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Short Communication

Absence of Association between the *Fcγ Receptor IIIA-176F/V* Polymorphism and the Severity of Malaria in Thai

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SUMMARY: Human Fcγ receptor (*FcγR*) genes form a clustered gene family, which consists of *FcγRIIA*, *IIB*, *IIC*, *IIIA*, and *IIIB* genes, on chromosome 1q23. We previously reported that the *FcγRIIA-131H/H* genotype in combination with the *FcγRIIIB-NA2* allele is associated with susceptibility to cerebral malaria, and that such an association can be caused by linkage disequilibrium (LD) between these polymorphisms and the primary associated gene(s) in this region. *FcγRIIIA* is known to exhibit the genetic polymorphism *FcγRIIIA-176F/V* coded for different affinity to IgG1 and IgG3. In this study, we examined a possible association between *FcγRIIIA-176F/V* polymorphism and severity of malaria in 462 adult Thai patients with *Plasmodium falciparum* malaria. The frequencies of *FcγRIIIA-176V* among patients with mild malaria, with non-cerebral severe malaria, and with cerebral malaria were 32.7%, 29.9%, and 36.3%, respectively. This polymorphism showed neither positive nor negative association with the severity of malaria. Thus, we concluded that the association of *FcγRIIA-131H/R* and *FcγRIIIB-NA1/NA2* polymorphisms with cerebral malaria in Thailand is not due to the LD caused by *FcγRIIIA-176F/V*.

Fcγ receptors (FcγR) act as a crucial link between the humoral and cellular immune responses. The major function of FcγR is the activation of accessory cells against pathogens, making FcγR an essential molecule in the host defense against infection. Human *FcγR* is constituted by a gene family clustered on chromosome 1q23 (1). There are three families of *FcγR* (*FcγRI*, *FcγRII*, and *FcγRIII*), each containing multiple distinct genes and alternative splicing variants (2). FcγRIIA and FcγRIIIB exhibit the genetic polymorphisms *FcγRIIA-131H/R* and *FcγRIIIB-NA1/NA2*, coded for different capacities for IgG binding and phagocytosis (3,4). Recently, we found that the *FcγRIIA-131H/H* genotype in combination with the *FcγRIIIB-NA2* allele is associated with susceptibility to cerebral malaria in Thai patients (5). However, in Kenyan infants, the *FcγRIIA-131H/H* genotype was not associated with high-density *Plasmodium falciparum* infection, although those with *FcγRIIA-131R/R* were found to be less at risk than those with *FcγRIIA-131H/R* (6). These discrepant findings among different populations raise the prospect that another gene(s) in this chromosomal region, in linkage disequilibrium (LD) with *FcγRIIA* and *FcγRIIIB*, may be primarily associated with the severity of malaria.

The *FcγRIIIA* gene is located proximate to *FcγRIIA* and *FcγRIIIB*, and LD has been found among these three genes in the Japanese population (7,8). A dimorphism was recently reported at amino acid position 176 (F/V) of FcγRIIIA, and found to influence the binding of IgG1, IgG3, and IgG4. *FcγRIIIA-176V*, compared with *FcγRIIIA-176F*, was shown to have a higher affinity for IgG1 and IgG3 (9). In *P. falciparum* malaria, IgG1 and IgG3 have been known to be associated with lower parasitemia (10) or lower risk of malaria infection (11). Therefore, *FcγRIIIA-176F/V* is considered an important candidate polymorphism for influencing the pathogenesis of severe malaria. The aim of this study was to examine a possible association between *FcγRIIIA-176F/V* polymorphism and severity of malaria in Thai patients. The association between *FcγR* polymorphisms and susceptibility to severe malaria is only beginning to be investigated. To our knowledge, this study is the first to examine a possible association between the *FcγRIIIA-176F/V* polymorphism and severity of malaria.

A total of 260 adult patients with severe *P. falciparum* malaria (106 cerebral malaria and 154 non-cerebral severe malaria patients) and 202 adult patients with mild malaria (controls) living in northwest Thailand were analyzed in this study. All thse patients underwent treatment at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University. Clinical manifestations of malaria were classified as follows. Cerebral malaria was defined as unrousable coma, positive asexual form of *P. falciparum* in blood smear, and exclusion of other causes of coma. Severe malaria but not cerebral (non-cerebral severe malaria) was characterized by any one of the following signs: high parasitemia (>100,000 parasite/ml), hypoglycemia (glucose <22 nmol/l), severe anemia (hematocrit <20% or hemoglobin <7.0 g/dl), and increased serum level of creatinine more than 3.0 mg/dl. Mild malaria was characterized by a positive blood smear, presence of fever and absence of other causes of infection, and none of the manifestations of severe malaria described above. All individuals were 13 years of age or older, and the mean ages for severe malaria and mild malaria were 25.5 and 25.5 years, respectively. This study was approved by the institute review board of the Faculty of Tropical Medicine, Mahidol University, and informed consent was obtained from all patients. Genomic DNA was extracted from peripheral blood leukocytes

