The southeast asian 12.5 kb (DELTA BETA)° deletion: a common beta-thalassemia in mon-khmer groups (lao theung) of south laos

Seven patients with unexplained anemia and mild thalassemic features were ascertained during a survey of hemoglobinopathies in the Sekong Province in South Laos. These patients belong to the Austroasiatic (Mon-Khmer) population of South Laos (official designation Lao Theung). Hemoglobin electrophoresis on cellulose acetate showed absence of Hb A and two bands in the positions of Hb E and Hb F respectively. Sequencing of DNA isolated from venous blood revealed the codon 26 G→A mutation characteristic of the HBB*E gene, but none of the common Southeast Asian beta-thalassemia mutations were found. Detailed studies in four of the seven subjects identified a 12.5 kb deletion encompassing part of the delta-globin gene and the entire beta-globin gene. We conclude that this deletion is a common, and possibly the predominant beta-thalassemia mutation of the Austroasiatic Lao Theung population. Similar deletions reported in single individuals in Laos, Thailand and Vietnam are probably due to migrational spreading to areas adjacent to South Laos.

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Thalassemia intermedia comprises a group of patients who have mild anemia and a clinical picture of survival without regular blood transfusions. The majority of the patients are homozygous or compound heterozygous for mild beta -thalassemia mutation(s).[1] A mild clinical picture is also observed in some patients with delta-beta-thalassemia, which is characterized by increased production of HbF in adult life. In a recent survey of hemoglobinopathies in the Lao Theung of South Laos, populations belonging to the Austroasiatic (Mon-Khmer) language group, seven patients, registered at a health center of the Sekong Province, were diagnosed with unexplained anemia and mild thalassemic features. Four of these individuals were presented for further diagnostic examinations. We report here the clinical, hematological and genomic data on these patients.

Material and Methods

The four probands belong to the ethnic groups Alak and Ngeh, which are members of the numerous Mon-Khmer speaking populations of South Laos (official designation Lao Theung)[2] and come from four villages in the Thateng District of Sekong Province. They live in a tropical, formerly highly malarious environment and economically depend on subsistence farming.

Hematological data were obtained by an automatic counter and routine methods. The HPLC and DNA sequencing techniques applied at the Thalassemia Unit of Chiangmai University Hospital have been described earlier.[3] The beta-globin gene associated 'framework' is determined by single nucleotide polymorphisms at five sites in exon 1 and IVS2, codon (cd) 2 nucleotide (nt) 3, IVS2 nt16, nt74, nt 81, and nt 666.[4]

DNA was purified from blood leukocytes by the salting out procedure.[5] Southern analysis was carried out on DNA digested with the restriction enzymes XbaI, AvaII and EcoRI and hybridized with a delta-globin IVS-II-specific 574 bp probe synthesized by PCR: primer (pr delta 5′) 5′-TGCATACCAGCTCTCACCT-3′ (forward) and (pr delta 3′) 5′-CTGAGAAACTGAGCCAACACC-3′ (reverse) at position 55497 to 55516 and 56070 to 56050, respectively (according HSHBB sequence in the GenBank). After hybridization with the DIG labelled probe the specific DNA fragments were detected with DIG non-radioactive nucleic acid labelling system (Roche).

For amplification of the fragment that covers the deletion we chose the same forward primer (pr delta 5′) whereas the reverse primer (prL1) 5′-GCTGCCCT TAA-CATTTTTTCC-3′ was located in the L1 (KpnI) element at nucleotide 68795-68775. PCR-conditions were as follows: 100 ng of genomic DNA was used for each PCR reaction (total volume 25 µL). The amplification buffer consisted of 1.5 mM $MgCl_2$, 10 mM Tris-HCl pH 8.4, 50 mM KCl, 200 µM dNTPs and 1.25 U recombinant Taq polymerase (Invitrogene). 32 cycles of 94°C-30 s, 62°C-1 min and 72°C-1 min were performed. The approximately 700 bp deletion-specific PCR product was subjected to sequencing analysis using the ABI PRISM 310 (Applied Biosystems).

