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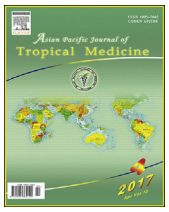

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Endogamous marriage and the prevalence of hemoglobin E in ethnic groups of northern Thailand

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ABSTRACT

Objective: To investigate the impact of the endogamous marriage culture on the prevalence of the hemoglobin E (HbE) recessive variant.
Methods: The prevalence of the hemoglobin E (HbE) recessive variant was determined by dot-blot hybridization in 4 endogamous villages (1 Mlabri and 3 Htin ethnic groups) in comparison with 9 other nearby non-endogamous populations.
Results: Although the overall HbE prevalence in the population studied (8.44%, 33/391) was not significantly different from that of the general southeast Asian population, a high prevalence and individuals with homozygous HbE were observed in two villages, the Mlabri from Wiang Sa district and the Htin from Thung Chang district of Nan province (26.3% and 26.9%, respectively). The low HbE allelic frequency noticed in some endogamous populations suggests that not only endogamy but also other evolutionary forces, such as founder effect and HbE/β-thalassemia negative selection may have an effect on the distribution of the HbE trait.
Conclusion: Our study strongly documents that cultural impact has to be considered in the extensive prevalence studies for genetic disorders in the ethnic groups of northern Thailand.

1. Introduction

Hemoglobin E (HbE) is one of the hemoglobin variants and is caused by a single base substitution at codon 26 of the beta globin gene, from GAG to AAG. As a consequence, the corresponding amino acid is changed from a glutamic acid (Glu) to a lysine (Lys). This mutation, in combination with other hemoglobinopathies such as β-thalassemia results in heterogeneous phenotypes ranging from mild to severe clinical symptoms [1]. The frequency of the HbE allele varies amongst distinct ethnic groups of the world but high prevalence is usually observed in populations living in Southeast Asia, ranging from 0.05 to 0.10 and as high as 0.50 in some groups of Cambodia and Thailand [2,3].

Although a previous study documented that the prevalence of HbE in the Thai population of northern Thailand was approximately 12% [4], this figure may not apply to ethnic groups whose genetic background is distinct from the majority of northern Thai population [5,6]. Moreover, some groups still practice endogamous marriage, and offspring of endogamous unions are at increased risk of some certain diseases [7] such as heart defect, gastro-intestinal disorders, hypertension, congenital abnormalities, vision loss, hearing deficit and diabetes mellitus. The closer the biological relationship is between the parents, the greater the probability that their offspring will inherit identical detrimental recessive genes [8]. In populations with high endogamous rates and common inherited blood disorders, community programs for premarital screening to detect carriers of hemoglobinopathies are usually in place, as for example in Saudi Arabia [9] and Turkey [10]. Carrier detection and genetic counseling programs have been very successful in reducing the birth prevalence of inherited disorders in some populations [8].

Although the prevalence of HbE has been surveyed in some ethnic groups of Thailand [3,11], very limited information is available about the possible role of endogamous culture on the

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frequency of this recessive gene. In this work, the HbE prevalence in two groups that practice endogamous marriage, the Mlabri and Htin, of northern Thailand was surveyed in comparison with other nearby non-endogamous marriage groups.

The Mlabri are a small group of nomadic hunter-gatherers inhabiting northern Thailand, with "Mlabri" literally meaning "forest people". Outsiders refer to the Mlabri as "Phi Tong Lueang" (spirit of yellow banana leaves) which refers to the Mlabri's tradition of building their shelters with fresh banana leaves. When the leaves turn yellow, the Mlabri people rapidly move to build a new shelter at another location. Nowadays, the Mlabri people live in scattered areas of both Laos and Thailand. The Mlabri's language is grouped in the Mon-Khmer subfamily of the Austroasiatic family [12]. The Mlabri have a strong tradition of endogamy, despite the fact that there has long been casual contact between the mountain-dwelling Mlabri and other remote groups of northern Thailand [13].

