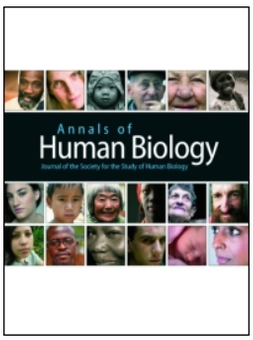

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SHORT REPORT

Molecular analysis of haemoglobin E in Southeast Asian populations

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ABSTRACT

Haemoglobin (Hb) E is the most common Hb variant in Asia where its gene frequency approaches 0.3 in some areas. We studied genetic background of Hb E genes among Southeast Asian populations. This study examined β-globin gene haplotypes linked to haemoglobin E (Hb E) in diverse groups of Southeast Asian populations. The study was conducted on southern Thai (22 alleles), Cambodian (84 alleles), Laotian (120 alleles), Vietnamese (87 alleles) and Burmese (one allele) subjects. Results were compared with those of previous studies in northeast Thailand, the Yunnan of China, West India and Europe. Ten different haplotypes were observed. The four most common haplotypes were haplotypes 1 (−+−+++−) and 2 (+−−−−+−) on chromosomes with framework 2 and haplotypes 6 (−+−+−+) and 7 (+−−−−−+) on chromosomes with framework 3 variety. Phylogenetic analysis indicated that haplotype 1 is a relatively recent haplotype found in all populations, whereas haplotype 6 is found predominately in Cambodians. The results indicate that at least two genetic origins of Hb E are responsible for the high prevalence and spread of Hb E among Southeast Asian populations.

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Haemoglobin variant; beta globin gene haplotype; phylogenetic tree

Introduction

Haemoglobin (Hb) E is the most common Hb variant found among Southeast Asian populations (Fucharoen & Winichagoon, 1987). It results from a mutation at codon 26 (GAG to AAG) of the β-globin gene, which changes an amino acid from glutamic acid to lysine. The mutation creates an abnormal splicing of the β-globin gene leading to a β^{+}-thalassaemia phenotype. Hb E may be present in the heterozygote, homozygote or compound heterozygote state with other haemoglobinopathies leading to Hb E/β thalassaemia, EABart's disease, EFBart's disease and other complex $\alpha\beta$-thalassaemia syndromes. The distribution of Hb E in areas with endemic malaria in Southeast Asia leads to an acceptance of the malaria hypothesis and that Hb E may have advantages against malaria infection in the region (Weatherall, 2008). Using β-globin gene haplotype analysis, it has been proposed that there are multiple origins of Hb E among the world population (Kazazian et al., 1984). A prevalence of as high as 50% Hb E has been documented in some ethnic minorities in northeast Thailand, where β-globin gene haplotype analysis has confirmed at least two independent origins (Fucharoen et al., 2002). Although there is a similar prevalence of Hb E, little is known about the genetic background of Hb E genes among other Southeast Asian populations. We have addressed this for the first time by investigating a large cohort of Hb E genes among southern Thai, Cambodian, Laotian, Vietnamese and Myanmar populations and the results are compared with those of Hb E genes identified in other populations.

Subjects and methods

Subjects and specimens

Ethical approval of the study protocol was obtained from the Institution Review Board (IRB) of Khon Kaen University, Thailand (HE562275). Left-over DNA specimens were obtained from subjects with homozygous Hb E, including 11 Thai individuals from Narathiwat province of southern Thailand, 42 Cambodian subjects from Phnom Penh, Cambodia, 13 Laotian subjects attending the Maria Teresa Hospital, Vientiane, Lao People's Democratic Republic (Lao PDR) and 47 Laotian subjects from Khammouane province, Lao PDR. In addition, 78 DNA specimens were also obtained from Vietnamese subjects in Thua Thien Hue in the middle of Vietnam and a Myanmar student at Khon Kaen University, Thailand, who was Hb E heterozygote.

