POLYMORPHISMS OF *CD36* IN THAI MALARIA PATIENTS

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Abstract. The human protein CD36 is a major endothelial receptor for *Plasmodium falciparum* parasitized erythrocytes. Several polymorphisms causing CD36 deficiency have been identified to date: T1264G in Kenyan and Gambian patients, and C478T, 539delAC, and 1159insA in Japanese patients. The T1264G polymorphism is reportedly associated with protection from severe malaria in Kenyans, although there is a contradictory report suggesting the susceptibility of T1264G to severe malaria. The polymorphism of *CD36* has not been thoroughly studied in Asian malaria patients. In this study, nucleotide sequence variations in exons 4, 5, 6, and 10 of *CD36* were investigated in mild and cerebral malaria patients living in northwest Thailand. A novel synonymous substitution T1168C was detected in exon 10, whereas no variation was found in exons 4 and 6. The 539delAC allele in exon 5 was detected in Thai malaria patients, while T1264G, C478T, and 1159insA were not found. The 539delAC allele was observed in three cerebral malaria patients (3/107), but not in mild malaria patients (0/203). The frequency of 539delAC was significantly higher in cerebral malaria patients than in mild malaria patients ($p = 0.040$, Fisher's exact test). Although independent studies should be performed in order to confirm our findings, the 539delAC allele might be a high-risk variant for cerebral malaria in Thai.

INTRODUCTION

CD36 is an 88kDa glycoprotein involved in the cytoadherence of *Plasmodium falciparum* parasitized erythrocytes to endothelial cells. CD36 is a major receptor for *Plasmodium falciparum* parasitized erythrocytes (Ockenhouse *et al*, 1989; 1991; Newbold *et al*, 1997) and amino acids 139-184 (encoded by exons 5 and 6) of CD36 form the adhesion region for *Plasmodium falciparum* parasitized erythrocytes (Baruch *et al*, 1999). Adhesion of parasitized erythrocytes to CD36 contributes to the sequestration of parasitized erythrocytes (Ockenhouse *et al*, 1989; 1991) and the inhibition of the immune response to malaria parasites (Urban *et al*, 1999). In addition, since individuals deficient in CD36 expression are apparently healthy, CD36 deficiency is expected to protect against malaria infection. The frequency of CD36 deficiency is reported to be around 2-11% in Asians and Africans (Urwijitaroon *et al*, 1995; Curtis and Aster, 1996; Yanai *et al*, 2000), while CD36 deficiency is scarcely found in Caucasians (Simsek *et al*, 1993). Several variants of the *CD36* gene causing CD36 deficiency have been reported to date. Among the Japanese, three mutations responsible for CD36 deficiency have been reported: C478T in exon 4, 539delAC in exon 5, and 1159insA in exon 10 (Kashiwagi *et al*, 1994; 1995; 1996). In Africa, the T1264G stop mutation in exon 10 is known to be the major cause of CD36 deficiency (Aitman *et al*, 2000; Pain *et al*, 2001). Recently, the 1264G allele was found to be associated with protection from severe malaria in Africa (Pain *et al*, 2001), although there is a contradictory report showing a positive association of the 1264G allele with the severity of malaria (Aitman *et al*, 2000). However, nucleotide variations of *CD36* have not been thoroughly studied in Asian malaria patients. In this study, variations in exons 4, 5, 6, and 10 of *CD36* gene were investigated in malaria patients living in northwest Thailand. To our knowledge, this is the first study of polymorphisms of *CD36* in Asian malaria patients.

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MATERIALS AND METHODS

Study subjects

Two hundred and three adult patients with mild malaria and 107 adult patients with cerebral malaria living in northwest of Thailand were recruited in this study after receiving informed consent. Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp blood kit (Qiagen, Hilden, Germany). The study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University.

