Letter to the Editor

Genetic compound heterozygosity for Southeast Asian ovalocytosis and thalassemia in Thailand: prevalence and phenotypic analysis

To the Editor:

Southeast Asian ovalocytosis (SAO) is one of the red blood cell (Rbc) disorders characterized by a genomic deletion of 27 bp encoding amino acids 400–408 of the transmembrane domain of band 3 protein. Interaction of SAO with anion exchanger 1 (AE1) mutations could lead to a distal renal tubular acidosis. It might be a cause of anemia and hyperbilirubinemia in neonates.

![Map of Thailand showing the areas where subjects were recruited. Southeast Asian ovalocytosis (SAO) gene frequencies observed in Thailand and neighboring countries are depicted (▲ indicates catchment areas being studied, ● indicates other areas in neighboring countries where SAO gene frequencies have been reported). (b) A representative gel electrophoresis for identification of SAO mutation by polymerase chain reaction. The amplified fragments of 175 and 148 bp represent normal and mutant alleles, respectively. Lane 1 is normal control; lane 2 is heterozygous for SAO and lanes 3–6 are normal subjects. M represents the HyperLadder V markers (Bioline Ltd, London, UK).]

Fig. 1.
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Table 1. Hematological parameters of 402 subjects with various forms of thalassemia and abnormal Hb with and without SAO

<table>
<thead>
<tr>
<th>Type of thalassemia</th>
<th>SAO</th>
<th>N</th>
<th>Rbc (×10^{12}/l)</th>
<th>Hb (g/dl)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>RDW-CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-thalassemia</td>
<td>−</td>
<td>203</td>
<td>4.1 ± 0.5</td>
<td>12.0 ± 1.3</td>
<td>87.2 ± 4.6</td>
<td>29.5 ± 1.7</td>
<td>13.1 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>20</td>
<td>4.0 ± 0.5</td>
<td>12.6 ± 1.6</td>
<td>91.5 ± 3.8</td>
<td>31.4 ± 1.7</td>
<td>14.6 ± 1.4</td>
</tr>
<tr>
<td>Hb E trait</td>
<td>−</td>
<td>47</td>
<td>4.6 ± 0.7</td>
<td>11.8 ± 1.6</td>
<td>77.3 ± 3.3</td>
<td>25.5 ± 1.4</td>
<td>14.2 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>4</td>
<td>4.6 ± 0.9</td>
<td>12.8 ± 2.6</td>
<td>81.3 ± 1.9</td>
<td>27.6 ± 1.4</td>
<td>15.3 ± 2.2</td>
</tr>
<tr>
<td>β-Thalassemia trait</td>
<td>−</td>
<td>28</td>
<td>5.3 ± 1.0</td>
<td>11.0 ± 1.9</td>
<td>65.5 ± 5.8</td>
<td>20.9 ± 1.8</td>
<td>16.1 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>4</td>
<td>4.1 ± 1.1</td>
<td>9.3 ± 0.9</td>
<td>71.3 ± 8.6</td>
<td>23.0 ± 3.2</td>
<td>15.7 ± 1.1</td>
</tr>
<tr>
<td>α-Thalassemia 1 trait</td>
<td>−</td>
<td>41</td>
<td>5.5 ± 0.7</td>
<td>11.2 ± 1.8</td>
<td>64.6 ± 6.1</td>
<td>20.4 ± 2.0</td>
<td>16.3 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2</td>
<td>4.6, 5.1</td>
<td>9.5, 11.5</td>
<td>65.9, 72.0</td>
<td>20.5, 22.6</td>
<td>16.7, 13.4</td>
</tr>
<tr>
<td>Hb H disease</td>
<td>−</td>
<td>28</td>
<td>4.2 ± 1.3</td>
<td>7.4 ± 1.9</td>
<td>59.0 ± 8.4</td>
<td>18.1 ± 2.2</td>
<td>25.3 ± 4.9</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1</td>
<td>4.8</td>
<td>8.6</td>
<td>61.3</td>
<td>17.9</td>
<td>24.0</td>
</tr>
<tr>
<td>Hb constant spring trait</td>
<td>−</td>
<td>8</td>
<td>3.9 ± 0.8</td>
<td>10.0 ± 2.1</td>
<td>79.4 ± 6.4</td>
<td>26.0 ± 2.5</td>
<td>16.2 ± 6.6</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1</td>
<td>3.7</td>
<td>11.5</td>
<td>93.6</td>
<td>31</td>
<td>15.6</td>
</tr>
<tr>
<td>Homozygous α-thalassemia 2</td>
<td>−</td>
<td>5</td>
<td>4.6 ± 0.5</td>
<td>10.0 ± 1.6</td>
<td>68.7 ± 7.8</td>
<td>22.0 ± 3.6</td>
<td>15.6 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1</td>
<td>5.3</td>
<td>12.2</td>
<td>73.5</td>
<td>22.9</td>
<td>13.7</td>
</tr>
<tr>
<td>Hb D-Punjab trait</td>
<td>−</td>
<td>6</td>
<td>4.4 ± 0.5</td>
<td>11.9 ± 3.3</td>
<td>81.3 ± 15.4</td>
<td>26.4 ± 5.6</td>
<td>14.7 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2</td>
<td>3.5, 4.6</td>
<td>11.3, 13.1</td>
<td>88.2, 83.0</td>
<td>32.1, 28.5</td>
<td>13.7, 13.4</td>
</tr>
</tbody>
</table>