Results

The four patients exhibited only mild signs of thalassemia. There was slight pallor of the mucous membranes, without jaundice, facial or skull deformities. Liver and spleen were palpable in two subjects. The hematological data are summarized in Table 1. There is mild to moderate anemia and pronounced microcytosis, but the mean corpuscular hemoglobin concentration is normal or only slightly reduced. The percentage of Hb F is near the mean of the range observed in beta-thalassemia/Hb E subjects.[6]

Hemoglobin electrophoresis on cellulose acetate at alkaline pH showed a slow-moving fraction in the position of Hb E and an intense band in the position of Hb F. Hb F values ranged from 31 to 47 percent. Quantification of the hemoglobin fractions by HPLC confirmed the absence of Hb A.

DNA sequencing of the beta-globin gene revealed apparent homozygosity for the G→A transition in the first nucleotide of codon 26 in all samples. This mutation is characteristic for the Hb E gene (HBB*E). None of the common point mutations or small deletions causing beta-thalassemia in Southeast Asian populations was found. Although the subjects had only mild clinical signs of thalassemia, we presumed that these findings were most likely due to compound heterozygosity for HBB*E and a deletional beta-thalassemia mutation.

The Southern analysis revealed in all subjects abnormal bands characteristic for the Laotian (delta beta)o-deletion as described before.[7] The obtained patterns were identical in all four subjects (*data not shown*). Using amplification and sequencing of the bridging fragment we showed that the 5′-breakpoint of the deletion is located in delta-IVS-II between nucleotides 758/759 (nt 55990/55991, HSHBB) and the 3′-breakpoint occurs 57 nucleotides upstream of the PstI site in the L1 repeat element at nt 68574/68575. The deletion is thus 12.585 bp long and is identical to the delta beta o deletion described by Fucharoen *et al.*, 2001.[8]

A summary of the genomic data is shown in Table 2. In all four subjects the sequence of the framework nucleotides was C-C-T-C-T indicating framework 2, the most common framework on *HBB*E* chromosomes.[9, 10, 11]

Several factors assumed to ameliorate the phenotypic expression of beta-thalassemia mutations were exam-

Table 1. Hematological data on four subjects with (delta-beta)° thalassemia/HbE.

Protocol Number	Age[a] /Sex	Hb g/dl	PCV l/l	RBC -/pl	MCV fl	MCH pg	MCHC g/dl	HbE[b] %	HbF %	HbF g/dl
378/64	25/F	10.1	27.8	3.86	72.2	26.2	36.3	52.2	43.5	4.4
446/59	35/M	10.6	34.3	4.57	75.1	23.2	30.9	52.0	44.1	4.7
476/60	40/F	8.6	27.9	4.34	64.3	19.8	30.8	53.0	45.3	3.9
490/52	30/M	n.d.	n.d.	n.d.	-	-	-	63.9	31.2	-

n.d. not determined; a estimated age; b HbE plus an expectedly small complement of Hb A2 which cannot be separated with the employed methods

Table 2. Genomic data of four subjects with (delta-beta)° thalassemia/HbE.

Protocol Number	beta-globin genotype	*HBB*E* framework[a]	-158 $^{G}_{gamma}$[b]	alpha-globin genotype
378/64	(delta-beta)°/*HBB*E*	C-C-T-C-T – 2	T/T	n.d.
446/59	(delta-beta)°/*HBB*E*	C-C-T-C-T – 2	T/T	alpha alpha/-alpha$^{3.7}$
476/60	(delta-beta)°/*HBB*E*	C-C-T-C-T – 2	T/T	alpha alpha/--^{SEA}
490/52	(delta-beta)°/*HBB*E*	C-C-T-C-T – 2	T/T	alpha alpha/-alpha$^{3.7}$

