

Original Article

Polymorphisms of the *HLA-B* and *HLA-DRB1* Genes in Thai Malaria Patients

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SUMMARY: The high degree of polymorphism of human leukocyte antigen (*HLA*) genes has been suggested to result from natural selection against susceptibility to a variety of infectious pathogens, including malaria. *HLA* molecules are considered to play a crucial role in the defense of the host against malarial infection, and different *HLA* class I and class II alleles have been reported to be associated with reduced susceptibility to malaria or severity of malaria in different populations. To test for associations between *HLA* alleles and severity of malaria in a Thai population, polymorphisms of *HLA-B* and *HLA-DRB1* genes were investigated in 472 adult patients in northwest Thailand with *Plasmodium falciparum* malaria. In this study, malaria patients were classified into three groups: mild malaria, non-cerebral severe malaria, and cerebral malaria. Our results revealed that the allele frequencies of *HLA-B46*, *-B56*, and *-DRB1*1001* were statistically different between non-cerebral severe malaria and cerebral malaria ($P = 0.005$), between mild malaria and cerebral malaria ($P = 0.032$), and between mild malaria and non-cerebral malaria ($P = 0.007$). However, our results may be showing false positives due to multiple testing. Thus, further study with a larger sample size must be conducted to obtain conclusive evidence of the association of these *HLA-B* and *DRB1* alleles with severity of malaria in Thailand.

INTRODUCTION

Malaria infection caused by *Plasmodium falciparum* is one of several common fatal diseases in humans. It remains a major cause of morbidity and mortality in tropical countries, affecting 300 million people and causing more than 2 million deaths annually. Malaria infection is variable; not all infected patients have severe or cerebral symptoms. The reasons for this variability have not yet been well documented, but variability between individuals' genetic background has long been proposed as a possible cause. Human leukocyte antigen (*HLA*) genes have been prominent candidates for investigation, as they have a remarkable degree of genetic polymorphism. A large number of alleles have been identified at *HLA* class I and II loci: 490 *HLA-B* (class I) and 315 *HLA-DRB1* (class II) alleles were reported in 2002 (1). Because the primary role of *HLA* molecules is to present peptides derived from infectious pathogens to T cells in the immune response against infection, the high degree of polymorphism of the *HLA* genes may have been attained and maintained through natural selection imposed by infectious organisms (2,3).

In the case of malaria, the first convincing association study was carried out in West Africans, and the frequent *HLA-Bw53* allele and the special *DRB1*1302-DQB1*0501* haplotype were found to be independently associated with reduced susceptibility to severe malaria (4). Furthermore, *HLA-Bw53*-restricted cytotoxic T lymphocytes are reported to recognize a conserved epitope in *P. falciparum* liver-stage antigen type

I (5). The evidence clearly demonstrates the advantage of these alleles, which are common in the sub-Saharan region, in fighting against fatal malaria that is highly endemic. However, different ethnic groups and populations have different allelic distributions, and epidemiological studies have found different alleles to be associated with malaria or with the severity of malaria in different populations (6-8). Therefore, research on the associations between *HLA* alleles and malaria in other diverse regions remains necessary. In the present study we examined whether *HLA* alleles in a Thai population confer either protection against or susceptibility to severe malaria. This study investigated the *HLA-B* and *-DRB1* alleles in mild and severe malaria patients living in northwest Thailand.

MATERIALS AND METHODS

Study sample: Included in this study were 202 mild malaria, 161 non-cerebral severe malaria, and 109 cerebral malaria patients living in northwest Thailand near the border with Myanmar. All patients underwent treatment at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University. For all patients, malarial infection by *P. falciparum* was confirmed by a blood smear positive for the asexual form of *P. falciparum*. The clinical manifestations of severe and mild malaria have been described elsewhere (9,10), but note that patients with cerebral malaria were not included in the severe malaria class in the present study. Thus, patients were classified into three malaria groups: mild malaria, non-cerebral severe malaria, and cerebral malaria. All individuals were 13 years old or older, and the mean ages of patients with mild malaria, non-cerebral severe malaria, and cerebral malaria were 25.5, 23.8, and 28.6 years, respectively. This study was approved by the institute review board of the Faculty of Tropical Medicine, Mahidol University, and

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informed consent was obtained from all patients.

Genomic DNA: Genomic DNAs from all patients were purified from peripheral blood leukocytes using a commercially available kit (QIAmp blood kit; Qiagen, Hilden, Germany).

Allele typing of *HLA-B* and *-DRB1* alleles: Typing for the *HLA-DRB1* and *-B* genes was performed using the PCR-microtiter plate hybridization (MPH) method as previously described (11).

