ASSOCIATION OF NRAMP1 POLYMORPHISM WITH LEPROSY TYPE BUT NOT SUSCEPTIBILITY TO LEPROSY PER SE IN WEST AFRICANS

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Abstract. Twin and family studies indicate that host genetic factors influence susceptibility to leprosy and, possibly, leprosy type. Murine studies have suggested a role for the natural resistance–associated macrophage protein 1 (Nramp1) gene, which can influence cellular immune responses to intracellular pathogens. We evaluated a variation in the human homolog, NRAMP1, recently associated with tuberculosis susceptibility in West Africa. A total of 273 patients with leprosy and 201 controls from Mali were genotyped for NRAMP1 polymorphisms previously associated with tuberculosis. No association was found with leprosy per se ($P = 0.83$), but the NRAMP1 3′-untranslated region 4-bp insertion/deletion polymorphism was associated with leprosy type ($P = 0.007$). Heterozygotes were more frequent among multibacillary than paucibacillary leprosy cases. Thus, variation in or near the NRAMP1 gene may exert an influence on the clinical presentation of leprosy, possibly by influencing cellular immune response type.

INTRODUCTION

Several types of study support a role for host genetics in susceptibility to leprosy. Significantly higher concordance rates for leprosy in monozygotic than dizygotic twins were found in an Indian population. In a New Guinean highland population where children live apart from their parents, it was possible to distinguish and document genetic and environmental contributions to leprosy familial clustering. Most complex segregation analyses of leprosy pedigrees have supported a genetic contribution to susceptibility and suggested the presence of major gene loci.

The relevance of particular non-HLA genes has been less clear. Although recent studies have demonstrated associations between variants of the tumor necrosis factor and vitamin D receptor gene and leprosy susceptibility in Indian populations, neither of these loci was associated with susceptibility to leprosy per se. A well-studied locus in the mouse, originally termed Lsh and Ity and later termed Bcg, encodes the murine Nramp1 gene, which has been found to influence susceptibility to several intracellular infections in mice, including Mycobacterium lepraemurium. Susceptible mice are homozygous for a nonconservative glycine to aspartic acid substitution in the fourth transmembrane domain of Nramp1. Several polymorphisms have now been identified in the human homolog NRAMP1, but the functional significance of these is uncertain.

In a family linkage study of NRAMP1 in 20 Vietnamese families, Abel and others found evidence that this gene may be linked to leprosy susceptibility and suggested a causative role for this locus. However, no evidence of association with leprosy was found. In a much larger study of tuberculosis in The Gambia, West Africa, we recently identified a clear association between variants of the NRAMP1 gene and susceptibility to this mycobacterial disease. Here we searched for a possible association between these variants and both leprosy per se and leprosy type in another West African population.

MATERIALS AND METHODS

Case-patients and controls were recruited at the Institut Marchoux, Bamako, Mali, the national leprology center, after receiving local ethical approval. Leprosy is an infectious disease with a broad clinical spectrum, ranging from the paucibacillary tuberculoid form, in which a TH1-type response predominates, to the multibacillary lepromatous form, which is associated with a TH2-type immune response. Cases of all clinical types were recruited from among outpatients, inpatients, and former patients of the leprology unit from April to June 1997. Leprosy or its complications were the main medical problems in these patients. Patients were classified as having paucibacillary or multibacillary leprosy on the basis of the presence of typical skin lesions accompanied by anesthesia, thickening of the nerves, and a positive split skin smear, biopsy results, or both. Controls were people without leprosy, including staff members and nonleprosy patients attending the general medical or infectious diseases outpatient clinic.

We extracted DNA from venous blood samples by means of Nucleon kits (Scotlab, Glasgow, Scotland). Details of age, sex, ethnic group, and place of residency were obtained by questioning the patients at the time of recruitment. The NRAMP1 polymorphisms typed were 1) the 3′-untranslated region (3′-UTR), also denoted 1729-55del4, a TGTG deletion in the 3′-UTR; 2) a single nucleotide transversion in intron 4 (469+14G/C); and 3) a (CA)n microsatellite in the 5′ region of the gene. These were genotyped exactly as previously described.

