GENETIC VARIANTS OF β-GLOBIN GENE IN THAI MALARIA PATIENTS

Izumi Naka[1], Jun Ohashi[1], Jintana Patarapotikul[2], Hathairad Hananantachai[2], Sornchai Looareesuwan[2], and Katsushi Tokunaga[1]

[1]Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; [2]Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Abstract. Hemoglobin E (E26K variant of β-globin gene) causing hemoglobinopathy is commonly observed in parts of Thailand, regardless of the hematologic disadvantage of the homozygotes. In order to detect further variants of the β-globin gene, we performed variation screening for exon 1 of the β-globin gene in 64 adult patients with *P. falciparum* malaria, living in northwest Thailand. We identified E26K and two novel variants, 59C>T and IVS+1G>T. IVS+1G>T lies on the splice donor site, and a substitution of A for G at the same site (IVS+1G>A) is known to be linked to β-thalassemia. Thus, the biological significance of IVS+1G>T and its association with malarial infection should be clarified in future studies.

INTRODUCTION

Thalassemias and hemoglobinopathies, caused by genetic variants of α- and β-globins, are commonly observed in parts of malaria-endemic areas of Southeast Asia, such as Cambodia, Thailand and Myanmar, regardless of the hematologic disadvantage. The plausible explanation for this is that heterozygotes of the causative variants have the advantage of protection from malarial infection, as suggested by JBS Haldane (*eg*, Weatherall, 1987). The overall prevalence of hemoglobinopathies has been reported to be 39% in Phitsanulok, a province in the southern part of northern Thailand, and the prevalence of hemoglobin E (Hb E; E26K variant of β-globin gene) in this province was 25% (Pravatmuang *et al*, 1995). If such a variant can be maintained by natural selection due to the protection from malarial infection, there may be other variants causing thalassemias or hemoglobinopathies in malaria-endemic areas of Thailand. In order to detect further variants, we performed variation screening for exon 1 of β-globin gene in adult patients with malaria in northwest Thailand.

MATERIALS AND METHODS

Patients

A total of 64 adult patients with *P. falciparum* malaria living in northwest Thailand was enrolled in this study. All of them underwent treatment at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University. All patients were 13 years of age or older. This study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University and informed consent was obtained from all patients.

Correspondence: Dr Jun Ohashi, Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.
Tel: +81-3-5841-3693; Fax: +81-3-5802-8619;
E-mail: juno@m.u-tokyo.ac.jp

DNA extraction

Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp Blood Kit (Qiagen, Hilden, Germany).

PCR-direct sequencing

PCR-direct sequencing was performed for exon 1 (including surrounding regions) of the β-globin gene with an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). PCR was performed using the following primers: a 5´ primer HBBex1F: 5´-AGGAGCAGGGAGGGCAGGA-3´ and a 3´primer HBBex1R: 5´-TCCAAGGGTAGACCACCAGC-3´. The amplification conditions 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 a thermal cycler (GeneAmp® PCR system 9700, Applied Biosystems, USA).

Statistical analysis

A Hardy-Weinberg equilibrium test was performed for the detected variants. The significance level was set at 0.05 in this study.

RESULTS

Fig 1 shows variants detected in the β-globin gene

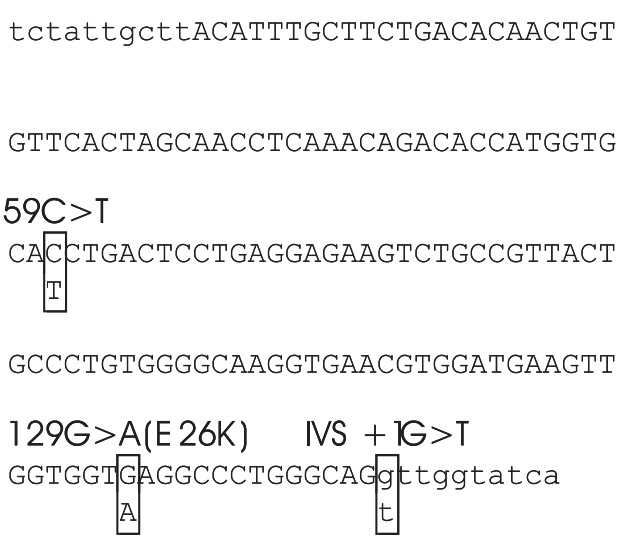

Fig 1- Schematic representation of variants detected in the β-globin gene in 64 Thai malaria patients.

