses denote a nonrelevant parameter in the model; FC, familial correlations; MG, major gene; PO, parent-offspring; SS, sibling-sibling.

\(^a\) Genotypic mean for genotype \(i\) \((\text{AA, Aa, or AA})\), adjusted for covariate effects.

\(^b\) Frequency of allele A, which predisposes to high Mitsuda values.

\(^c\) Probability of transmitting allele A for a parent \(i\) \((\text{AA, Aa, or AA})\). These parameters are used to test the hypothesis of Mendelian transmission \((\tau_{AAA} = 1, \tau_{Aa} = 0.5, \text{and} \tau_{aa} = 0)\) against alternative hypotheses, such as general transmission (free \(\tau\) values) and the absence of parent-offspring transmission (equal \(\tau\) values).

\(^d\) Residual variance of the phenotype.

\(^e\) Multivariate regression coefficient of covariate \(i\). Covariates are as follows: sex \((0, \text{male}; 1, \text{female})\) and ethnic group \(\text{"eth"}\) \((0, \text{Vietnamese}; 1, \text{Chinese})\).

\(^f\) Corresponding to twice the logarithm of the likelihood \((2\ln L)\) of the best-fitting model (model VI).

\(^g\) Parameter estimated in the model.

\(^h\) To test for gene-ethnicity interaction, the \(\beta_{PO\times eth}\) parameter is split into 3 parameters, depending on genotype \(\beta_{PO\times AA}, \beta_{PO\times Aa}, \text{and} \beta_{PO\times aa}\); the hypothesis of no interaction corresponds to \(\beta_{PO\times AA} = \beta_{PO\times Aa} = \beta_{PO\times aa}\).
Both the parent-offspring and the sibling-sibling correlations significantly improved the likelihood of the model. In the presence of those familial correlations, strong evidence of a major gene was observed (model IIC vs. IVB; $\chi^2 = 71; 3 \text{ df}; P < 10^{-7}$). When the model assumed the presence of a major gene, the codominant model always tended towards the recessive model IVC ($\mu_{aa}$ is fixed at its bound $\mu_{ar}$, and the codominant model is strictly equivalent to the recessive model). The dominant model was rejected (model IVa vs. IVB; $\chi^2 = 8.2; 1 \text{ df}; P = .005$). In the presence of a major effect, residual parent-offspring ($\rho_{PO}$ = 0.29) and sibling-sibling ($\rho_{SS}$ = 0.43) correlations remained significant (model III vs. IVC; $\chi^2 = 68.5; 2 \text{ df}; P < 10^{-7}$). No significant interaction between ethnic group and major gene was observed (model IVC vs. IVD; $\chi^2 = 0.1; 1 \text{ df}; P = .95$). Finally, the transmission of the recessive major effect was compatible with the Mendelian transmission (model IVC vs. VI; $\chi^2 = 2.5; 3 \text{ df}; P = .47$), whereas the hypothesis of no parent-offspring transmission was rejected (model V vs. VI; $\chi^2 = 54; 2 \text{ df}; P < 10^{-7}$). In conclusion, there was strong evidence of the presence of a recessive major gene controlling the Mitsuda reaction, and the estimated frequency of the recessive allele $A$—that is, the allele predisposing to high Mitsuda values—was 0.34. In the Vietnamese (Chinese) males, the genotypic means were 5.52 (6.37) mm for aa and Aa subjects and 10.59 (11.44) mm for AA subjects (11.6% of the individuals). Means for female subjects can be obtained by adding $\beta_{sex}$ (i.e., $-0.29$) to these values. The proportion of phenotypic variance explained by this genetic model, also denoted as heritability, was 0.29.

We then repeated the previous analysis, first studying the healthy population only and then including leprosy status as an explanatory covariate in the whole population. Following the previous steps, we reached similar conclusions in both analyses. In the presence of parent-offspring and sibling-sibling correlations, there was evidence of a recessive major gene ($P < 10^{-7}$ in both analyses). No gene-ethnicity interaction was observed, and strong residual familial correlations persisted. Mendelian transmission of the major effect was compatible with the data ($P = .5$ and $P = .3$, when the healthy population was studied and when leprosy status was included as a covariate in the study of the whole population, respectively), and the hypothesis of nontransmission was rejected against the general model of transmission ($P < 10^{-7}$ and $P < 10^{-7}$, respectively). In both analyses, the best-fitting model was a recessive major gene with residual parent-offspring and sibling-sibling correlations. Parameter estimates were close to those found in the “naive” analysis, in terms of allele frequency, genotypic means (table 3), and heritability (0.31 and 0.36, respectively). Then, we conducted a stratified analysis separating families into 2 subsamples with the proband either as a parent (80 families) or as a child (88 families). Again, the best-fitting model of segregation analysis was a recessive major gene with residual familial correlations, and parameter estimates were close to those obtained in the 2 previous analyses (table 3).


