

STUDY ON GENETIC POLYMORPHISMS OF HEME OXYGENASE-1 IN DIFFERENT ETHNIC GROUPS OF MALARIA PATIENTS

Jiraporn Kuesap¹ Kenji Hirayama² Mihoko Kikuchi² Ronnatrai Ruangweerayut³

Kesara Na-Bangchang^{1,*}

¹Pharmacology and Toxicology Unit, Graduate Program in Biomedical Sciences, Faculty of Allied Health Sciences, Thammasat University, Pathumthani, Thailand ²Department of Molecular Immunogenetics, Institute of Tropical Medicine, Nagasaki University, Japan ³Mae Sot General Hospital, Tak Province, Thailand

ABSTRACT: The study in genetic polymorphism of heme oxygenase-1 (HO-1) in different ethnic groups of malaria patients, we analyzed the frequencies of (GT)_n alleles in a total of 200 Thai, Burmese and Karen patients. In Thai patients, the frequencies of short (21-27), medium (28-33) and long (34-39) (GT)_n repeats of HO-1 gene were 89 (50.6%), 75 (42.6%), and 12 (6.8%), respectively. The corresponding values observed in Burmese patients were 82 (45.0%), 84 (46.2%), and 16 (8.8%), whereas that in Karen patients were 24 (57.1%), 12 (28.6%), 6 (14.3%), respectively. The SL genotype of Thais was significantly different from Burmese. This finding may suggest that difference in the expression of HO-1 genotype in different ethnic groups may associate to malaria disease.

Keywords: heme oxygenase, malaria, *Plasmodium falciparum*

INTRODUCTION: Malaria is an infectious disease that causes enormous medical, economic and emotional burdens in developing countries. Malaria infection causes a range of clinical symptoms from asymptomatic or mild flu-like illness to the rarer complications of severe manifestation. In Thailand, malaria is still problematic in particular areas, especially in the forest and forest fringes and along international borders where there is significant population movement. The highest incidence of malaria cases in 2006 was reported from Tak Province, where Mae Sot District is located. They have carried the largest burden of malaria diagnosis and treatment for foreigners, the majority of whom are from Myanmar¹⁾ In the late 1980s, resistance to sulfadoxine - pyrimethamine and mefloquine was reported from the Thai-Cambodian and Thai-Myanmar borders, rendering them established multidrug resistant (MDR) areas²⁾.

Recently, host's heme oxygenase (HO) enzyme has

been proposed as one of the factors that may play significant role in pathogenesis including susceptibility and severity of malaria infection^{3,4)}. It is a microsomal enzyme which exists in two isoforms, HO-1 and HO-2. HO-1 and HO-2 play important role in heme degradation process, which is eventually yielding biliverdin/bilirubin, carbonmonoxide (CO) and iron^{3,5,6,7)}. Expression of HO-1 is inducible or repressible, depending on cell types or cellular microenvironments⁴⁾. The human HO-1 gene promoter contains the (GT)_n repeat polymorphism which may contribute to the fine tuning of the transcription. Long (GT)_n alleles have been found associated with susceptibility to smoking-induced emphysema or coronary artery disease, while they may be linked to resistance to cerebral malaria^{8,9,10)}. In this study, we analyzed (GT)_n repeat polymorphism in the promoter region of the inducible HO-1 in malaria patients (Thais, Burmese and Karens), to investigate ethnic difference in HO-1 polymorphism.

* To whom correspondence should be addressed.
E-mail: kesaratmu@yahoo.com,
Tel. 66 2986 9213 ext.7271, Fax. 66 2516 5379

MATERIALS AND METHODS:

Subjects and blood collection

Malaria patients (n=200; mean age 26.5 years; parasitemia, 1,200-800,000 / μ l) consisting of 88 (44%) Thais, 91 (45.5%) Burmese and 21 (10.5%) Karens who were presented at malaria clinic and Mae Sot General Hospital, Mae Sot, Thailand were recruited into the study. Blood samples (1 ml) were collected from all patients for analysis of genetic polymorphism of HO-1. These samples consisted of 190 *P.falciparum* (189 uncomplicated malaria and 1 cerebral malaria) and 10 *P.vivax* cases. The protocol, including the informed consent form, was approved by the Ethics Committee of the Ministry of Public Health, Thailand.

Extraction of genomic DNA

Genomic DNA was extracted from 1 ml of peripheral venous blood with 0.05 EDTA using standard phenol-chloroform extraction technique.