Statistical analysis was conducted in a stepwise manner. Initially, the overall significance levels for a variant was assessed in a 3 × 2 chi-square test, and if the significance level was < 0.05, further analysis of particular genotypes was undertaken by 2 × 2 chi-square tests. Allowance was made for age, sex, ethnic group, and region of residence differences by logistic regression and the software package SPSS version 7.0 (SPSS Inc., Chicago, IL).

RESULTS

A total of 273 patients with leprosy and 201 controls from Mali were genotyped. Among the patients with leprosy, 70%...
were men (71% of the multibacillary cases and 67% of the paucibacillary group), with a mean age of 45 years. By use of the Ridley-Jopling classification, 47% of the patients with leprosy were lepromatous, 19% borderline lepromatous, 17% tuberculoid, and 17% borderline tuberculoid.17 The latter 2 groups are combined as paucibacillary and the former 2 as multibacillary.16 The controls had a mean age of 33 years, and 37% were men. In view of the findings in the Gambian tuberculosis study,15 we genotyped 3 particular NRAMP1 polymorphisms in this case-control study: a microsatellite in the promoter of the gene with 4 alleles, a single base pair polymorphism in the 3'-untranslated region of the gene (3'-UTR). There was no significant association between the frequencies of any of these polymorphisms and ethnic group (Table 1), area of residence, or sex (data not shown). In particular, as shown in Table 1, the frequency of the 3'-UTR alleles did not vary significantly between different ethnic groups.

In the Gambian tuberculosis study, the strongest allelic association was found with the 3'-UTR 4-bp insertion/deletion polymorphism.15 The presence or absence of this TGTG sequence was determined by allele-specific nucleotide hybridization after polymerase chain reaction amplification. The control genotypes were in Hardy-Weinberg equilibrium. No significant association was found between leprosy per se and this 3'-UTR genotype (Table 2). But multibacillary leprosy genotypes differed significantly from paucibacillary genotypes (3 chi-square = 8.83, P = 0.003). There were more heterozygotes among the multibacillary cases and more deletion allele homozygotes among the paucibacillary cases (2 chi-square = 8.88, P = 0.003, odds ratio = 5.79, 95% confidence interval, 1.46–24.61). TGTG and del refer to the 2 NRAMP1 3'-UTR alleles.

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>Allele frequency of 3'-UTR</th>
<th>Multibacillary leprosy, n (%)</th>
<th>Paucibacillary leprosy, n (%)</th>
<th>Controls, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bambara</td>
<td>0.75</td>
<td>79 (44)</td>
<td>39 (42)</td>
<td>71 (35)</td>
</tr>
<tr>
<td>Malinke</td>
<td>0.79</td>
<td>16 (9)</td>
<td>11 (12)</td>
<td>37 (18)</td>
</tr>
<tr>
<td>Peuhl/Fula</td>
<td>0.73</td>
<td>36 (20)</td>
<td>25 (27)</td>
<td>35 (17)</td>
</tr>
<tr>
<td>Sarakole</td>
<td>0.84</td>
<td>18 (10)</td>
<td>7 (8)</td>
<td>24 (12)</td>
</tr>
<tr>
<td>Others</td>
<td>0.78</td>
<td>32 (18)</td>
<td>10 (11)</td>
<td>34 (17)</td>
</tr>
</tbody>
</table>

* UTR = untranslated region. There was no significant difference between the TGTG allele frequencies in the various ethnic groups (chi-square < 0.1, P = 0.97). The number and percentage of subjects of each ethnic group among the 2 types of leprosy and controls is shown. Several ethnic groups with small numbers of subjects are grouped as “other.”

**Table 1** Ethnic group distribution of the NRAMP1 3'-UTR TGTG allele among the 474 Malians studied according to ethnic group.

**Table 2** NRAMP1 3'-UTR genotypes in multibacillary and paucibacillary leprosy.

<table>
<thead>
<tr>
<th>NRAMP1 3'-UTR genotype</th>
<th>Multibacillary leprosy, n (%)</th>
<th>Paucibacillary leprosy, n (%)</th>
<th>All leprosy, n (%)</th>
<th>Controls, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGTG/TGTG</td>
<td>105 (58.0)</td>
<td>55 (59.8)</td>
<td>160 (58.6)</td>
<td>118 (58.7)</td>
</tr>
<tr>
<td>TGTG/del</td>
<td>72 (39.8)</td>
<td>28 (30.4)</td>
<td>100 (36.6)</td>
<td>71 (35.3)</td>
</tr>
<tr>
<td>Del/del</td>
<td>4 (2.2)</td>
<td>9 (9.8)</td>
<td>13 (4.8)</td>
<td>12 (6.0)</td>
</tr>
<tr>
<td>Total</td>
<td>181</td>
<td>92</td>
<td>273</td>
<td>201</td>
</tr>
</tbody>
</table>