Hb E gene and Haplotype analysis

Hb E mutation (β^{26}; GAG-AAG) was confirmed in all cases using allele specific polymerase chain reaction (PCR), as described previously (Fucharoen et al., 1994). β-Globin gene

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haplotypes based on seven polymorphic sites including rs77526129 (*Hinc* II-5ε), rs2070972 (*Hind* III-$^{G}\gamma$), rs28440105 (*Hind* III-$^{A}\gamma$), rs10128556 (*Hinc* II-$\psi\beta$), rs968857 (*Hinc* II-3′$\psi\beta$), rs10768683 (*Ava* II–β) and rs9704069 (*BamH* I-β3′) were determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as previously described (Fucharoen et al., 2002). Samples with known genotype at each polymorphic site were included in each PCR-RFLP assay. Haplotypes associated with β^{E} globin genes were readily obtained in all homozygous subjects. For Vietnamese heterozygous subjects, Hb E associated haplotypes were defined using the HAPLOVIEW 4.2 software (Barrett et al., 2005).

Phylogenetic analysis

A phylogenetic tree was generated with haplotype data using the DendroUPGMA (http://genomes.urv.ca/UPGMA/) applying Jaccard (Tanimoto) coefficient with default settings and the FigTree v.1.4.0 programme was used to design a graphical view of the phylogenetic tree (Santiago et al., 1999).

Results

Hb and DNA analyses confirmed the Hb E mutation (β^{26}; GAG-AAG) in all cases. All Thai subjects, Cambodians and Laotians were homozygous for Hb E. The Vietnamese subjects included nine homozygous and 69 heterozygous for Hb E. A Myanmar subject was Hb E heterozygote. Haplotypic segregation was possible for a total of 22 chromosomes from Thai subjects, 26 chromosomes from Loatians from Vientiane (VT) and 94 chromosomes from subjects from Khammouane (KM) of Lao PDR, 84 chromosomes from Cambodians, 87 chromosomes from Vietnamese and one chromosome of Myanmar origin.

Table 1 summarises the 10 haplotypes associated with β^{E} globin genes observed in these populations. For comparison, previously reported data from northeast Thai, the Yunnanese of China, Indians from west Bengal and Manipur and Europeans were also listed. The β^{A} haplotypes among northeast Thai were also provided (Bandyopadhyay et al., 1999; Fucharoen et al., 2002; Kazazian et al., 1984; Maishnam et al., 2011). Two β^{E} haplotypes were found in Laotians (haplotypes 1, 2), five in Cambodians (haplotypes 1, 2, 3, 6, 7), four in Vietnamese (haplotypes 1, 2, 7, 9), six in Thai subjects (haplotypes 1, 2, 6, 7, 8, 10) and one in Burmese subjects. Haplotypes 1 (−+−+++−) and 2 (+−−−−+−), both of which are of framework 2 chromosome (*Ava* II +, *BamH* I −), are the two most common β^{E} haplotypes in these Southeast Asian populations. However, in Cambodians, high proportions of β^{E} genes were associated with haplotypes 6 (−+−+++−) and 7 (+−−++−+) on chromosomes with framework 3 variety (*Ava* II −, *BamH* I +).

Figure 1 shows a phylogenetic tree constructed using haplotype data and the DendroUPGMA software (http://genomes.urv.cat/UPGMA/) applying Jaccard (Tanimoto) coefficient, Cophenetic Correlation Coefficient (CP) = 0.7836 and the distance matrix based on the Jaccard (Tanimoto) coefficient. This represents the evolution of Hb E in Southeast Asians as compared to other populations. The oldest Hb E haplotype in the region seems to be related to framework 3

Table 1. Beta-globin gene haplotypes linked to Hb E genes in various populations.

