รายงานวิจัยฉบับสมบูรณ์

โครงการ Association Study of Human Genetics to Cerebral Malaria in Thai Patients Infected *Plasmodium falciparum*: Genetic Polymorphism of *Interferon Inducible Protein Tetratricopeptide Repeat* (*IFIT*) Gene Family

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สนับสนุนโดยสำนักงานคณะกรรมการการอุดมศึกษา สำนักงานกองทุนสนับสนุนการวิจัย
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บทคัดย่อ

รหัสโครงการ: MRG5480062

ชื่อโครงการ: Association Study of Human Genetics to Cerebral Malaria in Thai Patients Infected Plasmodium falciparum: Genetic Polymorphism of *Interferon Inducible Protein Tetratricopeptide Repeat* (*IFIT*) Gene Family

ชื่อนักวิจัย และสถาบัน

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ระยะเวลาโครงการ: 2011-2013

บทคัดย่อ:

Objective: โรคมาลาเรียชนิดขึ้นสมองเกิดจากการติดเชื้อ *P. falciparum* ซึ่งมีอาการแสดงทางคลินิกที่รุนแรงจนถึงขั้นเสียชีวิตได้ กลไกในการเกิดโรคเกิดจากตัวเชื้อกระตุ้นระบบภูมิคุ้มกันทำให้มีการสร้าง interferon-gamma (IFN-γ) มากว่าปกติ จากการศึกษาในหนูพบว่า interferon-induced protein with tetratricopeptide repeats (Ifit1) มีระดับการแสดงออกที่สูงในหนูที่เป็นมาลาเรียขึ้นสมองโดยเปรียบเทียบกับหนูที่ไม่เป็นมาลาเรียขึ้นสมอง โครงการวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาความหลากหลายทางพันธุกรรมของมนุษย์ในยีน IFIT1 ซึ่งอาจส่งผลต่อความไวในการเกิดโรคมาลาเรียขึ้นสมอง

Method: โครงการวิจัยนี้เป็นแบบ case-control association study โดยศึกษาในกลุ่มตัวอย่างผู้ป่วยที่ติดเชื้อมาลาเรียชนิด P. falciparum จำนวน 314 ราย โดยแบ่งเป็น 2 กลุ่มตามอาการทางคลิกนิก คือ 1) มาลาเรียชนิดขึ้นสมอง 110 ราย 2) มาลาเรียชนิดไม่รุนแรง จำนวน 204 ราย ทำการศึกษา tag SNP จำนวน 5 ตำแหน่ง จำนวน 5 ตำแหน่ง ดังต่อไปนี้ 1) rs304478 2) rs303217 3) rs303215 4) rs11203109 5) rs304485 โดยวิธี endpoint genotyping

Result: ไม่พบความสัมพันธ์ระหว่าง genotype frequencies ของ tag SNP ทั้ง 5 ตำแหน่งกับการเกิดมาลาเรียขึ้นสมอง อย่างไรก็ตามพบความสัมพันธ์ระหว่าง allele frequency ของ SNP (rs 11203109) กับการป้องกันการเกิดมาลาเรียขึ้นสมอง (Odd ratio = 0.62, 95% Confidence Interval = 0.38-0.99, P = 0.048). จากการวิเคราะห์เพิ่มเติมทาง Bioinformatics พบว่า SNP ดังกล่าว (rs 11203109) มีความเชื่อมโยงทางพันธุศาสตร์ (linkage disequilibrium) กับ SNP อีก 2 ตำแหน่ง (rs5786868 และ rs57941432)

Conclusion: จากผลการศึกษาดังกล่าวชี้ให้เห็นว่า SNP (rs11203109) สามารถใช้เป็นตัวบ่งชี้ทางพันธุกรรมของการเกิดมาลาเรียขึ้นสมองในผู้ป่วยไทยได้

