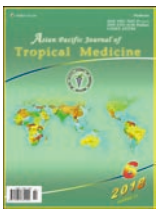

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Interferon-induced protein with tetratricopeptide repeats 1 (*IFIT1*) polymorphism as a genetic marker of cerebral malaria in Thai population

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ABSTRACT

Objective: To know whether the effect of interferon-induced protein with tetratricopeptide repeats (*IFIT*) 1 polymorphism influences the susceptibility of cerebral malaria outcome. **Methods:** Case-control association study was performed among 314 Thai patients (110 with cerebral malaria and 204 with uncomplicated malaria) infected with *Plasmodium falciparum*. Genotyping for five tag-single nucleotide polymorphisms of *IFIT1* was performed by endpoint genotyping. **Results:** Genotype frequencies of all tag-SNPs (single nucleotide polymorphisms) showed no association with malaria outcome. However, C allele of rs11203109 was associated with the protection from cerebral malaria (*OR*=0.62, 95% *CI*=0.38–0.99, *P*=0.048). Two single nucleotide polymorphisms (rs5786868 and rs57941432) were in linkage disequilibrium with rs11203109. **Conclusions:** This suggests that our associated single nucleotide polymorphism (rs11203109) might be a genetic marker of cerebral malaria progression in the Thai population.

1. Introduction

Malaria is a considerable global health burden among 91 countries and territories at risk especially in tropical regions. In the year 2016, World Health Organization estimated that approximately 216 million people worldwide were infected with malaria and of them 445 000 people died. Cerebral malaria (CM) is the major cause of death and is most frequently encountered in African children[1]. It is hypothesized that the mechanism of CM relates excessive sequestration of parasitized red cells in the brain's microvasculature and local overproduction of inflammatory cytokines such as interferon (IFN)-γ and tissue necrotic factor-α. Despite the exact pathophysiology of CM is still unclear, it is believed that human genetic factors predispose to this clinical outcome[2].

IFN-γ, encoded by the *IFN*-γ gene, affects a critical aspect in the pathogenesis of CM. The polymorphisms in *IFN*-γ gene and IFN-γ receptor 1 gene are associated with the clinical manifestations of malaria infection[3,4]. Although the role of IFN-α/β in malaria has not been investigated extensively, the polymorphism in IFN-α/β receptor 1 is associated with decreased risk of CM in the Gambia[5]. In addition, a study in an experimental malaria mouse model shows that expression of

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the IFN-stimulated gene (*Isg*, also called *Ifit 1–3*) is remarkably upregulated in CM-susceptible mice compared to levels in resistant mice. The researchers suggest that the *Ifit* gene family is potentially a CM susceptibility locus. Among them, *Ifit1/Isg56* expression is predominantly upregulated[6]. These observations support the hypothesis that downstream variants in the IFN signalling pathway [IFN-induced protein with tetratricopeptide repeats (*IFIT*) 1 in humans] may influence whether or not CM develops. To date, there are no reports of an association between polymorphisms of *IFIT1* and CM. The objective of our study was to access the relationship of single nucleotide polymorphisms (SNP) in the *IFIT1* gene with the development of CM in Thai population.

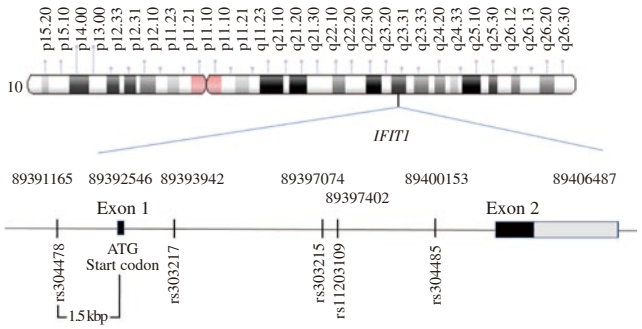

Figure 1. Chromosomal location of candidate single nucleotide polymorphisms (SNPs) in interferon-induced protein with tetratricopeptide repeats 1 (*IFIT1*) gene.