DNA restriction sites studied							Haplotypes	Frameworks	β^{E}-globin gene											β^{A}-globin gene
									Asia								Europe			
									Southeast Asia						China	India				
									Laos		Cambodia (Phnom Penh)	Vietnam (Hue)	Myanmar (Yangon)	Thai						
Hinc II	*Hind* III	*Hind* III	*Hinc* II	*Hinc* II	*Ava* II	*BamH* I			Vientiane	Khammouane				Southern	Northeastern[a]	Yunnan[b]	West Bengal[c]	Manipur[d]	European[e]	Northeast Thai[a]
-	+	-	+	-	+	-	1	2	20	81	26	20	1	14	262	12	8	-	1	33
+	-	-	-	-	+	-	2		6	13	11	59	-	3	83	27	31	5	-	183
-	-	-	+	+	+	-	3		-	-	1	-	-	-	-	5	-	-	-	-
-	+	+	+	+	+	-	4		-	-	-	-	-	-	-	80	-	-	-	-
+	+	-	-	-	+	-	5		-	-	-	-	-	-	-	5	-		-	-
-	+	-	+	+	-	+	6	3	-	-	36	-	-	2	21	-	-	-	-	23
+	-	-	-	-	-	+	7		-	-	10	6	-	3	-	-	-	-	-	166
-	-	-	-	-	-	+	8		-	-	-	-	-	-	14	-	-	-	-	-
-	+	+	+	+	-	+	9		-	-	-	2	-	-	-	-	-	-	-	-
+	-	-	-	-	+	+	10	1	-	-	-	-	-	-	2	-	-	-	1	79
Total									26	94	84	87	1	22	382	129	39	5	2	484

Recombination hot spot

Number indicates the number of chromosomes observed in each population. Plus (+) and minus (–) indicate the presence and absence of each restriction site. β^{A}-Globin gene associated haplotypes in the northeast Thai population are also presented.
[a]Data from Fucharoen et al. (2002); [b]data from Liu et al. (2016); [c]data from Bandyopadhyay et al. (1999); [d]data from Maishnam et al. (2011); [e]data from Kazazian et al. (1984).