Abstract

Project Code : MRG5480062

Project Title : Association Study of Human Genetics to Cerebral Malaria in Thai Patients Infected Plasmodium falciparum: Genetic Polymorphism of Interferon Inducible Protein.Tetratricopeptide Repeat (IFIT) Gene Family

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Project Period : 2011-2013

Abstract:

Objective: Cerebral malaria is the most severe clinical manifestation of *Plasmodium falciparum* infection. The malaria parasites stimulate host immune response leading to the overproduction of interferon (IFN)-γ that contributes to the pathophysiology of cerebral malaria. In the IFN signaling pathway, interferon-induced protein with tetratricopeptide repeats (Ifit)1 expression is increased in a cerebral malaria mouse model compared to resistant controls. To elucidate the effect of IFIT1 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 *P. falciparum*. Genotyping for five tag-single nucleotide polymorphisms of IFIT1 was performed by endpoint genotyping.

Results: Genotype frequencies of all tag-SNPs showed no association with cerebral malaria outcome. However, C allele of rs11203109 was associated with the protection from cerebral malaria (Odd ratio = 0.62, 95% Confidence Interval = 0.38-0.99, P = 0.048). Two SNPs (rs5786868 and rs57941432) were in linkage disequilibrium with rs11203109.

Conclusion: This suggested that our associated SNP (rs11203109) might be a genetic marker for development of cerebral malaria in the Thai population.

Keywords : *IFIT1*; polymorphisms; cerebral malaria; Thai; rs11203109

เนื้อหางานวิจัยประกอบด้วย

1. บทคัดย่อภาษาไทย และภาษาอังกฤษ (ตามเอกสารแนบหมายเลข 2/1 และ 2/2)

2. บทสรุปผู้บริหาร (Executive Summary) ประกอบด้วย:

ที่มาและความสำคัญของปัญหา-ทบทวนวรรณกรรม

Malaria is a major global health problem with 91 countries and territories at risk especially in tropical regions. In the year 2015, approximately 214 million people worldwide were infected with malaria and of them 429,000 people died [1]. Cerebral malaria (CM), the most severe complication of *Plasmodium falciparum* infection, is the major cause of death and is most frequently encountered in African children (19% case fatality rate)[2]. It is hypothesized that the mechanism of cerebral malaria involves excessive sequestration of parasitized red cells in the brain's microvasculature and local overproduction of inflammatory cytokines such as IFN-γ and tissue necrotic factor-α [3-5]. Although the exact pathogenesis of cerebral malaria is still unclear, it is believed that human genetic factors predispose to this clinical outcome [6].

IFN-γ, encoded by the INF-γ gene (*IFNG*), plays an important role in the pathogenesis of cerebral malaria. The polymorphisms in *IFNG* and *IFN-γ* receptor 1 (*IFNGR1*) gene are associated with the clinical manifestations of malaria infection [7, 8]. Although the role of IFN-α/β in malaria has not been investigated extensively, an interferon alpha/beta receptor-1 (IFNAR1) variant is associated with protection against cerebral malaria in the Gambia [9]. In addition, a study in an experimental malaria mouse model shows that expression of the interferon-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 cerebral malaria susceptibility locus. Among them, *Ifit1/Isg56* expression is predominantly upregulated [10]. These observations support the hypothesis that

downstream variants in the interferon signalling pathway (*IFIT1* in humans) may influence whether or not CM develops. To date, there are no reports of an association between polymorphisms of *IFIT1* and cerebral malaria. The objective of our study was to access the relationship of single nucleotide polymorphisms (SNP) in the *IFIT1* gene with the development of cerebral malaria in Thai malaria patients.