discussion

The present study is the first large familial survey of quantitative Mitsuda reactivity aimed at identifying the factors influencing the extent of the granulomatosus reaction. From an immunological point of view, the Mitsuda reaction reflects the host’s ability to develop an immune granuloma in response to $M. leprae$ antigens [4]. Of all skin tests used for diagnosis or research purposes in infectious diseases (e.g., tuberculin, histoplasm, toxoplasmin, and candidin), the Mitsuda test is the only one that induces the formation of a granuloma. Two characteristics of the Mitsuda test could explain this property: (1) the injected infectious stimulus is an entire microbial agent (not only microbial proteins), and (2) the reading is taken very late (28–30 days after injection), whereas the other skin tests

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**Table 3. Best-fitting models obtained by segregation analysis of quantitative Mitsuda values in (1) the whole population, without adjustment for leprosy status (model IVC in table 2), (2) the healthy population, (3) the whole population, adjusted for leprosy class, and (4) families stratified according to the type of proband (i.e., parent or child).**

<table>
<thead>
<tr>
<th>Population</th>
<th>Sample size</th>
<th>$\mu_{AA}$</th>
<th>$\mu_{Aa}$</th>
<th>$\mu_{aa}$</th>
<th>$\sigma^2$</th>
<th>$\rho_{PO}$</th>
<th>$\rho_{SS}$</th>
<th>$\beta_{sex}$</th>
<th>$\beta_{lep}$</th>
<th>$\beta_{sex}$</th>
<th>$\beta_{lep}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole population</td>
<td>1118</td>
<td>10.59</td>
<td>5.32</td>
<td>0.34</td>
<td>7.33</td>
<td>0.29</td>
<td>0.43</td>
<td>$-0.29$</td>
<td>0.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy population</td>
<td>870</td>
<td>10.18</td>
<td>5.13</td>
<td>0.37</td>
<td>5.60</td>
<td>0.37</td>
<td>0.56</td>
<td>$-0.73$</td>
<td>1.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole population, adjusted for leprosy status</td>
<td>1118</td>
<td>10.39</td>
<td>4.95</td>
<td>0.38</td>
<td>5.63</td>
<td>0.29</td>
<td>0.46</td>
<td>$-0.23$</td>
<td>$-4.40$</td>
<td>0.54</td>
<td>1.15</td>
</tr>
<tr>
<td>Families, stratified by type of proband</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parent</td>
<td>516</td>
<td>10.76</td>
<td>4.94</td>
<td>0.36</td>
<td>7.70</td>
<td>0.40</td>
<td>0.61</td>
<td>$-0.48$</td>
<td>0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child</td>
<td>602</td>
<td>10.58</td>
<td>4.50</td>
<td>0.31</td>
<td>7.77</td>
<td>0.23</td>
<td>0.34</td>
<td>$-0.19$</td>
<td>1.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Parameters are as defined in table 2, with 2 additional regression coefficients: $\beta_{sex}$ ("lep" is coded as follows: 0, healthy; 1, lepromatous) and $\beta_{sex}$ ("lep" is coded as follows: 0, healthy; 1, lepromatous).
are read 48–72 h after injection and explore the delayed hypersensitivity to a specific microbial antigen. Although the Mitsuda test has been developed to assess immune response to live M. leprae in natural conditions of infection, it has been established that other microbial agents influence the Mitsuda reaction, such as M. tuberculosis [30] and live bacille Calmette-Guérin (BCG) vaccine [21, 31]. Therefore, the Mitsuda test reflects the ability to develop an immune granuloma after mycobacterial infection but is not a marker for specific immune responses to the leprosy bacillus. This conclusion is supported by our present results, demonstrating genetic control of Mitsuda values independent of leprosy status.