The Htin was originally a native people of Northern Laos before some of them migrated to the Nan province of Thailand about 60–80 years ago. There are two subgroups of the Htin people, the Mal and the Prai, who are divided by their different spoken dialects. Endogamy is a part of the Htin life which supports the transmission of cultural continuity. Most of the Htin prefer to marry people from the same village, and follow the matrilocal residence tradition [14].

2. Materials and methods

2.1. Population sampling and DNA extraction

The populations used in this study were 391 volunteers belonging to 13 different ethnic populations of northern Thailand. The criteria for population sampling and informed consent were as described elsewhere [5,6,15]. All populations were selected from non-malaria endemic areas based on the prevalence of malaria infection in blood-smear surveys undertaken between 2007 and 2012 [16], to avoid the effect of possible HbE positive selection by malaria parasites.

Total genomic DNA was extracted from white blood cell lysates using an inorganic salting out protocol [17]. Quality and quantity of extracted genomic DNAs were checked by agarose gel electrophoresis and spectrophotometry, respectively.

2.2. PCR amplification and dot-blot hybridization

A fragment between exons 1 and 2 of the beta-globin gene was amplified by the Polymerase Chain Reaction (PCR) technique using two primers (modified from Winichagoon *et al.*, 1989 [11]) which are China 1F (5′ GTA CGG CTG TCA TCA CTT AGA CCT CA 3′) and China 2R (5′ TGC AGC TTG TCA CAG TGC AGC TCA CT 3′).

The 25 μL reaction contained 150 ng of the DNA template and a mixture of 1× PCR buffer, 0.2 mM dNTP, 10 μM of each primers, 1.25U RBC *Taq* DNA polymerase, and sterile deionized water. The PCR reaction was performed with the GeneAmp PCR system 2004 (Perkin Elmer) as follows: one initial cycle at 95 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s and extension at 72 °C for 45 s, then one cycle of final extension at 72 °C for 10 min. The amplified DNA products were analyzed on 2.0% agarose gels and visualized under UV light after ethidium bromide staining. The expected product size was 602 bp.

The amplified PCR products of each samples were denatured, spotted onto nylon membranes (Porablot NY plus, Pacific Science), and then hybridized with each of specific normal and mutant probes (modified from Winichagoon *et al.*, 1989 [11]) which are normal probe (5′-Biotin-CAG GGC CTC ACC ACC A-3′) and mutant probe (5′-Biotin-TTG GTG GTA AGG CCC T-3′). Positive controls of normal, HbE heterozygous (AE) and HbE homozygous (EE) samples were simultaneously analyzed. A positive signal shows as a blue color spot generated from the enzyme substrate reaction. Membranes were cut vertically and compared to known genotypes controls to verify the results.

3. Results

Among 391 individuals studied, 33 (8.44%) possessed the HbE trait. Twenty-nine (7.42%) samples were hemoglobin E carriers and 4 (1.02%) subjects were homozygous HbE (Table 1). The HbE allelic frequencies were different amongst the ethnic groups, and varied from the highest in the Htin2 (19.23%) to the lowest in the Htin1, Blang, Lawa, and Yong (0%) (Table 1 and Figure 1). The HbE trait was found in every Tai-Kadai speaking populations except the Yong. However, the

Table 1
General information of the studied ethnic groups, number of samples, number of observed HbE individuals and alleles.