วัตถุประสงค์

To elucidate the association between *IFIT1* SNP and cerebral malaria development in Thai patients infected with *P.falciparum*

วิธีทดลอง

Subjects

We recruited 314 Thai adult patients (13 years or older) infected with *P. falciparum* who were living in the north-west area of Thailand near the border with Myanmar. Clinical manifestations of malaria were classified according to the criteria of the World Health Organization. We selected 110 cerebral malaria patients who suffering unarousable coma with blood smear positive for asexual forms of *P. falciparum* and other causes of coma excluded. Also, we selected a control group of 204 patients with uncomplicated malaria (UM) who also had blood smears positive for asexual forms of *P. falciparum* along with symptoms of febrile illness without any other cause of infection. For the control group, we excluded all patients who had any signs of severe malaria or evidence of vital organ dysfunction 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 underwent treatment at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Thailand. Informed consent was obtained from all patients. The study was approved by the Institutional Review Board of the Faculty of Tropical Medicine, Mahidol University, Thailand.

SNP Selection

IFIT1 is situated on the long arm of chromosome 10 (10q23.31) and 13,942 nucleotides in length (Figure 1). 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 association between *IFIT1* and cerebral malaria, tag-SNPs located in the *IFIT1* gene were selected based on genotypic data of the Asian HapMap samples, 45 Japanese from Tokyo, Japan (JPT) and 45 Han Chinese from Beijing, China (CHB) [11, 12]. Tagger algorithm was implemented in the Haploview Software, version 4.2 with the default settings of pairwise tagging only with linkage disequilibrium ($r^2 \geq 0.8$). Among five tag-SNPs, one SNP (rs304478) was situated about 1.5 kb 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 2).

Genotyping

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

Statistical Analysis

To determine the extent of deviation from Hardy–Weinberg Equilibrium (HWE), 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 considered statistically significant. The association between the genotypes and allele frequency for the risk of CM was analyzed by calculating odds ratios (ORs) and 95% confidence intervals (CIs) in various models. To know the extent of linkage disequilibrium (LD) between SNPs, we calculated the pairwise linkage disequilibrium coefficient by using Haploview Software, version 4.2 (13). The haplotype frequency and its association with cerebral malaria was analyzed by using Haploview Software, version 4.2 [13].

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. To access whether the associated SNP had LD with a nearby functional SNP, we retrieved the SNPs in the region of 10 kb 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 1000 Genomes Project [14]. After obtaining 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 predicted by PROMO database (version 3.0.2). It is a virtual laboratory for the identification of putative transcription factor binding sites in DNA sequences from a species or group of species of interest. TFBS defined in the TRANSFAC database (version 8.3) are used to construct specific binding site weight matrices for TFBS prediction [15, 16]. For SNP located in the 3' untranslated region (UTR) of the *IFIT1* gene, prediction of microRNA binding sites was determined with an miRDB database that uses a bioinformatics tool, MirTarget, developed by analyzing thousands of miRNA-target interactions from high-throughput sequencing experiments [17].

แผนการดำเนินงาน

- Bioinformatics analysis for tag SNP selection
- Molecular Genotyping
- Statistical Analysis
- Manuscript Submission

3. ผลการทดลอง

To examine possible associations between *IFIT1* polymorphism and cerebral malaria, 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 agreement with Hardy-Weinberg equilibrium ($P > 0.05$). The genotype frequencies of all SNPs in various genetic models were comparable between the two malaria groups ($P > 0.05$). Association analysis revealed that the minor C allele of rs11203109 was associated with protection from cerebral malaria (OR = 0.62, 95% CI = 0.38-0.99, P = 0.048) (Table 1). Four major haplotypes consisting of five *IFIT1* SNPs showed no association with cerebral malaria. (Table 2)

To find the SNPs that are in LD with rs11203109, we retrieved the SNPs located within 10 kb 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. (Fig. 3) To determine whether the linking SNPs have a functional effect, we performed computational analysis for transcription factor binding sites for the SNPs located in the 5’ region and microRNA binding sites for the SNPs located in the 3’ UTR of the *IFIT1*. Table 3 shows the SNP that are in LD with rs11203109 and results of the computational prediction of transcription factor binding sites and microRNA binding sites.

