DNA haplotypes and frameworks linked to the β-globin locus in an Austro-Asiatic population with a high prevalence of hemoglobin E

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Summary. DNA haplotypes (HT) and frameworks (FW) linked to the β-globin locus were determined by restriction fragment analysis using eight restriction enzymes on chromosomes bearing the HbA gene (HBB*A) or the HbE gene (HBB*E) in the So, an Austro-Asiatic population of northeast Thailand with an HBB*E frequency near 0.5. All HBB*E genes were present with FW2, and only two haplotypes were observed (25 HT 27-2, +++++++; 10 HT 41-2, +++++++). In a control group from the general population of Northeast Thailand the HT distribution was more diverse, and 2 of 20 HBB*E genes were present in FW 3. High frequencies of HBB*E in FW 3 in Southeast Asia are apparently limited to the Khmer population of Cambodia. There were no differences in the hematologic parameters in subjects homozygous for HBB*E/FW2 or HBB*E/FW3.

Introduction

The problems connected with the peculiar distribution of the hemoglobin E gene (HBB*E) in Southeast Asia have been accentuated by the detection of linkage disequilibria of DNA restriction polymorphisms in and around the β-globin gene complex (Antonarakis et al. 1982; Kazazian et al. 1984; Hundrieser et al. 1988a, b). The DNA haplotypes linked to the β-globin locus (HBB) are characterized by several polymorphic restriction sites 5' to the β-globin gene and by additional sites in and 3' to the β-globin gene that indicate nucleotide exchanges forming HBB frameworks as defined by Antonarakis et al. (1982). In all examined Southeast Asian populations, the HbA gene (HBB*A) is preferentially linked to the 5' haplotype +++++++ (41 in the present nomenclature), whereas HBB*E is most frequently linked to the 5' haplotype −+++++ (27). In contrast to HBB*A, which is found with all three observed DNA frameworks (1, 2, and 3 Asian), HBB*E is exclusively connected with framework 2 in most Southeast Asian populations. High frequencies of HBB*E in framework 3 (henceforth abbreviated HBB*E/FW3) were only found in the Khmer population of Cambodia (Hundrieser et al. 1988b).

In most regions of Southeast Asia there is a correlation between high frequencies of HBB*E and ethnicity, in particular with the Mon-Khmer or, in a wider sense, Austro-Asiatic language group (Flatz 1967). To test the hypothesis of an association between Mon-Khmer affiliation and HBB*E/FW3, we have examined the general population in the northern part of Northeast Thailand and the So, a group of people speaking an ancient Mon-Khmer language who have settled on both banks of the Mekong River in Northeast Thailand and Laos.

Material and methods

Description of the examined populations

The population of Northeast Thailand, the area in the arch formed by the Mekong River and the Thai-Cambodian border, is ethnically diverse. The northern and central parts are inhabited by Lao-speaking Thai people. Along the Thai-Cambodian border in the south of the area there is a substantial Khmer- and Soui-speaking population, and other Austro-Asiatic (Mon-Khmer) groups are located in isolated settlements in the eastern part of Northeast Thailand.

The So of Thailand, with approximately 50000 people the largest of these groups, are scattered over the provinces Sakon Nakhon and Nakhon Phanom (see Fig. 1). They speak a language related to Khmer (Cambodian), but more closely to Old Khmer and Soui, languages listed with So in the Katuic subgroup of Mon-Khmer by Voegelin and Voegelin (1977). The So in Northeast Thailand are derived from the large So population in the southern part of Laos and moved to the west bank of the Mekong River around the middle of the nineteenth century. They originated in the region with the highest reported frequencies of HBB*E in Southeast Asia (Livingstone 1985) and are the only population in this area for which an HBB*E frequency near 0.5 has been reported (Sriboonlue et al. 1985).

Test subjects, hematologic and DNA examinations

A total of 118 school children of the So ethnic group aged 10 to 14 years from three village groups of the Kusuman District in Sakon Nakhon Province in Northeast Thailand (Fig. 1) were screened for hemoglobinopathies with cellulose acetate electrophoresis at pH 8.7. Blood samples of 10ml were drawn from 19 children showing only hemoglobin in the position of Hb A2/HbE (presumed HBB*E homozygotes), one thalassemic child with an Hb E/Hb F pattern (presumed β-thalas-
The present field study was performed in Northeast Thailand. The areas inhabited by the So people are limited to the area of origin of the samples from the general population of this region. The purpose of the study was to examine the distribution of abnormal hemoglobins and the frequencies of the beta-globin gene cluster among So children, for the study of beta-globin linked haplotypes and frameworks.

