Lack of association of the HbE variant with protection from cerebral malaria in Thailand

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

[1]Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

[2]Department of Microimmunology and Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Key Words: Hemoglobin E; cerebral malaria; Thai

Address for 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-tky@umin.ac.jp

ABSTRACT: Hemoglobin E (*HbE*; $^{\beta}$26Glu->Lys) is the most common variant of β-globin gene in Southeast Asia, and has been suggested to confer resistance against *Plasmodium falciparum* malaria. In this study 306 adult patients with *P falciparum* malaria (198 mild and 108 cerebral malaria patients) living in northwest Thailand were investigated to examine whether the *HbE* variant is associated with protection from cerebral malaria. Our results revealed that the sample allele frequency of *HbE* was not significantly different between mild (7.3%) and cerebral malaria (7.4%) patients. Thus, the *HbA*/*HbE* polymorphism would not be a major genetic factor influencing the onset of cerebral malaria in Thailand.

INTRODUCTION

Plasmodium falciparum malaria is the most serious infectious diseases for humans. Approximately 400 million people are attacked by malaria annually, and ~ 3 million people succumb to the illness. It has been suggested that hemoglobin disorders confer resistance against malarial infection based on the observation that the geographical distribution of hemoglobin disorders globally and locally overlaps malaria endemic region. Hemoglobin E (*HbE*; $^{\beta}$26Glu->Lys), which causes the HbE disease and the HbE trait, is the most common variant of β-globin gene (*HBB*; OMIM 141900) in malaria endemic regions of Southeast Asia.

In Thailand, the overall allele frequency of *HbE* is approximately 13 %, and the frequency reaches 50 % in some northeastern parts of this country (Fucharoen *et al*., 1998). The high population frequency of *HbE* is suggested to have been attained rapidly by positive selection against malarial infection (Ohashi *et al*., 2004). In vitro studies have shown the inhibition of the growth of *P. falciparum* in HbE erythrocytes (Chotivanich *et al*., 2002; Vernes *et al*., 1986; Yuthavong *et al*., 1987). Added to these, malaria patients with the HbE trait were reported to have a severe complication malaria less frequently compared to reference patients (Hutagalung *et al*., 1999). Although there are many severe symptoms in malaria, the major cause of death due to malarial infection is cerebral malaria. It is therefore important, from the clinical point of view, to assess whether the *HbE* variant is associated with protection from cerebral malaria.

MATERIALS AND METHODS

Subjects

To examine a possible association of *HbE* with protection from cerebral malaria, we genotyped the *HbA*/*HbE* polymorphism for 306 patients with *falciparum* malaria living in northwest Thailand: 198 mild malaria and 108 cerebral malaria patients. All patients underwent treatment at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University. For all patients, malarial infection by *P. falciparum* was confirmed by a positive blood smear for the asexual form of *P. falciparum*. Clinical manifestations of malaria were classified according to the definitions and associated criteria by the World Health Organization. Cerebral malaria was defined as unrousable coma, a positive result in tests for the presence of an asexual form of *P. falciparum*, and exclusion of other causes of coma. Mild malaria was defined as having a positive blood smear and fever without other causes of infection and no signs indicating severe malaria as following: high parasitemia (> 100,000 parasites/ml), hypoglycemia (glucose level < 22 nmol/L), severe anemia (hematocrit < 20% or hemoglobin level < 7.0 g/dl) or increased serum level of creatinine (> 3.0 mg/dl). All individuals were 13 years old or older, and the mean ages of patients with mild malaria and cerebral malaria patients were 25.6 and 28.6 respectively. This study was approved by the institutional review board of the Faculty of Tropical Medicine, Mahidol University, and the Research Ethics Committee of the Faculty of Medicine, The University of Tokyo. Informed consent was obtained from all patients.

DNA Extraction, Amplification, and Sequencing

Genomic DNAs from all the patients were purified from peripheral blood leukocytes using a commercially available kit (QIAmp blood kit; Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) was performed for the HbE variant using a 5’ primer HBBex1F: 5’-AGGAGCAGGGAGGGCAGGA-3’ and a 3’ primer HBBex1R:

5’-TCCAAGGGTAGACCACCAGC-3’. The amplification condition consisted of an initial denaturation at 96 C for 10 min, followed by 35 cycles of denaturation at 96 C for 30 sec, annealing at 60 C for 30 sec, and extension at 72 C for 30 sec were applied using a thermal cycler (GeneAmp PCR system 9700; Perkin-Elmer Applied Biosystems). PCR products were sequenced for all samples using an automated sequencer (ABI Prism 3100; Perkin-Elmer Applied Biosystems).

