***IFNGR1* polymorphisms in Thai malaria patients**

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ABSTRACT: Interferon–γ (IFN–γ) has been suggested to play an important role in the pathogenesis of malaria. To examine possible association of the IFN–γ receptor 1 (*IFNGR1*) polymorphisms with cerebral malaria, 312 adult patients with *Plasmodium falciparum* malaria (203 mild and 109 cerebral malaria patients) living in northwest Thailand were genotyped for six single nucleotide polymorphisms (SNPs) including -56T/C (rs2234711) and a microsatellite marker in *IFNGR1*. A case-control association analysis failed to detect significant association between the *IFNGR1* polymorphisms and cerebral malaria, thus implying that the *IFNGR1* polymorphism may not be a major genetic factor influencing the development of cerebral malaria in the Thai population. These data also provide useful information for future genetic studies of *IFNG* polymorphisms in Thai patients.

Key Words: IFNGR1; polymorphisms; cerebral malaria; Thai

1. Introduction

Plasmodium falciparum malaria is one of the most deadly infectious diseases for humans. Among the severe complications of *P. falciparum* malaria infection, cerebral malaria accounts for vast majority of death from *P. falciparum* malaria. Sequestration of parasites in the small blood vessels of the brain has been suggested to be involved in cerebral malaria (Miller et al. 2002). Accumulation of infected erythrocytes induces inflammatory reactions mediated by pro-inflammatory cytokines, which damage cerebral endothelial cells and affect functions of the nervous system. Abundant production of interferon (IFN)–γ, a major pro-inflammatory cytokine, is observed during clinical episodes of malaria (Kwiatkowski et al. 1990). IFN-γ and its receptor therefore may play a crucial role in development of cerebral malaria.

The receptor for IFN-γ consists of α and β subunits. The α subunit (encoded by the gene *IFNGR1*; OMIM 107470) is essential for IFN-γ binding, receptor trafficking, and signal transduction (Bach et al. 1997). Two functional polymorphisms, *IFNGR1* -56T/C and *IFNGR1* -470insTT/delTT, have been identified in the promoter region of *IFNGR1* (Juliger et al. 2003; Koch et al. 2006; Rosenzweig et al. 2004). In Gambia, heterozygote of *IFNGR1* -56T/C is associated with protection against cerebral malaria, whereas *IFNGR1* -470delTT allele is associated with protection against severe malaria in general (Koch et al. 2002). Recently, significant association of haplotypes comprising the *IFNGR1* promoter polymorphisms with susceptibility to post-kala-azar dermal leishmaniasis was reported in Sudan (Salih et al. 2007). This study investigated whether the polymorphisms of *IFNGR1* influence the susceptibility to cerebral malaria in Thai malaria patients.

2. Materials and Methods

2.1 Subjects

A total of 312 adult patients with *P. falciparum* malaria (203 mild malaria and 109 cerebral malaria) living in northwest Thailand were analyzed in this study. All patients were self-reported Thais and underwent treatment at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University. Malarial infection by *P. falciparum* was confirmed in all patients by a positive blood smear for the asexual form of *P. falciparum*. Clinical manifestations of malaria were classified according to the definitions and associated criteria by the World Health Organization. Cerebral malaria was defined as unrousable coma, a positive result in tests for the presence of an asexual form of *P. falciparum*, and exclusion of other causes of coma. Mild malaria was defined as having a positive blood smear and fever without other causes of infection and no signs indicating severe malaria such as high parasitemia (> 100,000 parasites/ml), hypoglycemia (glucose level < 22 nmol/L), severe anemia (hematocrit < 20% or hemoglobin level < 7.0 g/dl) or increased serum level of creatinine (> 3.0 mg/dl). All individuals were 13 years old or older, and the mean ages of patients with mild malaria and cerebral malaria patients were 25.5 and 28.6 respectively. This study was approved by the institutional review board of the Faculty of Tropical Medicine, Mahidol University, and the Research Ethics Committee of the Graduate School of Comprehensive Human Sciences, University of Tsukuba. Informed consent was obtained from all patients.

