Linkage of mild malaria to the major histocompatibility complex in families living in Burkina Faso

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Tumor necrosis factor alpha (TNFα) is thought to be a critical mediator of malaria fever, and mild malaria was previously reported to be linked to the MHC region containing the tumor necrosis factor alpha gene (TNF). Thirty-four families from Burkina Faso were analyzed to test for linkage between polymorphisms within the MHC region and mild malaria using the maximum-likelihood-binomial (MLB) program. Two-point analysis indicated linkage of mild malaria to TNFd (LOD = 3.27; P = 5.44 × 10⁻⁵). Using multipoint analysis, we also found evidence for linkage of mild malaria to the MHC region, with a peak close to TNF (LOD = 3.86; P = 1.22 × 10⁻⁵). Our results support genes within the MHC region being involved in mild malaria. In particular, the genetic variation within TNF may influence susceptibility to mild malaria. Nevertheless, TNF-238, TNF-244 and TNF-308 polymorphisms are unlikely to explain linkage of mild malaria to the MHC region, and the causal mutations remain to be identified.

INTRODUCTION

A growing body of evidence suggests that the susceptibility to human malaria is influenced by genetic factors. Genetic factors have been shown to control mild malaria (1), blood infection levels (2–5) (MIM248310, Online Mendelian Inheritance in Man; www.ncbi.nlm.nih.gov/Omim), and to regulate anti-malarial immune responses (6,7). Case–control studies have detected associations between severe malaria and several candidate genes, including genes within the MHC. The class I antigen, HLA-B53 (MIM142830), and the class II haplotype, DRB1*1302-DQB1*0501 (MIM142857), were associated with resistance to severe malaria (8). Furthermore, high serum levels of tumor necrosis factor alpha (TNFα) and polymorphisms in the TNFα gene (MIM191160) have been associated with increased susceptibility to severe malaria (9–11). The importance of chromosome 6p21–p22 region, in the control of the disease was further supported by a linkage study conducted on Gambian twins: the genetic analysis of sibling pairs concordant for mild malaria showed evidence of linkage at the TNF locus (12). This result was consistent with clinical observations, indicating that TNFα is a critical mediator of malaria fever (13,14). We present here a strong linkage between the MHC region and mild malaria in an urban population living in an endemic area in Burkina Faso.

RESULTS

Two-point maximum likelihood binomial-LODs (MLB-LOD) for D6S276, TNFb and TNFd were 0.82, 0.71 and 3.27, respectively (Table 1). The other markers of the region showed no evidence of linkage (P > 0.05; Table 1). It should be stressed that heterozygosity and genetic information content were less than 16% for TNF-238, TNF-244 and TNF-308. The allele frequencies were 0.978 and 0.022 for TNF-238G and TNF-238A, 0.956 and 0.044 for TNF-244G and TNF-244A, and 0.917 and 0.083 for TNF-308G and TNF-308A.

Nevertheless, the information content of the inheritance pattern at each point of the 6p21–p22 region, computed with multipoint analysis, ranged from 80 to 97%. Multipoint linkage analysis in the whole region (Fig. 1) yielded an MLB-LOD of 3.86 (ZMLB = 4.22; P = 1.22 × 10⁻⁵), with a peak very close to the TNF markers. A MLB-LOD of 3.0 (ZMLB = 3.71; P = 0.0001) was achieved in the interval between D6S276 and the TNF markers.

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Table 1. Results of the two-point linkage analysis of mild malaria.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Alleles number</th>
<th>Heterozygosity</th>
<th>MLB-LOD</th>
<th>Z_{MLB}</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D68276</td>
<td>12</td>
<td>0.82</td>
<td>0.82</td>
<td>1.94</td>
<td>0.026</td>
</tr>
<tr>
<td>TNFb</td>
<td>6</td>
<td>0.69</td>
<td>0.71</td>
<td>1.81</td>
<td>0.035</td>
</tr>
<tr>
<td>TNF-308</td>
<td>2</td>
<td>0.15</td>
<td>0</td>
<td>0</td>
<td>0.05</td>
</tr>
<tr>
<td>TNF-244</td>
<td>2</td>
<td>0.08</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>TNF-238</td>
<td>2</td>
<td>0.04</td>
<td>0.50</td>
<td>1.51</td>
<td>0.06</td>
</tr>
<tr>
<td>TFNd</td>
<td>8</td>
<td>0.63</td>
<td>3.27</td>
<td>3.87</td>
<td>5.44 × 10^{-5}</td>
</tr>
<tr>
<td>D68291</td>
<td>11</td>
<td>0.87</td>
<td>0.17</td>
<td>0.89</td>
<td>0.19</td>
</tr>
</tbody>
</table>