Previous studies have shown that most infants (younger than 1 year of age) in Brazil present a negative Mitsuda test result [32], and that the proportion of positive test results rapidly increases until 5 years of age [4, 18, 33]. In our sample, however, we did not observe any effect of age on Mitsuda values. In particular, the mean Mitsuda values in the younger age class (2–9 years) were not different from those in older classes. Two facts may explain this discrepancy. First, our study included only 9 children younger than 5 years of age, which is not sufficient to reach definitive conclusions concerning young children. Second, most previous studies have been performed in noncontact (with respect to leprosy cases) and a priori non–BCG-vaccinated populations [32, 34]. By contrast, in our study, all children had been in contact with a patient with leprosy living in the same household, and BCG vaccination was widely performed in Vietnamese newborns, with an estimated coverage of 83% in Ho Chi Minh City from 1986 to 1991 [31].

Among the covariates we considered, leprosy status had the most significant effect on Mitsuda values, with much lower values observed in lepromatous patients. Indeed, from a clinical point of view, the main application of the Mitsuda test is the strong predictive value of negative test results for the development of a lepromatous form of leprosy in individuals infected with M. leprae [4, 18, 35]. Conversely, we found no difference in quantitative Mitsuda values between nonlepromatous patients and healthy subjects. This is consistent with the previous finding that the proportion of negative Mitsuda test results did not differ between tuberculoid patients and healthy individuals [4] and that a positive result did not have any protective effect for nonlepromatous leprosy [36]. So far, Mitsuda test outcomes have not been clearly associated with any other leprosy-related phenotype. It is noteworthy that the specific role of high Mitsuda values (>10 mm) has never been investigated. On the basis of our results, however, the relevance of this particular phenotype should be reconsidered.

The main result of our segregation analysis was the identification of a major gene influencing quantitative Mitsuda values as a recessive trait. The frequency of allele A, which predisposes to high Mitsuda values, was close to 0.35. Among Vietnamese subjects, the mean Mitsuda value was ~10 mm for AA homozygotes (12% of individuals) and 5 mm for other genotypes. The genetic model was similar in the Chinese population, with an overall increase of 1 mm in genotypic means. This difference was independent of the major-gene effect, since there was no significant interaction between major gene and ethnic group. The proportion of variance explained by the gene (heritability) was ~30%, indicating a substantial effect on Mitsuda values. Finally, we used several strategies of analysis to demonstrate that both the evidence of a major gene and the characteristics of the genetic model were independent of family ascertainment through a proband with leprosy. The consistent results obtained provide strong evidence that the genetic control of the Mitsuda reaction is independent of the one controlling leprosy clinical status.

To assess whether the major gene detected in the complex segregation analysis corresponds to NRAMP1, we performed a model-based linkage study of quantitative Mitsuda reactivity and the NRAMP1 region, using the same 20 families and haplotypic data as in a previous study [23]. We employed the classic LOD-score method [37], using the genetic model estimated by our segregation analysis. In this approach, the parameter of interest is the recombination rate (θ) between the major gene and the NRAMP1 haplotype. Under the hypothesis of no linkage, θ = 0.5, whereas the recombination rate is 0 ≤ θ ≤ 0.5 when the 2 loci are linked. The hypothesis of the major gene being confounded with NRAMP1 (i.e., θ = 0) was strongly rejected (LOD score, −4.04; P < 10^−4). However, when θ was estimated, there was some evidence of linkage between NRAMP1 and the major gene (θ = 0.24; LOD score, 0.79; P = .03). These observations are consistent with actual linkage of the Mitsuda reaction to NRAMP1, under the assumption of a genetic effect of NRAMP1 different from that of the present major gene—that is, the bias in the estimation of θ is due to a misspecification of the genetic model, as explained elsewhere [38]. In conclusion, although NRAMP1 does not correspond to the major gene we identified, it should play a role in the genetic control of the Mitsuda reaction and probably contributes to the strong residual familial correlations observed in our genetic model.

After having shown by segregation analysis in 1988 that leprosy is controlled by a major gene [6], we subsequently mapped the major locus [10] and identified the genetic variants [11]. Similarly, the present study paves the way for the identification of the Mitsuda reaction major gene. Indeed, taking advantage of the genetic model identified in the present study, a model-based genomewide scan to map this gene is ongoing. The identification of a major gene controlling the Mitsuda reaction will illuminate the immune responses to M. leprae by casting novel light on the molecular mechanisms of granuloma formation.
Acknowledgments

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