2.2 DNA extraction, variation screening, and genotyping

Genomic DNAs from all the patients were purified from peripheral blood leukocytes using a commercially available kit (QIAmp blood kit; Qiagen, Hilden, Germany). A variation screening for the entire seven exons of the *IFNGR1* gene was performed in 16 mild and

16 cerebral malaria patients (protocols and primer sequences are available upon request). In Fig. 1A, the region subjected to variation screening is indicated by shaded bar. Accordingly, six SNPs, -56C/T (rs2234711), IVS6-4A/G (rs3799488), 1050T/G (rs11914), 1400T/C (rs1887415), 1676A/G (rs1887416), and 1687T/A (rs1887417), were identified. These six SNPs and a microsatellite marker (corresponding to rs59020499) in intron 5 of *IFNGR1* were genotyped for the remaining malaria patients. The protocols and primer sequences are available upon request.

2.3 Statistical analysis

Deviation from Hardy-Weinberg equilibrium for each malaria group was assessed by the exact P-value obtained from a Monte Carlo simulation. The simulations were performed using the SNPAlyze software package ver. 7.0 (Dynacom). The differences in genotype and allele frequencies between cerebral and mild malaria patients were examined by Fisher's exact probability test. In association test, three modes of inheritance (i.e., dominance, recessive, and additive) were also evaluated by Fisher's exact probability test or trend test. For a microsatellite marker with multiple alleles, the P-value was calculated for each allele based on a 2 x 2 table (i.e., focused allele vs. the other alleles). Haplotype frequencies and pairwise linkage disequilibrium (LD) parameters, |D'| and r^2, between SNPs were estimated based on the expectation-maximization algorithm. The SNP haplotype frequencies were also compared between cerebral and mild malaria patients by a permutation test. The calculations of haplotype frequencies and LD parameters were performed with the Haploview software version 4.0 (Barrett et al. 2005). The power calculations were conducted as described elsewhere (Ohashi and Tokunaga 2002; Ohashi et al. 2001).

3. Results and Discussion

A variation screening for the *IFNGR1* gene identified six SNPs in 32 Thai patients with malaria (Fig. 1A). These SNPs were genotyped for all the patients. Table 1 shows the genotype and allele frequencies of the *IFNGR1* SNPs in cerebral and mild malaria patients. Association tests revealed that none of the SNPs was associated with cerebral malaria. According to previous studies (Juliger et al. 2003; Koch et al. 2002; Koch et al. 2006), among SNPs analyzed in this study, -56C/T is the most plausible candidate for showing the association with susceptibility to cerebral malaria. The power calculation suggested that the present sample size achieves a power of 0.8 under the assumption that the allele frequency is 0.35 and the allelic odds ratio is 1.61. Thus, it should be noted that the significant association is hard to be detected in this study if the -56T allele is truly but weakly associated with susceptibility to cerebral malaria in Thais. In Mandinka, the major Gambian ethnic group, heterozygote for -56C/T was found to be associated with protection against cerebral malaria (Koch et al. 2002). However, such a tendency or heterozygote advantage was not observed in the present study. The frequency of heterozygote was rather higher in cerebral malaria than in mild malaria (Table 1). Although this discrepancy may have been caused by ethnic difference or age difference of patients (i.e., children were studied in Mandinka), the association of -56C/T with cerebral malaria needs to be reexamined in further studies. A functional promoter polymorphism, *IFNGR1* -470insTT/delTT, previously shown to be associated with resistance to severe malaria (Koch et al. 2002; Koch et al. 2006) was not detected in the present variation screening.

Five major SNP haplotypes were observed in Thai malaria patients (Fig. 1B). The comparison of haplotype frequencies between cerebral and mild malaria patients revealed

no significant association of the *IFNGR1* haplotypes with cerebral malaria (data not shown). The analysis of LD in the *IFNGR1* gene showed that six SNPs were in absolute LD (|D'| = 1) and three of them (1400T/C, 1676A/G, and 1687T/A) were in perfect LD (r^2 = 1; Fig. 1C). Since four SNPs indicated by the arrow in Figure 1B can act as tag SNPs for *IFNGR1*, these SNPs should be analyzed in future association studies in the Thai population.

A total of 13 different alleles were observed at a microsatellite marker in intron 5 of *IFNGR1* in Thai malaria patients (Table 2). Although a microsatellite allele with 18 repeats showed P-value of less than 0.05, this should not be regarded as statistically significant because a number of association tests were conducted in this study. Neither SNPs nor microsatellite marker demonstrated a significant deviation from Hardy-Weinberg equilibrium.

In conclusion, we find no evidence for an association of *IFNGR1* polymorphisms with cerebral malaria in Thais.