Ethnic group	Location (sub-district, district, province)	Longitude	Latitude	Linguistic group	No. of samples	No. of HbE individuals (%)			No of HbE allele (%)
						AE[a]	EE[a]	Total	
Mlabri	Mae Ka Ning, Wiang Sa, Nan	100°54′	18°70′	Austroasiatic	19	4 (21.05)	1 (5.26)	5 (26.32)	6 (15.79)
Htin1 (Mal)	Pa Klang, Pua, Nan	100°55′	19°43′	Austroasiatic	40	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Htin2 (Prai)	Lae, Thung Chang, Nan	100°86′	19°08′	Austroasiatic	26	4 (15.38)	3 (11.54)	7 (26.92)	10 (19.23)
Htin3 (Prai)	Phra That, Chiang Klang, Nan	100°54′	19°19′	Austroasiatic	34	2 (5.88)	0 (0.00)	2 (5.88)	2 (2.94)
Blang	Wiang Pang Kham, Mae Sai, Chiang Rai	99°52′	20°25′	Austroasiatic	20	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Lawa	Huay Hom, Mae La Noi, Mae Hong Son	97°56	18°23	Austroasiatic	19	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Khamu	Ngop, Thung Chang, Nan	100°89′	19°48′	Austroasiatic	29	3 (10.34)	0 (0.00)	3 (10.34)	3 (5.17)
Khuen	Ban Pao, San Pa Thong, Chiang Mai	98°51′	18°38′	Tai-Kadai	20	1 (5.00)	0 (0.00)	1 (5.00)	1 (2.50)
Lue1	Silaleang, Pua, Nan	100°56′	19°09′	Tai-Kadai	51	5 (9.80)	0 (0.00)	5 (9.80)	5 (4.90)
Lue2	Nong Bua, Tha Wang Pha, Nan	100°47′	19°05′	Tai-Kadai	43	4 (9.30)	0 (0.00)	4 (9.30)	4 (4.65)
Lue3	Koh Chang, Mae Sai, Chiang Rai	99°53′	20°26′	Tai-Kadai	50	3 (6.00)	0 (0.00)	3 (6.00)	3 (3.00)
Yong	Makok, Pa Sang, Lamphun	98°56′	18°24′	Tai-Kadai	20	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Yuan	Mae Feak Mai, Sansai, Chiang Mai	98°59′	19°00′	Tai-Kadai	20	3 (15.00)	0 (0.00)	3 (15.00)	3 (7.50)
	Total				391	29 (7.42)	4 (1.02)	33 (8.44)	37 (4.73)

[a] AE-Hemoglobin E heterozygote; EE Hemoglobin E homozygote.

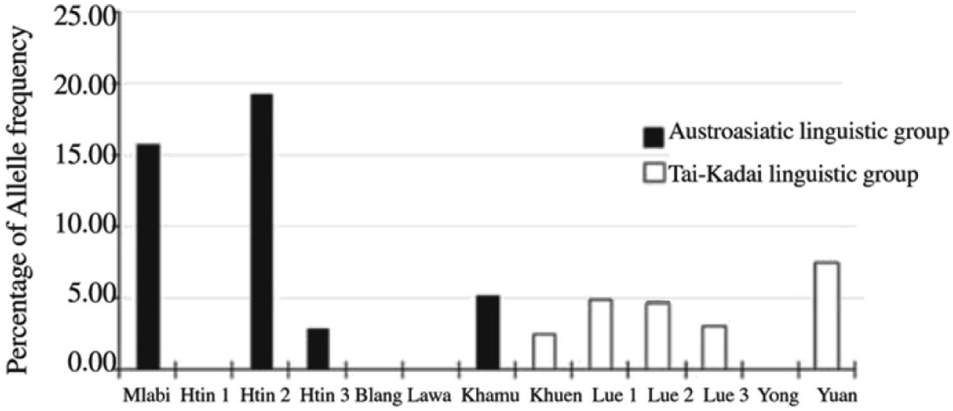

Figure 1. HbE prevalence in the ethnic groups of northern Thailand.

overall HbE allelic frequency of the Tai-Kadai (3.92%) was slightly lower than the Austroasiatic linguistic group (5.31%).

4. Discussion

The Mlabri and Htin are the descendants of the endogenous ethnic groups living in the area of present-day northern Thailand and northern Laos since prehistoric times. The Mlabri are hunter-gatherers while the Htin practice agriculture. Previous studies showed that both of the Mlabri and Htin had low genetic diversities as a consequence of founder effect and their endogamous marriage tradition [5,6,18]. Observation of high HbE prevalence and HbE homozygous individuals in the Mlabri and Htin2 have confirmed the impact of endogamous marriage which leads to the maintenance and incorporation of autosomal recessive genes inherited from close relative unions. Although unrelated volunteers were intended to be selected for our study, the sampling method used, self-reporting non-relative identification, has a limitation as the subjects recognized their predecessors to be unrelated after only a few generations, while consanguinity has probably been practiced over centuries.