2. Materials and methods

We recruited 314 Thai adult patients (13 years or older) infected with *Plasmodium falciparum* (*P. falciparum*) who were living in the north-west area of Thailand near Myanmar border. All subjects were ≥13 years old with a mean age of 25.5 and 28.6 years for uncomplicated malaria (UM) and CM, respectively. Clinical symptoms of malaria were categorized by the guidance of World Health Organization. We examined 110 CM patients who suffered from unarousable coma by excluding other causes of coma. For the control group, we selected 204 patients with UM with symptoms of febrile illness without any other cause of infection. Both groups of CM and UM were microscopically confirmed for asexual form of *P. falciparum*. For the control group, we excluded all patients who had any signs of severe malaria or manifestation of impairement in vital organ such as hypoglycaemia (glucose level <22 nmol/L), severe anaemia (hematocrit <20% or hemoglobin level <7 g/dL) or elevated serum creatinine (level >3.0 mg/dL), as well as high parasitaemia (>100 000 parasites/μL). All patients obtained treatment from the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Thailand. All patients were voluntarily involved in this study and informed consent was obtanied from all patients. The study was approved by the Institutional Review Board of the Faculty of Tropical Medicine, Mahidol University, Thailand.

IFIT1 is situated on the long arm of chromosome 10 (10q23.31) and has 13 942 nucleotides in length. It consists of two exons and one intron; the first exon encodes the start codon and the second exon encodes the rest of the mRNA. To investigate the relationship between *IFIT1* and CM, we selected tag-SNPs located in the *IFIT1* gene based on genotypic data of the Asian HapMap samples, 45 Japanese from Tokyo, Japan and 45 Han Chinese from Beijing, China[7,8]. Tagger algorithm was executed by the Haploview Software, version 4.2 with the default settings (*i.e.*, pairwise tagging only and linkage disequilibrium $r^2 \geq 0.8$). Among five tag-SNPs, one SNP (rs304478) was situated about 1.5 kbp upstream of the start codon (promoter) region of the *IFIT1* gene and the other four SNPs (rs303217, rs303215, rs11203109, rs304485) were located in the intron (Figure 1).

The genomic DNA was isolated from peripheral blood leukocyte samples using QIAamp Blood Kit (Qiagen, Hilden, Germany). TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA) was applied to genotype five SNPs in the *IFIT1* gene according to the manufacturer's instructions. LightCycler 480II (Roche Diagnostics, Mannheim, Germany) was used for PCR amplification and genotypic discrimination. SNP calling was determined by LightCycler 480II Endpoint Genotyping Software, version 1.5.0.39 (Roche Diagnostics, Mannheim, Germany).

To determine the extent of deviation from Hardy–Weinberg equilibrium, we compared the observed and expected frequencies of genotype in each malaria group by using χ^2 test. To compare the genotype and allele frequency among malaria groups, χ^2 test was used in the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA), version 18.0. A *P*-value less than 0.05 was indicated as statistically significant difference. The association between the genotypes and allele frequency for the risk of CM was analyzed by odds ratio (*OR*) and 95% confidence interval (95% *CI*) in various models. To know the extent of linkage disequilibrium (LD) between SNPs, we computed the pairwise linkage disequilibrium coefficient by using Haploview Software, version 4.2. The haplotype frequency and its association with CM was analyzed by using Haploview Software, version 4.2[9].

In a candidate gene association study, SNP association with the disease may be due either to the virtue of its functionality or to LD with a causal SNP in the neighbourhood[10]. To access whether the associated SNP had LD with a nearby functional SNP, we retrieved the SNPs in the region of 10 kbp upstream and downstream from the associated SNP. Then, LD was assessed by LDlink web-based application using genotype data of the East Asian population from phase 3 of the 1 000 Genomes Project[11]. After obtainment of the SNPs that are in LD with the associated SNP, their functional activity was predicted according to the location of the SNPs. For the SNP located in the 5' region near the *IFIT1* gene, transcription factor binding sites (TFBS) were computationally predicted by PROMO database (version 3.0.2). We inserted the 5' upstream region of

DNA sequences with different SNP alleles into the SearchSites of the PROMO database. The PROMO database determined the specific binding site weight matrices for TFBS prediction according to TRANSFAC database (version 8.3)[12,13]. For SNP localized in the 3' untranslated region (UTR) of the *IFIT1* gene, prediction of microRNA binding sites was determined using miRDB database. This database uses a bioinformatics tool, MirTarget, established from experimentally validated results of several miRNA-target interactions[14].