4. สรุปและวิจารณ์ผลการทดลอง และข้อเสนอแนะสำหรับงานวิจัยในอนาคต

To address the involvement of *IFIT1* gene polymorphism in cerebral malaria, 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 found to be associated with protection against cerebral malaria. 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 neighbouring causal SNP, (D’ or r^2 >0.8) based on analysis of the region spanning 10 kb upstream and downstream from rs11203109.

Gene expression may be influenced by the SNPs located within the coding region (cSNP), in the splice site (sSNP), in the non-coding regulatory region (rSNP) such as in promoters, flanking regions, 5’ or 3’ UTR, or in the intron [18]. The majority (~93%) of disease- and trait-associated variants are situated within the noncoding sequence and involve the transcriptional regulation mechanism by modulating promoter or enhancer sequences [19]. Since the development of chromatin immunoprecipitation (ChIP) accompanied by microarray (ChIP-chip) or massive parallel sequencing (ChIP-seq), evidence for the role of rSNP in regulating gene expression has increased. rSNPs may affect gene expression dramatically by eliminating or creating binding sites for transcription factors or by influencing the affinity of existing transcription factor binding sites [18]. There are several examples of rSNPs that influence the level or pattern of gene expression in association with various diseases. The polymorphism of *CELSR2* in 3’ UTR at 1p13 locus, the minor T

allele of rs12740374, creates a C/EBP transcription factor binding site and reduces the expression of the nearby *SORT1* gene in hepatocytes [20]. Genome-wide association analysis supposes that non-coding variants in the human genome may influence the risk of a common disease by regulating at a distance the acting transcriptional enhancers [21]. The common allele of the enhancer sequence located in intron 1 of the *RET* gene is associated with a twenty-fold increase in Hirschsprung disease susceptibility[22]. Our CM-associated SNP (rs11203109) is located in the intron. Although the genetic association observed in the present study may support the regulatory role of this *IFIT1* intronic SNP in the development of cerebral malaria, the functional significance of rs11203109 remains to be determined.

The explanation for the associated SNP (rs11203109) may be due to a LD with a nearby functional SNP. We therefore executed a computational prediction for the SNP functional effect. We predicted the location of TFBS near the linked SNP 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 (GR)-alpha and androgen receptor (AR), 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 cerebral malaria. Moreover, microRNA modulates gene expression either by transcriptional modification or translational repression in terms of microRNA sequences complementary with 3'UTR of the gene.[23] 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 *IFIT5* in liver tissue.[24] 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.[25] This information supports the concept that rs57941432 might have a functional role in cerebral malaria outcome by regulating the *IFIT* gene. So, our finding of an associated SNP (rs11203109) might

truly be a genetic marker for cerebral malaria outcome since the two LD SNPs (rs5786868 and rs57941432) are putatively functional.

In the haplotype analysis, four major haplotypes were observed in Thai malaria patients. The haplotype frequencies in cerebral and uncomplicated malaria patients were comparable, and no significant association of the *IFIT1* haplotypes with cerebral malaria was found. This suggests that there was no synergistic effect among the candidate SNPs on cerebral malaria outcome. Although we selected the candidate genes from tag-SNP based on the data of HapMap (CHB and JPT), we found high LD values (r^2 more than 0.8) among rs304487, rs303217 and rs304485. So, one of those three SNPs should be analysed in a future genetic association study of *IFIT1* in the Thai population.

Association studies of *IFIT1* polymorphism in other diseases are rare. *IFIT1* induction is high in viral infections and consequently there are polymorphism studies in hepatitis B and C infections. Xie DY et al. reported that rs11203109 C allele is associated with a better virological response after IFN-α treatment in chronic hepatitis B infection. [26] In chronic hepatitis C patients, A/A genotype of rs304478 is associated with a better therapeutic outcome, especially in patients infected with HCV-1.[27] 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 interferon signalling pathway. Additionally, the transcription of *IFIT1* is regulated by IFN-stimulated response elements (ISRE) and IFN regulatory factors (IRF). The stimuli for the transcription of *IFIT1* are IFN-α/β (major inducer), IFN-γ (weak inducer), and viral infections.[28] There is evidence that polymorphism in *IRF8* (rs11117432) is associated with decreased mRNA expression of *IFIT1* in European Caucasian. (29) Similarly, another *IRF8* polymorphism (rs17445836) is associated with decreased mRNA expression of *IFIT1* in anti-dsDNA negative SLE patients. (30) So, *IRF8* polymorphism should be considered for inclusion in further association studies of malaria.