Interestingly, there was a low frequency of HbE observed in two Htin villages, Htin1 and Htin3, who also practice within-village endogamous marriage. This observation suggests that endogamous marriage is not the only factor which shapes HbE prevalence. Two other possible evolutionary forces can be speculated to be responsible for the fluctuation of HbE prevalence amongst different Htin populations. The first is the founder effect which had been shown in the Htin by low Y-chromosomal and mitochondrial DNA diversities [5]. The founder effect is the genetic term for the establishment of a new population by a few original founders. The frequencies of the genes in the founding population usually differ from their ancestors as the founders carry a small fraction of the total genetic variation of their parental group. Prevalence of some specific characters may increase if new established population is isolated [19]. Random distribution of the HbE allele in each of the Htin founder groups leads to the possibility that none or only a few HbE alleles were carried and descended to the Htin1 and Htin3. Conversely, there was also a chance that a high number of HbE alleles were taken and rapidly increased in frequency through endogamous marriage, as with the Htin2. A disproportion of HbE alleles carried in the founder groups could have led to the distinctive dissimilarity of HbE prevalence amongst the present-day Htin populations. The second possibility is the loss of the HbE trait due to the negative selection pressure of a recessive allele. Although it is generally known that individuals who carry HbE alleles either as heterozygous or homozygous manifest no, or only mild clinical symptoms, the combination of HbE with other hemoglobin disorders such as thalassemia can result in a severe, transfusion dependent anemia [20]. In endogamous marriage societies, manifestations of homozygosity for recessive disorders or gene complexes are prominent [8]. High rates of endogamy in the Htin over multiple generations may lead to the elimination from the gene pool of the HbE alleles together with deleterious recessive genes. The rate of purifying selection in each Htin population would be different depending on two factors, the number of the hemoglobinopathies carried by their founders and the degree of endogamous marriage in the population.

Unlike the Mlabri and Htin, endogamy is generally uncommon and is perceived negatively in other ethnic groups of northern Thailand. Although the HbE allelic frequencies are slightly different amongst the studied non-endogamous subgroups, ranging from 0 to 7.5%, they are in the less-than-10% range in accord with previous estimations of Southeast Asian people [2]. However, it is noteworthy that the HbE alleles are observed in nearly all Tai-Kadai speaking populations whose genetic admixture has been reported [15,21]. The Tai-Kadai people are the majority of ethnic groups in present-day northern Thailand. Most of them emigrated from Southern China and gradually migrated southward through Laos and Myanmar into northern Thailand over the past hundreds of years [22]. Inter-ethnic marriages are common in the Tai-Kadai society in order to create outbound relationships for resources management. The genetic composition of each Tai-Kadai population and the close genetic relationship among them have inevitably been structured by the inter-ethnic genetic admixture [15,21]. Genetic variants, including HbE as shown here, have been introduced and transferred from one Tai-Kadai population to another continuously, resulting in the similar prevalences observed in the Tai-Kadai linguistic group.

In Thailand, while various ethnic groups still practice their unique way of life, public awareness regarding the impact of culture on genetic risk is generally low. Our report on HbE prevalence in endogamous marriage populations is an example showing how cultural effects shape the genetic structure of ethnic people. In other ethnicities cultural traits such as the strictly post-marital residence in the Karen or the inter-ethnic adoption in the Mien, may also have a significant influence on the distribution of genetic variants and needs to be further investigated. Monitoring the incidence of genetic disorders among the ethnic groups of Thailand should be taken as an

urgent issue. Genetic counseling and screening should be offered for the specific populations whose traditions can be linked with an increased risk of the genetic disorders.

Conflict of interest statement

We declare that we have no conflict of interest.

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