3. Results

To examine possible associations between *IFIT1* polymorphism and CM, genotyping of five candidate tag-SNPs was performed in 314 Thai patients infected with *P. falciparum*. All SNPs in each of the two malaria groups were in accordance with Hardy–Weinberg equilibrium. The genotype frequencies of all tag-SNPs in various genetic models showed no association with CM outcome. However, the minor C allele of rs11203109 was associated with decrease risk of CM (*OR*=0.62, 95% *CI*=0.38–0.99, *P*=0.048) (Tables 1 and 2). Four major haplotypes consisting of five *IFIT1* SNPs showed no association with CM (Table 3). LD analyzed by Haploview software version 4.2 indicates that the three SNPs (rs304487, rs303217 and rs304485) were in Linkage disequilibrium (r^2>0.8) (Figure 2).

Table 1

Genotype and allele frequencies of single nucleotide polymorphisms (SNPs) in interferon-induced protein with tetratricopeptide repeats (*IFIT*) 1.

SNP		Cerebral malaria (*n*=110) [*n*(%)]	Uncomplicated malaria (*n*=204) [*n*(%)]	*P* value
rs304478	TT	67(60.90)	131(64.20)	0.821
	TG	40(36.40)	67(32.80)	
	GG	3(2.70)	6(2.90)	
	T allele	174(0.79)	329(0.81)	0.643
	G allele	46(0.21)	79(0.19)	
rs303217	CC	65(59.10)	131(64.20)	0.484
	CT	43(39.10)	67(32.80)	
	TT	2(1.80)	6(2.90)	
	C allele	173(0.79)	329(0.81)	0.550
	T allele	47(0.21)	79(0.19)	
rs303215	TT	37(33.60)	70(34.30)	0.936
	TC	54(49.10)	102(50.00)	
	CC	19(17.30)	32(15.70)	
	T allele	128(0.58)	242(0.59)	0.783
	C allele	92(0.42)	166(0.41)	
rs11203109	TT	84(76.40)	136(66.70)	0.130
	TC	25(22.70)	61(29.90)	
	CC	1(0.90)	7(3.40)	
	T allele	193(0.88)	333(0.82)	0.048*
	C allele	27(0.12)	75(0.18)	
rs304485	AA	65(59.10)	131(64.20)	0.638
	AT	42(38.20)	67(32.80)	
	TT	3(2.70)	6(2.90)	
	A allele	172(0.78)	329(0.81)	0.464
	T allele	48(0.22)	79(0.19)	

*P<0.05.

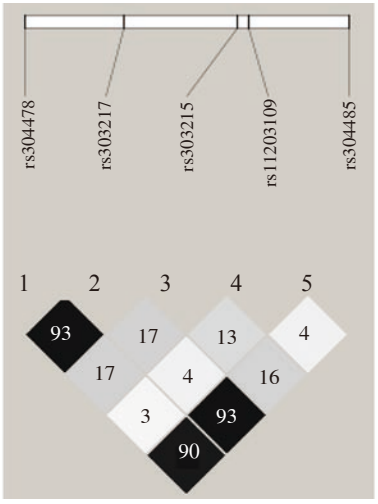

Figure 2. Linkage disequilibrium (LD) analysis of candidate SNPs by Haploview software version 4.2.

LD plot among five tag-SNPs that was determined in 314 Thai malaria patients. A pairwise r^2 value is represented in each square. The darken shade means higher r^2 value.

To find the SNPs that are in LD with rs11203109, we retrieved the SNPs located within 10 kbp upstream and downstream of rs11203109 that show minor allele frequency of ≥0.05 in the East Asian population (from phase 3 of 1000 Genomes Project). Among the SNPs that are in LD with rs11203109, we found one SNP located near the 5' region of *IFIT1* (rs5786868), four SNPs in the intron (rs11203105, rs147997420, rs11203106, rs10788642) and one SNP in the 3' UTR (rs57941432) of the *IFIT1* gene (Figure 3). To determine whether the linking SNPs have a functional effect, we performed computational analysis for TFBS for the SNPs located in the 5' region and microRNA binding sites for the SNPs located in the 3' UTR of the *IFIT1*. Table 4 shows the SNP that are in LD with rs11203109 and results of the computational prediction of TFBS and microRNA binding sites.

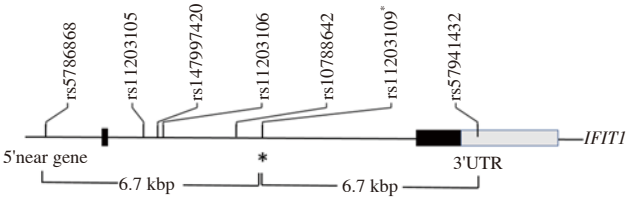

Figure 3. Chromosomal position of SNPs that are in LD with rs11203109.