In conclusion, this study found that a polymorphism in the intron of *IFIT1* (C allele of rs11203109) was associated with protection from cerebral malaria among the 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 cerebral malaria.

5. ภาคผนวก

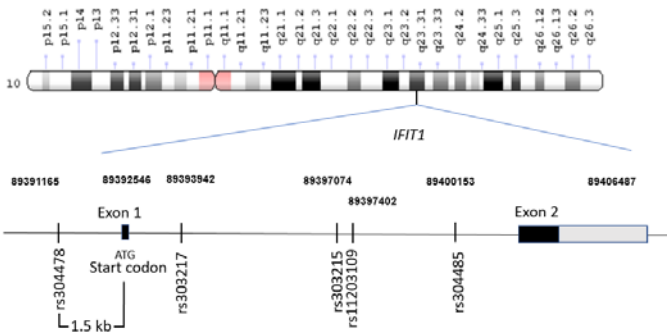

Figure 1. Chromosomal location of candidate SNPs in *IFIT1* gene

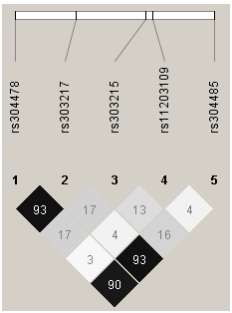

Figure 2. LD analysis of tag-SNPs by Haploview software version 4.2.
LD plot among five SNPs that were analysed in 314 Thai malaria patients.
A pairwise r^2 value is shown in each square. Darker shading indicates higher r^2 value

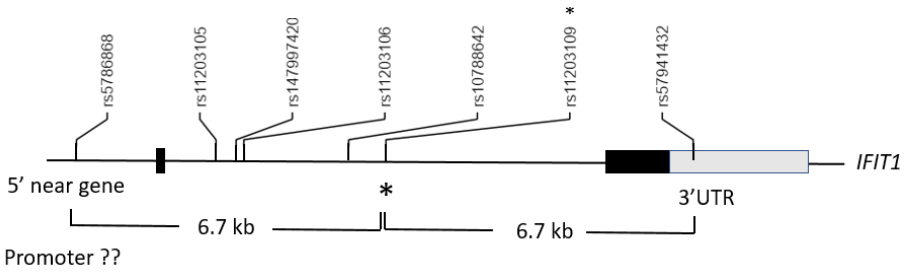

Figure 3. Chromosomal position of SNPs that are in LD with rs11203109 LD was assessed by LDlink web-based application (14) that used genotype data of the East

Asian from phase 3 of the 1000 Genomes Project and variant RS numbers are indexed based on dbSNP build 42. * indicates the position of the associated SNP.

Table 1. Genotype and allele frequencies of SNPs in *IFIT1* and their statistical association to risk of CM