A total of 106 apparently healthy adults from the northern part of Northeast Thailand (see Fig. 1) were similarly screened for abnormal hemoglobins, and 10-ml blood samples for DNA analysis were drawn from nine subjects homozygous for HBB*E. In addition, the haplotype of the HBB*E-carrying chromosome in four children with HbE-beta-thalassemia disease was determined by appropriate family examinations. Blood cell parameters were determined according to standard methods and using a Coulter Counter SplusQC in 38 healthy male HBB*E homozygotes from Northeast Thailand and Cambodia. The HBB-linked DNA haplotypes and the alpha-globin gene status of these subjects have been reported previously (Hundrieser et al. 1988a, b). The Hb Constant Spring gene was identified by hybridization with mutation-specific oligonucleotides according to a modification of the method of Zeff and Geliebter (1987). Because of possible effects of alpha-globin gene mutations on the hematologic parameters, subjects with alpha-globin gene deletions or Hb Constant Spring were excluded, leaving 26 HBB*E homozygotes with a normal complement of four alpha-globin genes.

**DNA restriction analysis**

DNA was isolated from frozen blood by phenol-chloroform extraction, and 3-6 µg DNA was digested with restriction enzymes under the conditions recommended by the manufacturer (Gibco-BRL). DNA fragments were separated by agarose gel electrophoresis and transferred to nitrocellulose filters according to Southern (1975). The filters were hybridized according to Law et al. (1984) with 32P-labeled alpha-, gamma-, and beta-specific sequences isolated from plasmid pC1.3, pJW151, pP3.9, and pHbetaI, constructed by Drs. J. Wilson, T. Maniatis, and R. A. Flavell.

**Beta-globin-gene-linked frameworks**

The beta-globin-gene-linked frameworks are characterized by several nucleotide exchanges and a specific restriction site pattern in and near the beta-globin gene (Antonarakis et al. 1982; cf. Fig. 2 in Hundrieser et al. 1988b). The frameworks were identified by the presence (+) or absence (−) of the AvalII restriction site in intron 2 of the beta-globin gene and of the BamHI site located 3' to the beta-globin gene. The nomenclature of Antonarakis et al. (1982) is used: ++, framework 1; +−, framework 2; and −+, framework 3. In examinations on more than 200 subjects in Southeast Asia (Hundrieser et al. 1988a, b) and in accordance with Antonarakis et al. (1982) the framework-associated restriction pattern −− was not observed. Therefore, subjects heterozygous at both the AvalII and the BamHI sites were classified as heterozygotes for frameworks 2 and 3.

**DNA haplotypes linked to the beta-globin gene cluster**

Six additional polymorphic restriction sites in the region of the beta-globin gene cluster were examined: (1) the HincII site 5' to the epsilon-globin gene, (2) the HindIII site in intron 2 of the gamma-globin gene (3) the HindIII site in intron 2 of the alpha-globin gene, (4) the HincII site in the gamma-globin gene, (5) the HincII site 3' to the gamma-globin gene, and (6) the HindI site 5' to the beta-globin gene. To permit comparison with our previous reports (Hundrieser et al. 1988a, b), the designation of the “5' haplotypes” (comprising the six above-described restriction sites) was retained: the first digit is the decimal equivalent of the binary representation of restriction sites 1-3, and the second digit, the same for restriction sites 4-6 (example: −−+++ is 5' haplotype 07).

**Results**

**Distribution of the HbE and beta-thalassemia genes**

The distribution of beta-globin genotypes among the So children, inferred from the electrophoretic and chromatographic phenotypes, is shown in Table 1 along with the gene frequencies and the Hardy-Weinberg expectations. The difference between observation and expectation is not significant (P = 0.545), and with respect to HBB*A and HBB*E the deviation from expectation is in the sense of an excess of heterozygotes. The control group of 106 subjects from the general population of the northern part of Northeast Thailand showed 57 HbA, 40 HbAE, and 9 HbE phenotypes, giving an HBB*E frequency of 0.274, which is in keeping with values previously reported from this area (Livingstone 1985).

**Table 1. Distribution of the beta-globin genotypes and gene frequencies in 118 So children from Kusuman District in Northeast Thailand.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>AA</th>
<th>AE</th>
<th>AT</th>
<th>EE</th>
<th>ET</th>
<th>TT</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
<td>26</td>
<td>62</td>
<td>2</td>
<td>27</td>
<td>1</td>
<td>0</td>
<td>118</td>
</tr>
<tr>
<td>Expected</td>
<td>28.5</td>
<td>57.5</td>
<td>1.5</td>
<td>29.0</td>
<td>1.5</td>
<td>0.0...</td>
<td>118</td>
</tr>
<tr>
<td>Gene frequencies</td>
<td>( p_A = 0.4915 )</td>
<td>( q_A = 0.4958 )</td>
<td>( r_T = 0.0127 )</td>
<td></td>
<td></td>
<td></td>
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</tbody>
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