IFIT1: interferon-induced protein with tetratricopeptide repeats1 gene. LD was assessed by LDlink web-based application[11] that used genotype data of the East Asian from phase 3 of the 1 000 Genomes Project and reference SNP accession numbers are indexed from dbSNP build 142. * indicates the position of the associated SNP.

Table 2

Association of SNPs in interferon-induced protein with *IFIT* 1 for risk of cerebral malaria.

SNP		*P* value	*OR* (95% *CI*)
rs304478	TG *vs.* TT	0.534	1.17(0.72–1.91)
	GG *vs.* TT	0.975	0.98(0.24–4.03)
	Dominant model (TT *vs.* TG+GG)	0.563	1.15(0.71–1.86)
	Over-dominant model (TT+GG *vs.* TG)	0.530	1.17(0.72–1.90)
	Recessive model (TT+TG *vs.* GG)	0.914	0.93(0.23–3.77)
	G *vs.* T allele	0.643	1.10(0.73–1.65)
rs303217	CT *vs.* CC	0.298	1.29(0.80–2.10)
	TT *vs.* CC	0.632	0.67(0.13–3.42)
	Dominant model (CC *vs.* CC+CT)	0.371	1.24(0.77–2.00)
	Over-dominant model (CC+TT *vs.* CT)	0.268	1.31(0.81–2.12)
	Recessive model (CC+CT *vs.* TT)	0.540	0.61(0.12–3.08)
	T *vs.* C allele	0.550	1.13(0.75–1.69)
rs303215	TC *vs.* TT	0.995	1.00(0.60–1.68)
	CC *vs.* TT	0.742	1.12(0.56–2.25)
	Dominant model (TT *vs.* TC+CC)	0.904	1.03(0.63–1.68)
	Over-dominant model (TT+CC *vs.* TC)	0.887	0.96(0.61–1.53)
	Recessive model (TT+TC *vs.* CC)	0.716	1.12(0.60–2.09)
	C *vs.* T allele	0.783	1.05(0.75–1.46)
rs11203109	TC *vs.* TT	0.135	0.66(0.39–1.14)
	CC *vs.* TT	0.174	0.23(0.03–1.91)
	Dominant model (TT *vs.* TT+TC)	0.074	0.62(0.37–1.05)
	Over-dominant model (TT+CC *vs.* TC)	0.174	0.69(0.40–1.18)
	Recessive model (TT+TC *vs.* CC)	0.144	0.26(0.03–2.13)
	C *vs.* T allele	0.048*	0.62(0.38–0.99)
rs304485	AT *vs.* AA	0.345	1.26(0.78–2.06)
	TT *vs.* AA	0.991	1.01(0.24–4.16)
	Dominant model (AA *vs.* AT+TT)	0.371	1.24(0.77–2.00)
	Over-dominant model (AA+TT *vs.* AT)	0.343	1.26(0.78–2.05)
	Recessive model (AA+AT *vs.* TT)	0.913	0.93(0.23–3.77)
	T *vs.* A allele	0.465	1.16(0.78–1.74)

*$P<0.05$.

Table 3

Haplotype analyses of rs304478, rs303217, rs303215, rs11203109 and rs304485 for the risk of cerebral malaria (CM).

Haplotype	Sequence	Cerebral malaria (*n*=110)	Mild malaria (*n*=204)	λ^2	*P* value
H1	TCCTA	0.409	0.404	0.013	0.909 2
H2	TCTTA	0.241	0.216	0.522	0.469 9
H3	GTTTT	0.200	0.186	0.174	0.676 4
H4	TCTCA	0.123	0.179	3.371	0.066 4

Four haplotypes (H1, H2, H3 and H4) in order of common types in Thai population.

4. Discussion

To address the involvement of *IFIT1* gene polymorphism in CM, we performed case-control association study in 314 Thai malaria patients infected with *P. falciparum*, grouped as having CM or UM. Out of five selected tag-SNPs in *IFIT1*, one intronic SNP (rs11203109-C allele) was associated with protection against CM. The basis of this association may be that the associated allele acts as a functional SNP and alters the transcription level of *IFIT1*. The functional evidence of this non-coding intronic SNP is unclear. No one has reported that this intronic SNP has a functional effect on the expression of the *IFIT1* gene nor of mRNA splicing. Another possibility is that the associated SNP is in linkage disequilibrium with a neighboring causal SNP (D' or r^2 >0.8) based on analysis of the region spanning 10 kbp upstream and downstream from rs11203109.