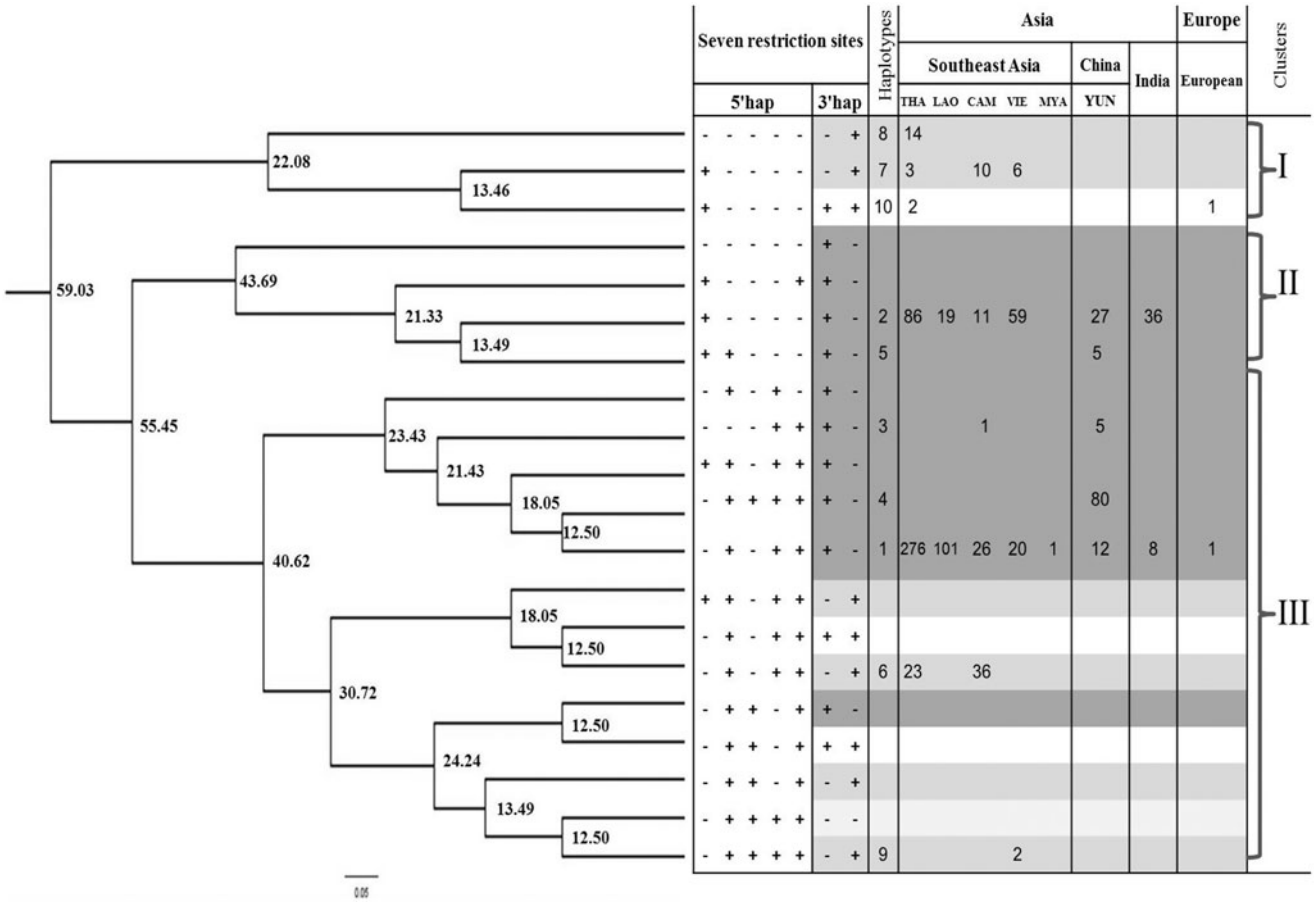

Figure 1. Phylogenetic tree constructed on Hb E haplotypes using the DendroUPGMA method. The Jaccard coefficient was used to compare between sets of variables and 100 bootstrap replicates have been generated. Haplotypes associated with Hb E are highlighted with dark grey (for framework 2), light grey (for framework 3) and no grey tone (for framework 1). Number indicates a total number of chromosomes observed in each population.

chromosome (haplotypes 8 & 7), found only in Thai people, Cambodians and Vietnamese. Although haplotypes 1 and 2 on the framework 2 variety are common Hb E haplotypes in the region, phylogenetic analysis indicates that they are from different clusters. Haplotype 4, a specific haplotype for the Yunnanese population, was found to be closely related with haplotype 1 of Southeast Asians, the data indicating the same origin.

Discussion

A study on linkage disequilibrium has suggested that Hb E in Southeast Asia arose ~1,240–4,440 years ago (Ohashi et al., 2004). Haplotype analysis shown in Table 1 reports that the four most common haplotypes found in the Southeast Asian population are haplotype 1 (−+ −−+++−) and haplotype 2 (+− − − −+−) on the framework 2 chromosome with (*Ava* II+, *BamH* I −) and haplotype 6 (−+−−+−+) and haplotype 7 (+− − − − −+) on framework 3 chromosome (*Ava* II −, *BamH* I+). It has been observed previously that Hb E on framework 3 chromosome is predominatly found in Cambodians (Antonarakis et al., 1982). Haplotypes 1 and 2 could have resulted from each other via a genetic recombination between the first five restriction sites (the 5′ haplotype) and the last two restriction sites (the 3′ haplotype). However, haplotypes 6 and 7 could only be explained by the independent origin of β^E globin gene.

The phylogenetic tree of Hb E haplotypes shown in Figure 1 depicts the three clusters. Cluster I comprises haplotypes 8 and 7 with the longest evolution and were found only in Thai, Cambodian and Vietnamese populations. These likely represent the ancestral haplotypes of Hb E in Southeast Asians. Cluster II comprises haplotypes on the chromosome with framework 2 variety. In this cluster, haplotype 2 is the most common one and is detected in all populations except for the Europeans. In cluster III, haplotype 1 is the most common haplotype found in all Southeast Asian populations, whereas haplotype 4 is found only in the Yunnanese of China. Both haplotypes 1 & 4 are closely related and share the same origin. These results confirm the multiple origins of Hb E gene in the Asian population. Multiple origins also exist for other common haemoglobinopathies like Hb Constant Spring (Jomoui et al., 2015) and Hb Q-Thailand (Singsanan et al., 2010). It is, therefore, likely that natural selection and multiple origins are responsible for a high prevalence and spread of Hb E in the region of Southeast Asia.

Disclosure statement

No potential conflict of interest was reported by the authors.

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