in 64 Thai malaria patients. We identified 129G>A (E26K) and two novel variants, 59C>T and IVS+1G>T. 59C>T is a synonymous substitution, and IVS+1G>T lies on the splice donor site of intron 1.

Genotype and allele frequencies of the detected variants are presented in Table 1. The observed genotype frequencies did not deviate from those expected from the Hardy-Weinberg equilibrium. Allele frequencies of 59T and 129A (26K) were 42.2% and 7.0%, respectively. The IVS+1T was found only in a malaria patient.

DISCUSSION

Although Hb C has been reported in Thailand (Wasi *et al*, 1980; Siriboon *et al*, 1993), the causative variant was not detected in this study, presumably because the present sample size is small and Hb C is very rare in Thailand.

A substitution of A for G at the same splice donor site (IVS+1G>A) has been suggested to be linked to β-thalassemia (Orkin *et al*, 1982), which raises a prospect that IVS+1G>T may cause β-thalassemia also through the abnormal splicing of intron 1. Although the frequency of IVS+1T was found to be very low in malaria patients, this variant may not be rare in the studied population, because healthy individuals free from malaria were not analyzed in this study. Thus, the biological significance of IVS+1G>T and its association with malarial infection remain to be studied.

Table 1
Genotype and allele frequencies of variants of the β-globin gene in Thai malaria patients.

Variant	No. (%)
Genotype[a]	
59C>T	
CC	24 (37.5)
CT	26 (40.6)
TT	14 (21.9)
129G>A (E26K)	
GG	56 (87.5)
GA	7 (10.9)
AA	1 (1.6)
IVS+1G>T	
GG	63 (98.4)
GT	1 (1.6)
TT	0 (0.0)
Allele	
59C>T	
C	74 (57.8)
T	54 (42.2)
129G>A (E26K)	
G	119 (93.0)
A	9 (7.0)
IVS+1G>T	
G	127 (99.2)
T	1 (0.8)

[a]Genotype frequencies did not deviate from those expected from the Hardy-Weinberg equilibrium.

ACKNOWLEDGEMENTS

We sincerely thank the patients who participated in this study. This study was supported by the Core University System Exchange Program under the Japan Society for the Promotion of Science, coordinated by the University of Tokyo and Mahidol University, and The National Research Council of Thailand.

REFERENCES

Orkin SH, Kazazian HH Jr, Antonarakis SE, *et al*. Linkage of β-thalassaemia mutations and β-globin gene polymorphisms with DNA polymorphisms in human β-globin gene cluster. *Nature* 1982;296:627-31.

Pravatmuang P, Tiloklurs M, Suannum M, Chaipat C. Phitsanulok population: the highest incidence of hemoglobin E in the northern provinces of Thailand and PND counseling. *Southeast Asian J Trop Med Public Health* 1995;26(suppl 1):266-70.

Siriboon W, Srisomsap C, Winichagoon P, Fucharoen S, Svasti J. Identification of Hb C [(β6(A3)Glu → Lys] in a Thai male. *Hemoglobin* 1993;17:419-25.

Wasi P, Pootrakul S, Pootrakul P, Pravatmuang P, Winichagoon P, Fucharoen S. Thalassemia in Thailand. *Ann NY Acad Sci* 1980;344:352-63.

Weatherall DJ. Common genetic disorders of the red cell and the "malaria hypothesis". *Ann Trop Med Parasitol* 1987;81:539-48.