* UTR = untranslated region. There was no significant difference between the leprosy and control genotypes (3 chi-square = 0.38, P = 0.83). But multibacillary leprosy genotypes differ from paucibacillary cases (3 chi-square = 8.83, P = 0.003). The association of NRAMP1 genotype with leprosy type showed a similar level of statistical significance using logistic regression analysis that allowed for ethnic group, sex, and area of residence (P = 0.002). Heterozygotes for the del allele were more common than homozygotes in the multibacillary compared with the paucibacillary cases (2 chi-square = 8.88, P = 0.003; odds ratio = 5.79, 95% confidence interval, 1.46–24.61). TGTG and del refer to the 2 NRAMP1 3'-UTR alleles.

**DISCUSSION**

On the basis of the strong influence of variation in the Nramp1 gene on susceptibility to BCG infection in inbred strains of mice, there has been considerable interest in the relevance of the human homolog NRAMP1 in susceptibility to human mycobacterial diseases.14 The largest reported study of tuberculosis found a clear association of heterozygosity for variants at both ends of the human NRAMP1 gene and with increased susceptibility to sputum-positive pulmonary disease.15 However, the magnitude of this effect is much smaller than that observed in mice.

Information on the role of NRAMP1 in susceptibility to leprosy has been limited. Family studies in Polynesia and Pakistan found no evidence for linkage of NRAMP1 to leprosy,19,20 but a more recent study of Vietnamese families found weak evidence for linkage.14 The small size of these studies was only sufficient to detect linkage to a major gene accounting for a substantial proportion of the genetic variance. However, it is perhaps unlikely that NRAMP1 variation has a major gene effect in any human disease comparable to that observed for particular infectious challenges in mice.21 More subtle effects may be observed in association rather than linkage studies.22 This study provides the first evidence that polymorphisms in NRAMP1 may be associated with clinical manifestations of leprosy. A modest but significant effect is observed for leprosy type in Mali, but not for susceptibility to leprosy per se.

The NRAMP1 genotype association for multibacillary leprosy is similar to that for sputum-positive tuberculosis in that heterozygotes but not homozygotes for the 3'-UTR variant appear susceptible.15 This contrasts with the findings in mice, where only homozygotes for the causative single amino acid mutation in Nramp1 are susceptible.12 It is possible that this is indeed a heterozygote effect in humans. Recent studies provide evidence that NRAMP1 is localized in the phagosomal membrane of macrophages, where it may act as an iron transporter and modulate intraphagosomal pH.23–25 Thus, we speculate that a heterozygote effect could relate to an optimum level of transporter activity or intraphagosomal ionic concentration for mycobacterial growth associated with this genotype. However, as the functionality of variation in the human NRAMP1 gene remains to be determined, such interpretations must await determination of the genotypic relationship of the functional susceptibility variants.
The finding of an NRAMP1 association with leprosy type is of particular interest in relation to the possible role of this gene in modulating the type of cellular immune response made to intracellular pathogens. It is clear that congenic mice differing at the Nramp1 locus display significant differences in their levels of macrophage activation and type of cellular immune response made after infection or immunization. For example, recently, Soo and others found that mice with the Nramp1 resistance allele mounted a predominantly TH1-type nonprotective cellular immune response after recombinant Salmonella immunization, whereas mice with the Nramp1 susceptibility allele generated a predominantly TH2-type nonprotective cellular immune response. The mechanism by which the Nramp1 molecule localized in the phagolysosomal membrane may modulate this immune response is unclear. Nonetheless, the parallel with the present results on NRAMP1 and leprosy type in humans is intriguing. Lepromatous leprosy has been reported to be associated with a predominantly TH2-type cellular immune response, and tuberculoid leprosy is associated with TH1-type immune responses. Thus, these data suggest the NRAMP1 may be one of the genes that can influence the type of cellular immune response made to foreign antigens in humans. Further analysis of NRAMP1 variation in other human autoimmune and infectious disease should be of interest.

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REFERENCES