The best explanation for the associated SNP (rs11203109) may be due to a LD with a nearby functional SNP[10]. We therefore executed a computational prediction for the SNP functional effect. We predicted TFBS near the linked SNP (rs5786868) that is located in the 5' region near the *IFIT1* (promoter region). Hence rs5786868 is an INDEL SNP and the allele with G creates binding sites for two transcription factors, glucocorticoid receptor- α and androgen receptor, compared to that of the allele with G deletion. This finding suggests that the rs5786868 SNP could alter the expression of the *IFIT1*. So, rs5786868 is probably a functional SNP that influences the susceptibility to develop CM. Moreover, microRNA modulates gene expression either by transcriptional modification or translational repression in terms of microRNA sequences complementary with 3'UTR of the gene[15]. So, we performed the prediction of microRNA binding sites in the 3'UTR of the *IFIT1* at the position of rs57941432. However, there were no predicted microRNA binding sites in either allele. For the remaining four LD SNPs located in the intron, we found no reports of functional evidence.

Interestingly, a scientific report stated that rs57941432 may behave as an expression quantitative trait locus (eQTL) of *IFIT*5 in liver tissue[16]. An eQTL is a locus that explains a portion of the genetic variance of a gene expression phenotype. It is still unresolved whether eQTL is involved in the regulatory control of expression in a tissue-specific manner. Moreover, the same regulatory regions and variants could be an eQTL for different genes in different tissues[17]. This information supports the concept that rs57941432 might have a

Table 4

Computational prediction for functional effect of SNPs that are in LD with rs11203109.

SNP	LD score (D' and r^2)	Functional position	Predicted effect
rs5786868	1.000, 0.989	5' near gene, 2 kbp upstream	insert G - create TFBS for GR-alpha and AR; delete G - lost TFBS for GR-alpha and AR
rs11203105	1.000, 0.989	Intron	NA
rs147997420	1.000, 0.989	Intron	NA
rs11203106	1.000, 0.989	Intron	NA
rs10788642	1.000, 1.000	Intron	NA
rs57941432	0.994, 0.983	3'UTR	No predicted binding sites for microRNA in both alleles

TFBS, transcription factor binding sites; GR, glucocorticoid receptor; AR, androgen receptor; NA, not attempted. LD assessed by LDlink web-based application[11]. TFBS prediction for promoter region was done by PROMO database (version 3.0.2)[12,13]. Prediction of microRNA binding sites in 3'UTR was performed by an miRDB database[14].

functional role in CM outcome by regulating the *IFIT* gene. So, our finding of an associated SNP (rs11203109) might truly be a genetic marker for CM outcome since the two LD SNPs (rs5786868 and rs57941432) are putatively functional.

In the haplotype analysis, four major haplotypes were found among Thai patients. The haplotype frequencies in CM and UM groups were comparable. No significant association of the *IFIT1* haplotypes with CM was found. This suggests that there was no synergistic effect among the candidate SNPs on CM outcome. Although we selected the candidate genes from tag-SNP based on the data of HapMap, we found high LD values (r^2>0.8) among rs304487, rs303217 and rs304485. So, one of those three SNPs should be considered for future research about *IFIT1* genetics in Thai population.

Association studies of *IFIT1* polymorphism in other diseases are rare. Xie *et al*. reported that rs11203109 C allele is associated with a better virological response after IFN-α treatment in chronic hepatitis B infection[18]. This finding appears to support our own that polymorphism in the *IFIT1* gene may give a beneficial effect to the host against malaria infection via triggering the IFN signalling pathway.

In conclusion, this study found that a polymorphism in the intron of *IFIT1* (C allele of rs11203109) was associated with protection from CM in Thai population. Computational predictions indicated that rs11203109 might be a genetic marker of cerebral malaria outcome, as it is in LD with SNPs which are probably functional. Experimental validation is necessary to confirm this and to understand the regulatory mechanisms of these functional SNPs. Nevertheless, our study highlights the potential role of *IFIT1* polymorphism in the development of CM.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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