Microsatellite polymorphism

To amplify the (GT)_n microsatellite located at position -270 of HO-1 gene the PCR assays were performed over 35 cycles of 30 s at 95°C, 30 s at 60°C, and 3 min at 72°C. A fluorescently labeled primer p1 (5'-AGAGCCTGCAGCTTCTCAGA-3') and an unlabeled anti-sense primer p2 (5'-ACAAAGTCTGGCCATAGGAC-3') was designed according to the published sequence¹⁰⁾. The PCR products were analyzed by a laser-based automated DNA sequencer, ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, Calif., USA).

Statistical analysis

All statistical analyses were performed using the SPSS statistical package (version 11.5). The association between prevalence of HO-1 genotypes in different ethnic groups was analyzed using chi-square test at a statistical significance level (α) of 0.05.

RESULTS: Up to now, there have been only two published studies investigating the association between the microsatellite polymorphism of HO-1 gene promoter and malaria disease. The first study reported a significant association between short (GT)_n alleles and incidence of cerebral malaria¹⁰⁾. In this study, the ethnic

groups of malaria patients were found to be associated with the HO-1 genotype especially SL genotype of Thais and Burmese.

The number of (GT)_n repeats of the HO-1 gene varied between 21 and 39 in all patients (Fig.1). The allele frequencies of (GT)_n allele were similar in the three ethnic groups. The repeat distribution of (GT)_n allele were trimodal, with peaks at 23, 30 and 37 in Thai and Burmese population and at 23, 30 and 38 in Karen population. We divided the alleles into three groups: short (S) with 21-27 repeats, intermediate (M) with 28-33 repeats and long (L) alleles with 34-39 repeats. The frequencies of the S, M and L in Thais were 0.51, 0.43 and 0.07 respectively. The corresponding frequencies of S, M and L in Burmese and Karen patients were 0.45, 0.46, and 0.09 and 0.57, 0.29, and 0.14, respectively. The allele frequency of the Burmese patients found in this study is in agreement with that has previously been reported in Karen patients with uncomplicated falciparum malaria (S=0.44, M=0.45 and L=0.11)¹⁰⁾. The allele frequencies in Thai, Burmese and Karen patients were not significantly different.

The frequencies of the genotypes (SS, SM, SL, MM, ML, LL) of (GT)_n repeats in Thai patients were 25 (28.4%), 36 (40.9%), 3 (3.4%), 16 (18.2%), 7 (8.0%), and 1 (1.1%), respectively, while they were 18 (19.8%), 34 (37.4%), 12 (13.2%), 24 (26.4%), 2 (2.2%), and 1 (1.1%) respectively in Burmese patients, and 9 (42.9%), 5 (23.8%), 1 (4.8%), 2 (9.5%), 3 (14.3%), and 1 (4.8%) respectively in Karen patients (table 1). The frequency of SL genotype in Thai patients was significantly different from Burmese patients ($p=0.036$). In a previous study in malaria disease, the frequency of homozygotes (SS) in cerebral malaria group was reported to be significantly higher than in the uncomplicated group¹⁰⁾. We investigated the association of parasite and HO-1 gene in three ethnic groups of patients. No significant difference between parasitemia and ethnic groups, genotype, allele and group of L and non-L allele, as well as between parasite type (*P.falciparum* and *P.vivax*) and allele including group of

L and non-L allele was found. However these results may be due to the limited number of samples and lack of the diversity of parasite in severity such as, cerebral malaria or other type of severe malaria.

Table 1 Distribution of HO-1 promoter genotypes and allele frequencies of the malaria patients

<i>n</i>	Ethnic		
	Thai	Burmese	Karen
Alleles, <i>n</i> (%)			
S	89 (50.6%)	82 (45.1%)	24 (57.1%)
M	75 (42.6%)	84 (46.2%)	12 (28.6%)
L	12 (6.8%)	16 (8.8%)	6 (14.3%)
Genotypes, <i>n</i> (%)			
S/S	25 (28.4%)	18 (19.8%)	9 (42.9%)
S/M	36 (40.9%)	34 (37.4%)	5 (23.8%)
S/L	3 (3.4%)	12 (13.2%)*	1 (4.8%)
M/M	16 (18.2%)	24 (26.4%)	2 (9.5%)
M/L	7 (8.0%)	2 (2.2%)	3 (14.3%)
L/L	1 (1.1%)	1 (1.1%)	1 (4.8%)

*The frequency of Burmese statistically significant higher than Thai population ($p=0.036$)

DISCUSSION: The (GT)_{*n*} repeat polymorphism in the HO-1 gene promoter has been demonstrated in various studies with different ethnic backgrounds. In Chinese population, the repeat number range from 16 to 38, with peak at (GT)₂₃ and (GT)₃₀; the allele frequencies were classified into S (14.3%), M (80.9%) and L (4.8%) in control subject with diabetes and S (10.9%), M (72.5%) and L (16.6%) in coronary artery disease (CAD) with diabetes and among type 2 diabetic patients¹¹⁾.