Statistical Analysis

The difference in genotype and allele frequencies between mild and cerebral malaria patients was examined by Fisher’s exact probability test.

RESULTS AND DISCUSSION

Table 1 shows the genotype and allele frequencies of the *HbA*/*HbE* polymorphism in mild and cerebral malaria patients. The sample frequencies of *HbE* in mild and cerebral malaria patients were 7.3% and 7.4%, respectively. The sample frequency in neither genotype nor allele was shown to be significantly different between mild and cerebral malaria patients by the Fisher’s exact probability test. The present results seem to be inconsistent with a previous study showing that the HbE trait is associated with non-possession of a severe complication malaria (Hutagalung *et al*., 1999). However, the present study focused only on cerebral malaria among severe symptoms. This may be a part of the reason of the inconsistency. Although a case-control study requires to be conducted to evaluate the association between the *HbE* variant and the other sever complications of malaria, we conclude that the *HbA*/*HbE* polymorphism is not a major genetic factor influencing the onset of cerebral malaria.

ACKNOWLEDGMENTS

We are grateful to the patients who participated in this study. We thank three anonymous reviewers for valuable comments and suggestions on a previous version of this manuscript. This study was supported by the Core University System Exchange Programme under Japan Society for the Promotion of Science, coordinated by the University of Tokyo and Mahidol University, The National Research Council of Thailand, a Grant-in-Aid for Scientific Research on Priority Areas (C) “Medical Science” from the Ministry of Education, Culture, Sports, Science and Technology, Japan, The Genetic Diversity Project supported by the New Energy and Industrial Technology Development Organization (NEDO), and NIH D43TW00620/CFDA#93.989.

REFERNCES

Chotivanich, K., Udomsangpetch, R., Pattanapanyasat, K., Chierakul, W., Simpson, J., Looareesuwan, S., and White, N. (2002) Hemoglobin E: a balanced polymorphism protective against high parasitemias and thus severe P falciparum malaria. *Blood* **100**:1172-1176.

Fucharoen, S., Winichagoon, P., Siritanaratkul, N., Chowthaworn, J., and Pootrakul, P. (1998) Alpha- and beta-thalassemia in Thailand. *Ann. N. Y. Acad. Sci.* **850**:412-414.

Hutagalung, R., Wilairatana, P., Looareesuwan, S., Brittenham, G. M., Aikawa, M., and Gordeuk, V. R. (1999) Influence of hemoglobin E trait on the severity of Falciparum malaria. *J. Infect. Dis*. **179**:283-286.

Ohashi, J., Naka, I., Patarapotikul, J., Hananantachai, H., Brittenham, G., Looareesuwan, S., Clark, A. G., and Tokunaga, K. (2004) Extended linkage disequilibrium surrounding the hemoglobin E variant due to malarial selection. *Am. J. Hum. Genet.* **74**:1198-1208.

Vernes, A. J., Haynes, J. D., Tang, D. B., Dutoit, E., and Diggs, C. L. (1986) Decreased growth of Plasmodium falciparum in red cells containing haemoglobin E, a role for oxidative stress, and a sero-epidemiological correlation. *Trans. R. Soc. Trop. Med. Hyg.* **80**:642-648.

Yuthavong, Y., Butthep, P., Bunyaratvej, A., and Fucharoen, S. (1987) Inhibitory effect of beta zero-thalassaemia/haemoglobin E erythrocytes on Plasmodium falciparum growth in vitro. *Trans. R. Soc. Trop. Med. Hyg.* **81**:903-906.

Table 1. *HbE* genotype and allele frequencies in Thai malaria patients

	Mild malaria (N = 198)	Cerebral malaria (N = 108)
Genotype frequency		
HbE/HbE	3 (1.5 %)	0 (0.0 %)
HbA/HbE	23 (11.6 %)	16 (14.8 %)
HbA/HbA	172 (86.9 %)	92 (85.2 %)
Allele frequency		
HbE	29 (7.3 %)	16 (7.4 %)
HbA	367 (92.3 %)	200 (92.6 %)

No significant difference was detected between mild and cerebral malaria patients by Fisher's exact probability test.