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using a QIAamp Blood Kit (Qiagen, Hilden, Germany). Genotyping for the *FcγRIIIA-176F/V* polymorphism was performed using PCR-single strand conformation polymorphism (PCR-SSCP) analysis (7). Samples whose genotype was confirmed by PCR-direct sequencing were used as references in PCR-SSCP analysis. The χ^2 test was used to compare genotype and allele frequencies of the *FcγRIIIA-176F/V* polymorphism among the three malaria groups. Conformity of genotype proportion to Hardy-Weinberg equilibrium was examined in each malaria group.

Genotype and allele frequencies of the *FcγRIIIA-176F/V* polymorphism in Thai malaria patients are shown in Table 1. *FcγRIIIA-176F/V* was a common polymorphism with a minor allele frequency of around 30 %. Neither positive nor negative association was observed between the *FcγRIIIA-176F/V* polymorphism and the severity of malaria in Thai patients. Genotype frequencies did not deviate from expectations of the Hardy-Weinberg equilibrium in each group. Table 2 shows the haplotype frequencies and degrees of LD between *FcγRIIIA-176F/V* and *FcγRIIA-131H/R*, and between *FcγRIIIA-176F/V* and *FcγRIIIB-NA1/NA2* in Thai patients. These polymorphisms were in linkage equilibrium in Thai patients with mild malaria. Since no LD was observed (Table 2), we can exclude the possibility that the association identified in our previous study is caused by LD with the *FcγRIIIA-176F/V* polymorphism. Thus, we conclude that the association between the combination of *FcγRIIA-131H/R* and *FcγRIIIB-NA1/NA2* polymorphisms with cerebral malaria in Thailand is not due to LD between these polymorphisms and *FcγRIIIA-176F/V*.

Our previous study revealed that, with the *FcγRIIIB-NA2* allele, the *FcγRIIA-131H/H* genotype was associated with susceptibility to cerebral malaria (5). FcγRIIA-131H binds IgG2, IgG1, and IgG3 efficiently (3). In areas where malaria is endemic, the predominance of IgG1 and IgG3 has been reported to be associated with protection against *P. falciparum* (12). If the efficient clearance of IgG1 and IgG3 plays a major role in the pathogenesis of malaria, *FcγRIIIA-176F/V* polymorphism would likely be associated with severe malaria. However, the *FcγRIIIA-176F/V* polymorphism showed no significant association with the severity of malaria in this study.

FcγRIIA-131H is known not to bind C-reactive protein (CRP) (13), which is involved in the hepatic development of parasites by preventing penetration of a sporozoite into a hepatocyte and by blocking parasite division (14). Shi et al. showed that the *FcγRIIA-131R* allele, which has a high affinity for CRP, is associated with protection against high-density *P. falciparum* infection in Kenyan infants (6). Those findings, taken together with our results, suggest that the binding of CRP to FcγRIIA may play a crucial role in the pathogenesis of severe malaria. Further population and functional studies will clarify this role.

Table 1. Genotype and allele frequencies of *FcγRIIIA-176F/V* polymorphism in Thai malaria patients

	Cerebral malaria (n = 106)	Non cerebral severe malaria (n = 154)	Mild malaria controls (n = 202)
Genotype frequency			
176V/V	14 (13.2%)	13 (8.4%)	21 (10.4%)
176V/F	49 (46.2%)	66 (42.9%)	90 (44.6%)
176F/F	43 (40.6%)	75 (48.7%)	91 (45.0%)
Allele frequency			
176V allele	77 (36.3%)	92 (29.9%)	132 (32.7%)
176F allele	135 (63.7%)	216 (70.1%)	272 (67.3%)

Table 2. Haplotype frequency and linkage disequilibrium between *FcγRIIIA-176F/V*, *FcγRIIA-131H/R* and *FcγRIIIB-NA1/NA2* polymorphisms in Thai mild malaria patients (n = 202)

Haplotype	HF[1)]	RLD[2)]
FcγRIIIA-IIA		
176F-131H	51.3	0.11
176F-131R	16.0	–0.11
176V-131H	21.9	–0.11
176V-131R	10.7	0.11
FcγRIIIA-IIIB		
176F-NA1	42.9	–0.09
176F-NA2	24.5	0.09
176V-NA1	22.2	0.09
176V-NA2	10.4	–0.09

[1)] HF: estimated Haplotype frequency based on an EM algorithm (ref. 15).
[2)] RLD: relative linkage disequilibrium.

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