DISCUSSION

We obtained strong evidence of linkage between mild malaria and the MHC region by using two-point and multipoint analyses, with a peak close to the TNF¢ gene. Our findings strengthen a previous two-point linkage analysis, which showed a linkage at the 0.001 significance level: twins concordant for mild malaria had an excess allele sharing at the TNF¢ locus (12). Since changes in blood TNF¢ levels closely precede the peak of fever (13), and since anti-TNF¢ therapy inhibits fever in children with cerebral malaria, TNF¢ is thought to be a critical factor in malaria fever (14). Our data, taken together with the clinical studies, suggest the existence of a causal mutation(s) within the TNF¢ gene. Nevertheless, the TNF-238 and TNF-308 alleles that are associated with severe malaria (11,15) are very low in the study population, and unlikely influence the risk of malaria attack in the population. This observation is consistent with the absence of association, in other studies, between the TNF-238 and TNF-308 polymorphisms and mild malaria (16,17). This raises the possibility that different mutations within the TNF¢ gene may cause mild malaria and severe malaria. In the same way, different TNF promoter alleles have been shown to be associated with severe malarial anemia and cerebral malaria (18).

Other genes in the same chromosomal region may also be involved in malaria pathogenesis. In particular, the tumor necrosis factor β gene, which is very close to TNF, presents DNA sequence homology with TNF. TNFβ cytokine is produced only by lymphocytes, and TNF¢ is produced by macrophages, NK cells, neutrophils, mast cells and lymphocytes. Both cytokines recognize the same cell surface receptors, and have numerous similar effects (19). Nevertheless, the role of TNFβ in human malarial disease remains to be studied (20). The HLA-B and HLA-DR loci were previously associated with severe malaria (8). They may also be involved in mild malaria, yet case-control studies failed to detect a clear-cut association (21).

Our data support that genes within the MHC region influence the outcome of malarial infection. In particular, TNF is probably involved in susceptibility to severe and mild malaria. However, it seems unlikely that TNF-238 and TNF-308 alleles associated with resistance to severe malaria strongly influence mild malaria. The question of whether the same alleles cause mild and severe forms of the disease remains an issue to be addressed.

MATERIALS AND METHODS

Subjects

We studied 34 families that included 197 individuals. The families had the following distribution of sibship sizes: 3, 12, 8, 2 and 1 sibships contained 2, 3, 4, 5, 6 and 7 sibs, respectively. The mean age of sibs was 12.1 ± 6.2 years (range 1–34 years). The study population and the area of parasite exposure have been described (4,5). The seasonal parasite transmission intensity was homogeneous within the area. The whole population volunteered to participate in the study, and all participants were clearly informed of the objective and the protocol. The Medical Authority of Burkina Faso approved the study protocol.

Clinical data and phenotype determination

Active case detection of febrile episodes was done once a week during the season of malaria transmission. All subjects filled out a questionnaire about symptoms that occurred during the week. The axillary temperature was recorded for every subject who complained of illness during the visit or the previous days. For patients with fever, a thick blood film was prepared following the standard procedures. Parasite determination and numerator were immediately established as described previously (22). A diagnosis of malaria attack was based on P. falciparum parasitemia and clinical symptoms, fever (axillary temperature more than 37.5°C) and the classical symptoms (headache, aching, vomiting or diarrhea in the children); in that case no threshold of parasitemia was used. In the absence of classical symptoms of malaria, and where other pathologies could not be eliminated, only children with more than 5000 infected red blood cells per µl and adults with more than 2000 infected red blood cells per µl were considered as having had a malaria attack. The successive malaria attacks separated by less than 3 weeks are not considered new ones. According to the recommendation of the CNLP (Centre National de Lutte Contre le Paludisme) of Burkina Faso, each episode of illness was treated with 25 mg/kg chloroquine during 3 days or until recovery. Parasitemia was checked at the end of the treatment.