*n.d. not determined a beta globin gene associated framework on the HBB*E chromosome according to Antonarakis et al. (1982) b Polymorphism at position 158 of the Ggamma globin gene (T/T=XmnI +/+, [Gilman et al., 1985]*

ined. Three subjects had α-thalassemia deletions - two alpha-thalassemia-2 (-alpha3.7) and one alpha-thalassemia-1 of the Southeast Asian type (--^{SEA}). All four were homozygous for T at position 158 of the ^{G}gamma-globin gene (XmnI +/+), a condition associated with a marked increase in HbF production in stress erythropoiesis.[12]

Discussion

A recurrent 12.585 bp deletion (12.5 kb deletion) with the breakpoints located at nt 55990/55991 of the δ-globin gene (5´) and nt 68574/68575 (3´of the β-globin gene) resulting in (δβ)° thalassemia was identified in four of seven subjects with a beta-thalassemia/Hb E phenotype of the Alak and Ngeh groups of Lao Theung. Similar deletions have been already described in single subjects heterozygous for the lesion in Vietnam,[13] Thailand[14] and Laos.[7] More recently, two Thai patients compound heterozygotes for *HBB*E* and the same 12.5 kb (delta beta)° thalassemia were reported.[8] In all quoted reports the ancestry and ethnicity of the probands is not specified. As the hitherto observed carriers of this deletion originate from the same geographic area, we assume that the deletion is identical by descent with that in our probands. The apparently high frequency of the 12.5 kb deletion in the Lao Theung indicates that the mutation may have been introduced to neighboring populations by migrations from Laos to Northeast Thailand[15] and Central Vietnam.[2] Our observations suggest that in contrast to Vietnam and Northeast Thailand, where only single cases were reported among a great number of different beta-thalassemia mutations, the 12.5 kb deletion is a common, perhaps the predominant beta-thalassemia mutation in the Lao Theung of South Laos.

The clinical expression of compound heterozygosity for a beta° thalassemia gene and HBB*E is variable and ranges between a severe transfusion-dependent thalassemia major and a mild anemia resembling thalassemia minor.[16] The clinical picture of our probands is of mild thalassemia intermedia. Several factors are assumed to alleviate the clinical expression of beta-thalassemia, in particular beta-thalassemia /Hb E disease. The excess of alpha-chains may cause oxidative damage to the erythrocyte membrane. Coinheritance of alpha-thalassemia would reduce the amount of unmatched alpha-chains. Thus, the alpha-globin gene deletions found in three of our subjects may explain in part the relatively mild expression. This is doubtful, however, because both of the probands reported by Fucharoen *et al.*[8] had a normal complement of four alpha-globin genes, but hemoglobin concentrations were much higher than in our subjects. Homozygosity for T at position 158 of the Ggamma-globin gene causes a marked increase in Ggamma-chain and concomitant HbF production.[12] The probands from Northeast Thailand[8] and our cases were homozygous for T. This may lead to high HbF production and a relatively mild clinical expression, but the HbF concentrations in our cases were distinctly lower than those reported for XmnI+/+ subjects with beta°-thalassemia/HbE.[6]

Other converse observations concerning the effect of alpha-thalassemia and the 158 Ggamma polymorphism[12, 17, 18] let it appear likely that hitherto undefined factors, e.g. erythrocyte proteolytic activity[19] or differences in splicing efficiency[20] are more important for the widely variable clinical expression of compound heterozygosity for a beta-thalassemia mutation and HBB*E.

Since the (delta beta)-thalassemia is one of the most frequent high HbF determinants in the South-east Asian populations, compound heterozygous of this mutation with beta-thalassemia or other hemoglobinopathies are not uncommon. The 12.5kb deletion is most probably the main cause of (delta beta)o-thalassemia and should be tested in all patients with mild to severe thalassemia intermedia to allow proper genetic counseling and prenatal diagnosis.

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