SNP	CM (n=110) N (%)	UM (n=204) N (%)	P value	OR (95% CI)
rs304478			0.821	
TT	67 (60.9)	131(64.2)		
TG	40 (36.4)	67 (32.8)	0.53 (TG Vs TT)	1.17 (0.72-1.91)
GG	3 (2.7)	6 (2.9)	0.98 (GG Vs TT)	0.98 (0.24-4.03)
Dominant model	43 (39.1)	73 (35.8)	0.56(TT Vs TG+GG)	1.15 (0.71-1.86)
Over-dominant model	70 (63.6)	137(67.2)	0.53(TT+GG Vs TG)	1.17 (0.72-1.9)
Recessive model	107(97.3)	198 (97.1)	0.91(TT+TG Vs GG)	0.93 (0.23-3.77)
T allele	174 (0.79)	329 (0.81)		
G allele	46 (0.21)	79 (0.19)	0.64 (G Vs T allele)	1.1(0.73-1.65)
HWE	0.29	0.46		
rs303217			0.484	
CC	65 (59.1)	131(64.2)		
CT	43 (39.1)	67 (32.8)	0.29 (CT Vs CC)	1.29 (0.8-2.1)
TT	2 (1.8)	6 (2.9)	0.63 (TT Vs CC)	0.67 (0.13-3.42)
Dominant model	45 (40.9)	73 (35.8)	0.37 (CC Vs CC+CT)	1.24 (0.77-2.0)
Over-dominant model	67 (60.)	137(67.2)	0.27 (CC+TT Vs CT)	1.31 (0.81-2.12)
Recessive model	108(98.2)	198(97.1)	0.54 (CC+CT Vs TT)	0.61 (0.12-3.08)
C allele	173 (0.79)	329 (0.81)		
T allele	47 (0.21)	79 (0.19)	0.55 (T Vs C allele)	1.13 (0.75-1.69)
HWE	0.086	0.46		
rs303215			0.936	
TT	37 (33.6)	70 (34.3)		
TC	54 (49.1)	102 (50)	0.99 (TC Vs TT)	1.0 (0.6-1.68)
CC	19 (17.3)	32 (15.7)	0.74 (CC Vs TT)	
Dominant model	73 (66.4)	137(65.7)	0.9 (TT Vs TC+CC)	1.12 (0.56-2.25)
Over-dominant model	56 (50.9)	102(50)	0.88(TT+CC Vs TC)	1.03 (0.63-1.68)
Recessive model	91(82.7)	172(84.3)	0.72(TT+TC Vs CC)	0.96 (0.61-1.53)
T allele	128 (0.58)	242 (0.59)		1.12 (0.60-2.09)
C allele	92 (0.42)	166 (0.41)	0.78 (C Vs T allele)	
HWE	0.93	0.61		1.05 (0.75-1.46)
rs11203109			0.13	
TT	84 (76.4)	136 (66.7)		
TC	25 (22.7)	61(29.9)	0.13 (TC Vs TT)	0.66 (0.39-1.14)
CC	1 (0.9)	7 (3.4)	0.17 (CC Vs TT)	0.23 (0.03-1.91)
Dominant model	26 (23.6)	68 (33.3)	0.07(TT Vs TT+TC)	0.62 (0.37-1.05)
Over-dominant model	85 (77.3)	143 (70.1)	0.17(TT+CC Vs TC)	0.69 (0.4-1.18)
Recessive model	109(99.1)	197(96.6)	0.14(TT+TC Vs CC)	0.26 (0.03-2.13)
T allele	193 (0.88)	333 (0.82)		
C allele	27 (0.12)	75 (0.18)	**0.048 (C Vs T allele)**	**0.62 (0.38-0.99)**
HWE	0.56	0.96		
rs304485			0.638	
AA	65 (59.1)	131(64.2)		
AT	42 (38.2)	67 (32.8)	0.34 (AT Vs AA)	1.26 (0.78-2.06)
TT	3 (2.7)	6 (2.9)	0.99 (TT Vs AA)	1.01 (0.24-4.16)
Dominant model	45 (40.9)	73 (35.8)	0.37(AA Vs AT+TT)	1.24 (0.77-2.0)
Over-dominant model	68 (61.8)	137(67.2)	0.34(AA+TT Vs AT)	1.26 (0.78-2.05)

Recessive model	107(97.3)	198(97.1)	0.91(AA+AT Vs TT)	0.93 (0.23-3.77)
A allele	172 (0.78)	329 (0.81)		
T allele	48 (0.22)	79 (0.19)	0.46 (T Vs A allele)	1.16 (0.78-1.74)
HWE	0.21	0.46		

Table 2. Haplotype analyses of rs304478, rs303217, rs303215, rs11203109 and rs304485 for the risk of CM