Statistical analysis: Allele frequencies were compared between pairs of malaria patient groups, using a chi-square test based on a 2×2 contingency table. In brief, when allele *A* was tested, all other alleles were regarded as non-*A* alleles for the 2×2 contingency table. Frequencies of haplotypes were estimated using the maximum likelihood method based on an EM algorithm (12), using ARLEQUIN software (13).

RESULTS

Table 1 shows *HLA-B* allele frequencies in patients with mild malaria, non-cerebral severe malaria, and cerebral malaria. In the population studied, 19 *HLA-B* alleles showed allele frequencies of more than 1%, and the most common allele was *HLA-B15* (allele frequency 20.5%). It should be noted that each type of *HLA-B* consists of several alleles that can be distinguished at the sequence level, but that were not distinguished by the hybridization assay used in this study. Therefore, the exact number of alleles in the studied population is almost certainly greater than 19. At the *HLA-B* locus, the allele frequencies of *HLA-B46* and *B56* were significantly different between the non-cerebral severe malaria and cerebral malaria groups ($P = 0.005$), and between the mild malaria and cerebral malaria groups ($P = 0.032$).

Table 2 shows *HLA-DRB1* allele frequencies in patients with mild malaria, non-cerebral severe malaria, and cerebral malaria. In the studied population, 18 *HLA-DRB1* alleles were found to have frequencies of more than 1%, and four alleles, *DRB1*0701*, *DRB1*1202*, *DRB1*1501*, and *DRB1*1502*, had population frequencies of more than 10%. Additionally, the *DRB1*1001* allele showed a difference in allele frequency between mild and non-cerebral severe malaria patients at a significance level of 5% ($P = 0.007$).

DISCUSSION

The polymorphisms of the *HLA-B* and *HLA-DRB1* genes were investigated in mild, non-cerebral severe, and cerebral malaria patients living in northwest Thailand. In this study, *HLA-B46* ($P = 0.005$), *-B56* ($P = 0.032$), and *-DRB1*1001* ($P = 0.007$) showed significant differences in alleles frequency between malaria groups. However, we examined a total of 37 alleles for three groups, resulting in 111 ($= 37 \times 3$) chi-square tests. Because such multiple testing results in an inflation of type I error, it is necessary to adjust the significance level. When the Bonferroni correction is applied to the present study, the significance level is 0.045% ($= 5/111\%$). After this correction, none of the three alleles mentioned above showed a statistically significant difference between groups. Thus, the associations of these *HLA-B* and *-DRB1* alleles with severity of malaria in the Thai population are not conclusive at present. Assuming that there is an authentic association, the influence of these alleles on malaria severity is not expected to be strong. This is not surprising for infectious diseases, in which different degrees of association are

Table 1. *HLA-B* allele frequencies in Thai patients with mild malaria, non-cerebral severe malaria, and cerebral malaria

<i>HLA-B</i>	Mild (2N = 404)	Non-cerebral severe (2N = 322)	Cerebral (2N = 218)
7	11 (2.7%)	6 (1.9%)	9 (4.1%)
13	19 (4.7%)	18 (5.6%)	13 (6.0%)
15	88 (21.8%)	66 (20.5%)	43 (19.7%)
18	16 (4.0%)	12 (3.7%)	5 (2.3%)
27	9 (2.2%)	6 (1.9%)	6 (2.8%)
35	22 (5.4%)	25 (7.8%)	12 (5.5%)
37	9 (2.2%)	7 (2.2%)	1 (0.1%)
38	17 (4.2%)	15 (4.7%)	11 (5.0%)
39	10 (2.5%)	5 (1.6%)	9 (4.1%)
40	29 (7.2%)	32 (9.9%)	18 (8.3%)
44	29 (7.2%)	21 (6.5%)	19 (8.7%)
46	37 (9.2%)	19 (5.9%)	28 (12.8%) ¹⁾
47	4 (1.0%)	5 (1.6%)	5 (2.3%)
48	7 (1.7%)	5 (1.6%)	2 (0.1%)
51	16 (4.0%)	16 (5.0%)	7 (3.2%)
52	26 (6.4%)	21 (6.5%)	8 (3.7%)
55	9 (2.2%)	5 (1.6%)	3 (1.4%)
56	19 (4.7%)	10 (3.1%)	3 (1.4%) ²⁾
58	20 (5.0%)	20 (6.2%)	15 (6.9%)
Others	7 (1.7%)	8 (2.5%)	1 (0.1%)

HLA, human leukocyte antigen.

P values were calculated by a chi-square test (comparing an allele *A* vs. all non-*A* alleles).

¹⁾: $P = 0.005$ (non-cerebral severe malaria vs. cerebral malaria).