Nucleotide sequencing

Nucleotide sequence variations of *CD36* were first analyzed by PCR-direct sequencing in randomly selected samples. The numbers of malaria patients

sequenced for exons 4, 5, 6, and 10 were 30 (15 mild and 15 cerebral malaria), 20 (10 mild and 10 cerebral malaria), 32 (16 mild and 16 cerebral malaria), and 52 (26 mild and 26 cerebral malaria), respectively. Each exon fragment amplified by polymerase chain reaction (PCR) from genomic DNA was used for the direct sequencing with an ABI PRISM^{TM} 310 Genetic Analyzer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA). The PCR primers used in this study are listed in Table 1.

Genotyping

The 539delAC allele was detected in Thai malaria patients by PCR-direct sequencing. For the typing of this mutation, the PCR-SSP (sequence specific primer) method was developed (Fig 1). Amplification consisted of an initial denaturation at 96˚C for 10 minutes, followed by 35 cycles of denaturation at 96˚C for 30 seconds, annealing at 60˚C for 30 seconds, and extension at 72˚C for 30 seconds, using GeneAmp reagents and AmpliTaq Gold DNA polymerase (Perkin-Elmer Applied Biosystems, Foster City, CA, USA). The primers are shown in Table 1.

RESULTS

A variation screening was performed for exons 4, 5, 6, and 10 of *CD36* by direct sequencing. As a result, two variations of *CD36* gene, 539delAC in exon 5 (Kashiwagi *et al*, 1994) as well as a novel synonymous substitution T1168C in exon 10, were detected in Thai malaria patients. However, the other known variations related to CD36 deficiency, T1264G, C478T, and 1159insA, were not detected. The frequencies of the 1168C allele in the screening were 3.8% (2/52) and 5.8% (3/52) in mild and cerebral malaria patients, respectively. The numbers of subjects possessing the 539delAC allele in all the available samples are shown in Table 2. The 539delAC allele was found only in cerebral malaria patients, but not in mild malaria patients. The number of individuals with the 539delAC allele was significantly increased in cerebral malaria patients, compared to mild malaria patients ($p = 0.040$, Fisher's exact test).

DISCUSSION

In this study, variations of *CD36* gene were investigated in exons 4, 5, 6, and 10, because several mutations causing CD36 deficiency have been reported in exons 4 and 10, and the adhesion region for *Plasmodium falciparum* parasitized erythrocytes is known to be encoded by exons 5 and 6. The T1264G and C478T substitutions related to CD36 deficiency have been described at allele frequencies of approximately 10% in Kenyan and 4% in Japanese, respectively (Aitman *et al*, 2000; Yanai *et al*, 2000; Pain *et al*, 2001). In contrast, these polymorphisms as well as 1159insA were not detected in Thai malaria patients in this study. Among the previously reported variations, 539delAC was detected in this study. 539delAC is known to cause CD36 deficiency due to

Table 1
Primers used in this study.

Name		Sequence
PCR-direct sequencing		
Exon 4	sense	5´-GAAGTGCCTGTACTTACTAC-3´
	antisense	5´-GAAATACATGGTCAAGGTAAG-3´
Exon 5 (Yanai *et al*, 2000)	sense	5´-AGATCTAATGTTCACATATG-3´
	antisense	5´-GATTAATTACATGAGTTCTAGAG-3´
Exon 6	sense	5´-TTTTGGCAGGATCTGGCAG-3´
	antisense	5´-TGCCTTGCCAATGCCATTG-3´
Exon 10 (Yanai *et al*, 2000)	sense	5´-AGTTCAGGTTCCTGGAATGC-3´
	antisense	5´-ATGGACTGTGCTACTGAGGT-3´
PCR-SSP for 539delAC		
For common allele	sense	5´-GACGCTGAGGACAACACG-3´
For 539delAC	sense	5´-GACGCTGAGGACAACGTC-3´
	antisense	5´-GTGGTCTTCTAATGCAGTCG-3´