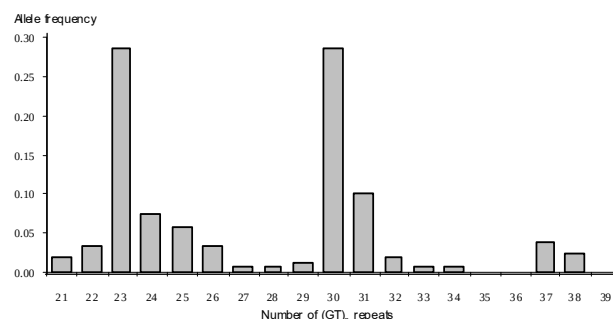
In Japanese population, The numbers of (GT)_{*n*} repeats in the human HO-1 gene showed a distribution of 16–38. The distribution of the numbers of (GT)_{*n*} repeats was trimodal, at 22, 27 and 30 GT repeats⁸⁾.

The number of (GT)_{*n*} repeats in the HO-1 gene promoter ranged from 11 to 41 in French population. The distribution of (GT)_{*n*} repeats was trimodal with

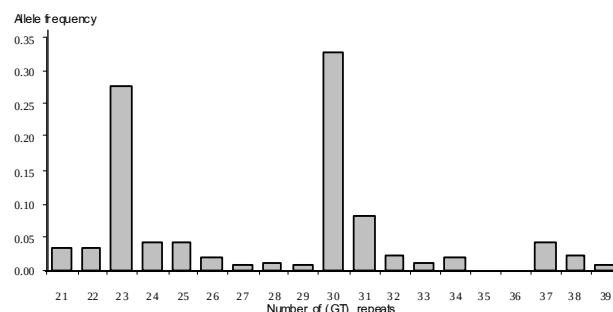
peaks at 23, 30, and 38 repeats. Alleles with (GT)_{*n*} repeats <27, 27–32 and >32 were categorised as S (38.1%), M (55.1%) and L (6.8%), respectively¹²⁾.

The findings support the hypothesis that ethnic specific are influence to genetic polymorphisms of HO-1 and might associate to malaria disease. The analysis of association between ethnic groups and genetic polymorphisms of HO-1 gene and malaria pathology in a larger number of patients with different degree of disease severity is required to definitely conclude on this hypothesis.

A. Frequency distribution of (GT)_{*n*} repeats in Thai patients



B. Frequency distribution of (GT)_{*n*} repeats in Burmese patients



C. Frequency distribution of (GT)_{*n*} repeats in Karen patients

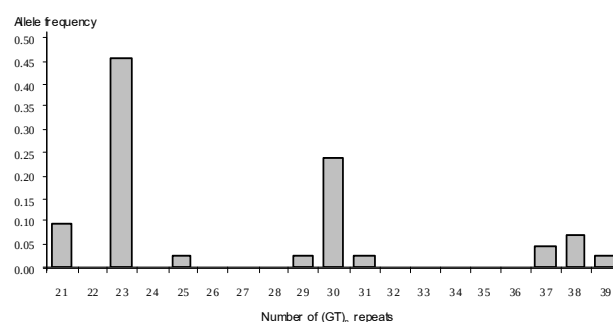


Figure 1 Frequency distribution of (GT)_{*n*} repeats in three ethnic groups of patients

REFERENCES:

1. Na-Bangchang K, Congpuong K. 2007. Current malaria status and distribution of drug resistance in East and Southeast Asia with special focus to Thailand. *Tohoku J Exp Med* 211(2): 99-113.
2. Wongsrichanalai C, Lin K, Pang LW, Faiz MA, Noedl H, Wimonwattawatee T, *et al.* 2001. In vitro susceptibility of *Plasmodium falciparum* isolates from Myanmar to antimalarial drugs. *Am J Trop Med Hyg* 65(5): 450-5.
3. Shibahara S, Kitamuro T, Takahashi K. 2002. Heme degradation and human disease: diversity is the soul of life. *Antioxid Redox Signal* 4(4): 593-602.
4. Shibahara S. 2003. The heme oxygenase dilemma in cellular homeostasis: new insights for the feedback regulation of heme catabolism. *Tohoku J Exp Med* 200(4): 167-86.
5. Tenhunen R, Marver HS, Schmid R. 1968. The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. *Proc Natl Acad Sci USA* 61(2): 748-55.
6. Tenhunen R, Marver HS, Schmid R. 1969. Microsomal heme oxygenase. Characterization of the enzyme. *J Biol Chem* 244(23): 6388-94.
7. Maines MD. 1988. Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. *FASEB J* 2(10): 2557-68.
8. Yamada N, Yamaya M, Okinaga S, Nakayama K, Sekizawa K, Shibahara S, *et al.* 2000. Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. *Am J Hum Genet* 66(1): 187-95.
9. Exner M, Schillinger M, Minar E, Mlekusch W, Schlerka G, Haumer M, *et al.* 2001. Heme oxygenase-1 gene promoter microsatellite polymorphism is associated with restenosis after percutaneous transluminal angioplasty. *J Endovasc Ther* 8(5): 433-40.
10. Takeda M, Kikuchi M, Ubalee R, Na-Bangchang K, Ruangweerayut R, Shibahara S, *et al.* 2005. Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to cerebral malaria in Myanmar. *Jpn J Infect Dis* 58(5): 268-71.
11. Chen YH, Lin SJ, Lin MW, Tsai HL, Kuo SS, Chen JW, *et al.* 2002. Microsatellite polymorphism in promoter of heme oxygenase-1 gene is associated with susceptibility to coronary artery disease in type 2 diabetic patients. *Hum Genet* 111(1): 1-8.
12. Guénégué A, Leynaert B, Bénessiano J, Pin I, Demoly P, Neukirch F, *et al.* 2006. Association of lung function decline with the heme oxygenase-1 gene promoter microsatellite polymorphism in a general population sample. Results from the European Community Respiratory Health Survey (ECRHS), France. *J Med Genet* 43(8): e43,.

การศึกษาความหลากหลายทางพันธุกรรมของเอนไซม์ฮีโมออกซิเจนเนส 1 ในผู้ป่วยมาลาเรียที่มีเชื้อชาติต่างกัน

จิราภรณ์ คัญทรัพย์¹ เคนจิ อิรายามา² มิโฮโกะ คิคุจิ² รณไตร เรืองวีรยุทธ³ เกศรา ณ บางช้าง^{1,*}

¹ภาควิชาเภสัชวิทยาและพิษวิทยา โครงการบัณฑิตศึกษา สาขาชีวเวชศาสตร์ คณะสหเวชศาสตร์ มหาวิทยาลัยธรรมศาสตร์

²สถาบันวิจัยเวชศาสตร์เขตร้อน มหาวิทยาลัยนาซากิ ประเทศญี่ปุ่น ³โรงพยาบาลแม่สอด จังหวัดตาก

บทคัดย่อ: ศึกษาความหลากหลายของจีโนมออกซิเจนเนส 1 ในผู้ป่วยมาลาเรียที่มีเชื้อชาติต่างกัน วิเคราะห์ความถี่ของ (GT)_n อัลลีล ในผู้ป่วยมาลาเรีย 200 คน ซึ่งเป็นคนไทย พม่า และกะเหรี่ยง โดยแบ่งความถี่ของ (GT)_n ออกเป็นสามกลุ่มคือ ขนาดสั้น 21-27 ขนาดกลาง 28-33 และขนาดยาว 34-39 ผลการวิเคราะห์ในกลุ่มผู้ป่วยชาวไทยมีขนาดสั้น 89 ราย (50.6%) กลาง 75 ราย (42.6%) และยาว 12 ราย (6.8%) สำหรับผู้ป่วยชาวพม่ามีขนาดสั้น 82 ราย (45.1%) กลาง 84 ราย (46.2%) และยาว 16 ราย (8.8%) ขณะที่ผู้ป่วยชาวกะเหรี่ยงมีความถี่ของ (GT)_n ขนาดสั้น กลาง และยาว เป็น 24 ราย (57.1%) 12 ราย (28.6%) และ 6 ราย (14.3%) ตามลำดับ นอกจากนี้ยังพบว่าจีโนไทป์ (genotype) ชนิด SL ของผู้ป่วยชาวไทย มีความแตกต่างอย่างมีนัยสำคัญกับผู้ป่วยชาวพม่า การแสดงออกของจีโนไทป์ของเอนไซม์ฮีโมออกซิเจนเนสที่มีความแตกต่างกันในประชากรต่างเชื้อชาติ อาจเป็นปัจจัยเกี่ยวข้องกับการเกิดโรคมาลาเรีย

คำสำคัญ: ฮีโมออกซิเจนเนส มาลาเรีย พลาสโมเดียม ฟัลซิพารัม

*ติดต่อได้ที่ kesaratmu@yahoo.com โทรศัพท์ 66 2986 9213 ต่อ 7271 โทรสาร 66 2516 5379