Haplotype	Sequence	Cerebral malaria (n=110)	Mild malaria (n=204)	χ^2	p value
H1	TCCTA	0.409	0.404	0.013	0.9092
H2	TCTTA	0.241	0.216	0.522	0.4699
H3	GTTTT	0.200	0.186	0.174	0.6764
H4	TCTCA	0.123	0.179	3.371	0.0664

Table 3. 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, 0.989	5' near gene, 2 kb upstream (Promoter ?)	insert G - create TFBS for GR-alpha and AR delete G - lost TFBS for GR-alpha and AR
rs11203105	1, 0.989	Intron	NA
rs147997420	1, 0.989	Intron	NA
rs11203106	1, 0.989	Intron	NA
rs10788642	1, 1	Intron	NA
rs57941432	0.994, 0.983	3'UTR	NA No predicted binding sites for microRNA in both allele

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Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ (ระบุชื่อผู้แต่ง ชื่อเรื่อง ชื่อวารสาร ปี เล่มที่ เลขที่ และหน้า) หรือผลงานตามที่คาดไว้ในสัญญาโครงการ

1. Wah ST, Hananantachai H, kerdpin U, Prachayasittikul V and **Nuchnoi P*.** Molecular Basis of Human Cerebral Malaria Development. Trop Med Health. 2016;44:33.
2. **Nuchnoi P***, Thongbus J, Srisarin A, Kerdpin U and Prachayasittikul V. Clinical and Laboratory Update on the DEL Variants. Lab Med.2014;285-290.
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5. Thongbut J, Kerdpin U, Sakuldamrongpanich T, Isarakura-Na-Ayudhya C and **Nuchnoi P*.** RHD Specific microRNA for Regulation of the DEL Blood Group: Integration of Computational and Experimental Approaches. Br J Biomed Sci. 2017;74(4):181-186.
6. Kandhro AH, Prachayasittikul V, Isarakura-Na-Ayudhya C and **Nuchnoi P*.** Prevalence of Thalassemia Traits and Iron Deficiency Anemia in Sindh, Pakistan. Hemoglobin. 2017;41(3):157-163.

การนำผลงานวิจัยไปใช้ประโยชน์เชิงวิชาการ(การพัฒนาการเรียนการสอน/สร้างนักวิจัยใหม่):

ทุนวิจัยที่ได้รับจากสกว.ได้ช่วนในการสร้างนักวิจัยใหม่ทั้งในระดับปริญญาโทและปริญญาเอก ซึ่งมีผศ.ดร.พรลดา นุชน้อย ผู้รับทุนจากสกว.เป็นอาจารย์ที่ปรึกษาหลัก ดังต่อไปนี้

ระดับปริญญาโท

นางสาวใจรัก ทองบุศย์ สำเร็จการศึกษาวิทยาศาสตรมหาบัณฑิต (เทคนิคการแพทย์) ปีการศึกษา 2558 ผลงานวิจัย:

1. **Nuchnoi P***, Thongbus J, Srisarin A, Kerdpin U and Prachayasittikul V. Clinical and Laboratory Update on the DEL Variants. Lab Med.2014;285-290.
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ระดับปริญญาเอก

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1.1 **Kandhro AH**, Shoombuatong W, Nantasenamart C, Prachayasittikul V and **Nuchnoi P*.** The MicroRNA Interaction Network of Lipid Diseases. Front Genet. 2017;8:116.

1.2 **Kandhro AH**, Shoombuatong W, Prachayasittikul V and **Nuchnoi P*.** New Bioinformatics-Bases Discrimination Formulas for Differentiation of Thalassemia Traits from Iron Deficiency Anemia. Lab Med. 2017;48(3):230-237

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2.1 **Wah ST**, Hananantachai H, kerdpin U, Prachayasittikul V and **Nuchnoi P*.** Molecular Basis of Human Cerebral Malaria Development. Trop Med Health. 2016;44:33.

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