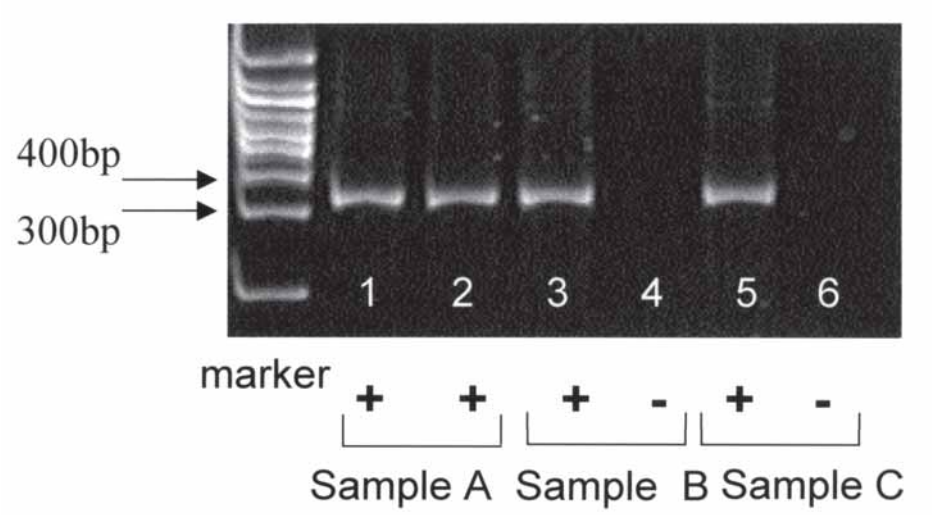

Fig 1- PCR-SSP typing for 539delAC. The specific primer set for the common allele was used in lanes 1, 3, and 5. The specific primer set for 539delAC was used in lanes 2, 4, and 6. Electrophoresis was performed using 10% polyacrylamide gel. Sample A is heterozygote for the 539delAC. Samples B and C are homozygotes for the common allele.

Table 2
Frequency of 539delAC in Thai malaria patients.

Genotype	All malaria (n = 310)	Mild malaria (n = 203)	Cerebral malaria (n = 107)	p-value[a]
Common/Common	307 (99.0)	203 (100.0)	104 (97.2)	0.040
Common/539delAC	3 (1.0)	0 (0.0)	3 (2.8)	

Numbers in parentheses indicate percentage.
[a]Mild malaria *vs* cerebral malaria patients by Fisher's exact test.

the frameshift leading to the appearance of a stop codon at 606 (Kashiwagi *et al*, 1994). The prevalence of CD36 deficiency has been reported to be less than 3% in Thai (Urwijitaroon *et al*, 1995). Thus, the major cause of CD36 deficiency in Thai would be neither T1264G nor C478T, but 539delAC or other unknown mutations.

The 539delAC allele was detected only in cerebral malaria patients. A recent study has revealed that CD36 on monocyte and macrophage plays a crucial role in the CD36-dependent phagocytosis of *Plasmodium falciparum* parasitized erythrocytes (McGilvay *et al*, 2000). Thus, 539delAC causing CD36 deficiency is expected to reduce the nonopsonic clearance of *Plasmodium falciparum* parasitized erythrocytes by monocyte and macrophage. Furthermore, in malaria patients with 539delAC or CD36 deficiency, other molecules such as the intercellular adhesion molecule (ICAM)-1 are considered to be involved in the cytoadherence of *Plasmodium falciparum* parasitized erythrocytes. Unlike CD36, ICAM-1 is expressed on cerebral microvasculature endothelial cells. Thus, the sequestration of *Plasmodium falciparum* parasitized erythrocytes in such vital sites is more likely to occur in malaria patients without CD36 than in those with CD36, since the parasitized erythrocytes do not adhere to CD36 in non-vital sites. Taken together, the 539delAC allele of *CD36* might be a high-risk allele for cerebral malaria in Thai malaria patients, although its frequency is relatively low. In order to confirm the association of CD36 deficiency with cerebral malaria, independent studies should be performed, and other exons of *CD36* gene are necessary to be investigated.

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