Fifty-nine of the 197 family members (55 sibs and four parents) presented at least one uncomplicated malaria attack during the survey. They were considered in the analysis as affected individuals. Sibs from five families were unaffected. Eleven, 12, 5 and 1 families contained 1, 2, 3 and 5 affected sibs, respectively.

To take into account the influence of known covariates on the phenotype, we performed logistic regression using the SPSS software (SPSS, Boulogne, France). Since age and hemoglobin genotype are known to influence the development of malaria attacks (23–25), we adjusted the logit of the probability P of malaria attack for age and hemoglobin genotype (25). The residual z of the logistic regression model was used in linkage analyses. All the sibs were included in linkage analyses.

Molecular methods

DNA microsatellite analysis was performed according to Vignal et al. (26) with DNA extracted from mononuclear cells separated by Ficoll–Hypaque density gradient. The DNA from the M134702 cell line was used as reference. The polymerase chain
reaction primers (GENSET) consisted of four highly polymorphic markers of the 6p21–p22 region (D6S276, TNFd, TNFb and D6S291; Genethon; www.genethon.fr) and of TNF promoter region (11,15,27,28). The TNFd and TNFb markers are, respectively, 9.7 and 7.2 kb distal to TNF. The biallelic typing of TNF was performed using the single-strand conformational polymorphisms method. A fragment of 139 bp, which includes a single base polymorphism at /C0 238 and /C0 244, and a fragment of 107 bp, which includes a single base polymorphism at −308, were amplified from DNA samples by PCR. The following oligonucleotides used as primers were chosen by means of the Primer program (Human Genome Mapping Project; http://menu.hgmp.mrc.ac.uk): 5’ ACACACAAATCAGTCAGTCCG 3’ and 5’ TCTCAGTTTCTCTCCATCG 3’ for TNF-238 and TNF-244 and 5’ AGGCATAGGTTTTGAGGGGCA 3’ and 5’ TCTCAGTTTCTCTCCATCG 3’ for TNF-308. The PCR products were separated by polyacrylamide gel electrophoresis (GENEPHOR, Amersham Pharmacia). The presence of the expected polymorphisms was checked by sequencing with the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Qiagen) and an ABI 310 automated fluorescent sequencer (PE Biosystems).

Statistical analyses

Nonparametric two-point and multipoint linkage analyses were performed with the MLBGH 2.0 program (29), which uses the general framework of Genehunter program (30). The maximum likelihood binomial (MLB) method, which is based on the binomial distribution of parental marker alleles among affected offspring, overcomes the common problem of multiple sibs by considering the sibship as a whole. The linkage analysis was based on marker allele frequencies from the study sample. The marker map position was based on a previously reported map (28,31), and on the available sequence (Human Genome Working Draft; http://genome.ucsc.edu for human DNA sequences). We used the MLB method in model-free linkage analysis of quantitative traits to assess linkage of the residual z to chromosome 6p21–p22. The quantitative trait method introduces a latent binary variable (0;1) that captures the linkage information between the observed quantitative trait and the marker. We used the fully non-parametric approach with no assumption on the distribution of the residual z. We divided the residual values into 10 consecutive equal subintervals with equal probabilities. The method is expected to specify the probability of the latent variable value according to the observed quantitative trait. We fixed the probability to have a 0 value at 0.95, 0.85, 0.75, 0.65, 0.55, 0.45, 0.35, 0.25, 0.15 and 0.05 for phenotypes belonging to the consecutive deciles (1–10). The MLB statistics was expressed in terms of a MLB-LOD and a one-sided standard normal deviate, denoted Z_{MLB}.

Figure 1. Results of the multipoint linkage analysis of mild malaria. The horizontal axis corresponds to the partial genetic map of chromosome 6p21–p22. Genetic distances between markers are expressed in centiMorgans. The vertical axis indicates the MLB-LOD scores obtained with the multipoint analysis at each point in the studied interval. The position of the markers relative to the MHC class I and class II regions is stated.
REFERENCES