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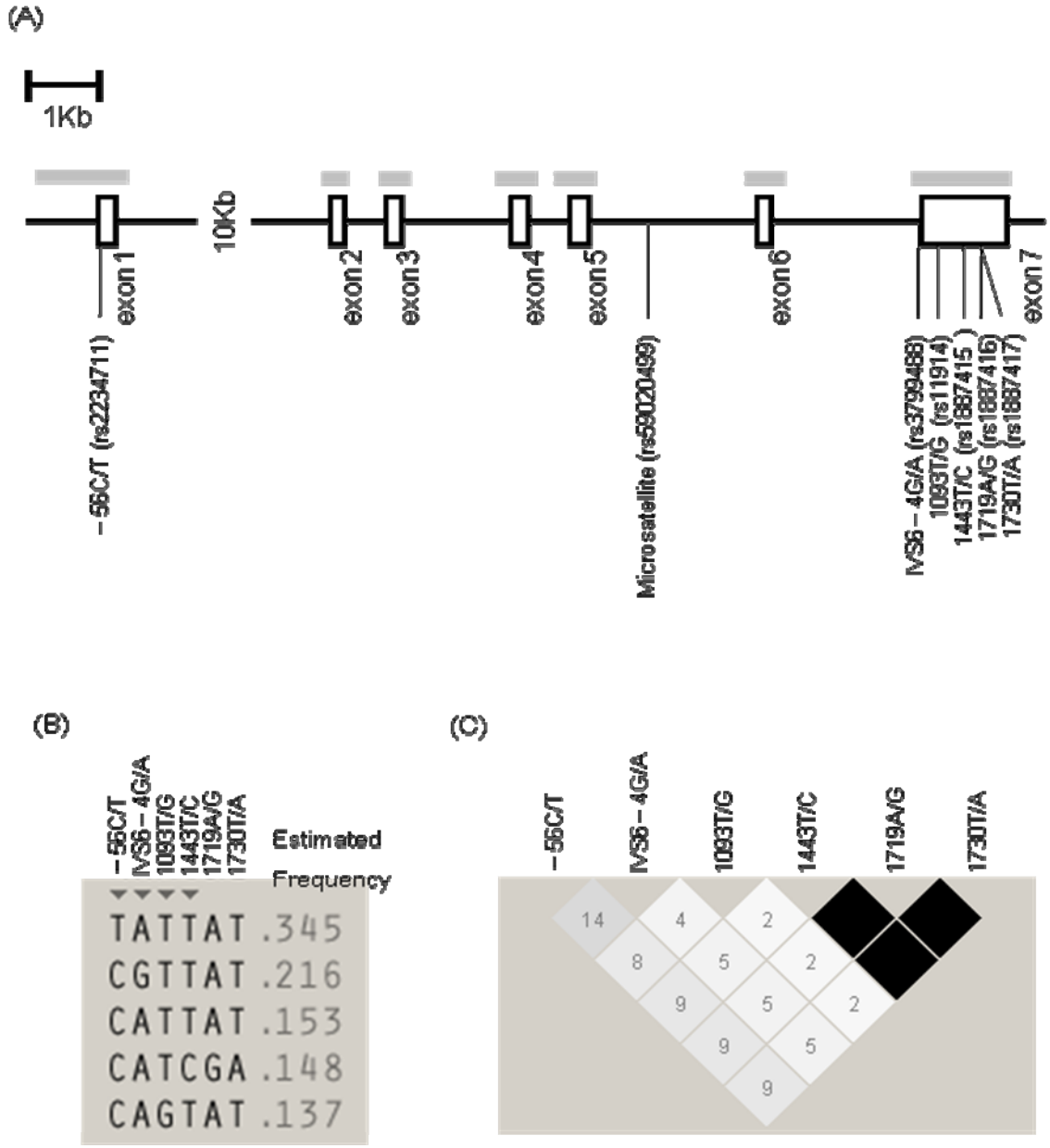
Figure legend

Figure 1. Polymorphisms, haplotypes, and LD structure of *IFNGR1*. (A) The studied polymorphisms of *IFNGR1* are herein presented. The shaded bar indicates the region subjected to variation screening. (B) The estimated frequencies of the haplotypes comprising six SNPs detected in this study. The arrow indicates tag SNP for *IFNGR1* in the Thai population. (C) The pairwise LD values between six SNPs measured by r^2.



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