²⁾: $P = 0.032$ (mild malaria vs. cerebral malaria).

Table 2. *HLA-DRB1* allele frequencies in Thai patients with mild malaria, non-cerebral severe malaria, and cerebral malaria

<i>HLA-DRB1</i>	Mild (2N = 388)	Non-cerebral severe (2N = 316)	Cerebral (2N = 204)
*0301	12 (3.1%)	13 (4.1%)	11 (5.4%)
*0403	4 (1.0%)	12 (3.8%)	3 (1.5%)
*0405	9 (2.3%)	15 (4.7%)	7 (3.4%)
*0406	5 (1.3%)	11 (3.5%)	3 (1.5%)
*0701	45 (11.6%)	36 (11.4%)	27 (13.2%)
*0803	5 (1.3%)	5 (1.6%)	4 (2.0%)
*0901	20 (5.2%)	23 (7.3%)	17 (8.3%)
*1001	21 (5.4%)	5 (1.6%) ¹⁾	6 (2.9%)
*1101	6 (1.5%)	8 (2.5%)	7 (3.4%)
*1104	5 (1.3%)	1 (0.3%)	4 (2.0%)
*1202	71 (18.3%)	50 (15.8%)	28 (13.7%)
*1301	3 (0.8%)	3 (0.9%)	4 (2.0%)
*1302	7 (1.8%)	3 (0.9%)	4 (2.0%)
*1401	16 (4.1%)	9 (2.8%)	10 (4.9%)
*1404	10 (2.6%)	10 (3.2%)	10 (4.9%)
*1501	47 (12.1%)	49 (15.5%)	21 (10.3%)
*1502	71 (18.3%)	48 (15.2%)	26 (12.7%)
*1602	13 (3.4%)	7 (2.2%)	6 (2.9%)
Others	18 (4.6%)	8 (2.5%)	6 (2.9%)

HLA, human leukocyte antigen.

P values were calculated by a chi-square test (comparing an allele *A* vs. all non-*A* alleles).

¹⁾: $P = 0.007$ (mild malaria vs. non-cerebral severe malaria).

found for different *HLA* alleles. In addition, the complex nature of pathogenesis may also play a role; the interaction of many genes, instead of a single gene, may influence disease severity.

Finally, it is possible that the sample size was too small to detect a statistically significant difference. For example, in order to obtain 80% discriminative power at a significance level of 0.045% for the examination of the difference in *HLA-B*46* allele frequencies, 631 non-cerebral severe malaria and 631 cerebral malaria patients would be required in a two-sided chi-square test (14,15).

In Gambia, the *HLA-B*53* and *-DRB1*1302* alleles are common and are associated with reduced susceptibility to severe malaria (4). However, *HLA-B*53* and *-DRB1*1302* were rarely found in our population. Alleles common in the Thai population were found to be different from those common in Gambia, and the common Thai alleles did not show any significant association with reduced susceptibility to severe malaria, even at a significance level of 5%. Our findings might imply that the threat of fatal malaria in Thailand is not as serious as in Africa, where malaria has been a major cause of death. In Africa, cerebral or severe malaria occurs primarily in young children, while in Thailand, cerebral malaria affects both children and adults. Thus, the intensity of selection by malaria may not be as strong in Thailand as it is in Africa, and the allele frequencies of common alleles in the population studied may not have been increased due to positive selection against severe malaria.

The tumor-necrosis factor-alpha (*TNFA*) gene is located between the *HLA-B* and *HLA-DRB1* genes. Although the promoter allele of *TNFA-308A*, was found to be associated with susceptibility to cerebral malaria in Gambia (16), this allele did not show any association in our previous study with the same samples (17). The *TNFA-308A* allele was inferred to be located on the *HLA-B*58 – DRB1*0301* haplotype in the studied population based on the estimated three-locus haplotype frequencies. *TNFA-308A* showed especially strong linkage disequilibrium with *HLA-B*58*.

Recently, we reported a significant association of the promoter polymorphism of *IL13, IL-13 -1055C>T*, with protection from non-cerebral severe malaria in the same samples (18). It therefore seemed possible that *HLA* alleles might show significant associations with severity of malaria within the subset of patients with the *IL-13 -1055C>T* allele, or within the subset lacking this allele. The present data were thus divided into two subsets according to possession or lack of *IL-13 -1055C>T*, and the allele frequencies of *HLA-B* and *-DRB1* were compared between mild, non-cerebral severe, and cerebral malaria groups within each subset. However, no statistically significant difference in allele frequency was detected after the Bonferroni correction was applied (data not shown). Thus, the possession of the protective allele *IL-13 -1055C>T* does not appear to strongly influence the association of *HLA-B* and *-DRB1* alleles with severe malaria.

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