Hb, hemoglobin; Rbc, red blood cell; SAO, Southeast Asian ovalocytosis; −, absence; +, presence.

*Significant difference from SAO negative group (Mann–Whitney U-test, *p* < 0.001).

(1, 2). However, the effects of SAO on hematological changes in thalassemia and thalassemia trait have rarely been documented. We have demonstrated this and examined the prevalence of SAO among the southern and northeastern Thai populations.

A total of 940 subjects were recruited from three representative areas in upper, middle and lower southern Thailand. For comparison, DNA samples were also obtained from 246 northeast Thai patients with hemoglobin (Hb) E-β-thalassemia including both thalassemia major (TM) and thalassemia intermedia (TI) from Khon Kaen Province (3) (Fig. 1a). Hematological parameters, Hb fractions and DNA diagnosis of thalassemia were performed in all cases. The SAO-specific 27 bp deletion in AE1 gene was examined by polymerase chain reaction (4) (Fig. 1b).

Figure 1a summarizes gene frequencies of SAO observed as compared with those described in other populations (5). Among 940 southern subjects, 41 (4.4%) were found to carry SAO mutation. The proportions of SAO showed an increasing trend from the upper toward the lower parts, i.e. 1.5% (gene frequency = 0.0075) in Nakhon Si Thammarat, 3.0% (gene frequency = 0.0173) in Yala and 6.2% (gene frequency = 0.0324) in Narathiwat Provinces. In contrast, no SAO mutation was detected among 246 patients from northeast Thailand. SAO is widespread in many regions of Southeast Asia and Melanesia where prevalence ranging from 0% to 30% has been reported based on blood film examination (5). Molecular survey of the mutation in Southeast Asian populations, however, showed much lower frequencies (6). Apparently, prevalence of SAO increases continually from upper to lower southern Thailand, approaching the prevalence seen in Malaysia, Indonesia and Papua New Guinea, where malaria is endemic (7). It is conceivable that the SAO mutation originated in insular Southeast Asia and Papua New Guinea and spread within the region including the Indonesian archipelago, the Malaysian peninsular and the south of Thailand.

Interaction of β-thalassemia with Hb E leads to Hb E-β-thalassemia disease with variable clinical severity (4). Ovalocytosis is occasionally observed on blood films of patients. It has been thought that SAO might be one of the phenotypic modifying factors. However, this is probably not the case for northeast Thai patients as no SAO mutation was detected among 246 patients investigated. The presence of ovalocytosis in some patients results secondarily from thalassemia rather than SAO. It is noteworthy among the southern Thai population, among whom a genetic compound heterozygosity for thalassemia and SAO might be encountered at a routine thalassemia screening. As shown in Table 1, SAO could significantly increase mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and red cell distribution width (RDW) in non-thalassemic but not thalassemic subjects. It is noteworthy that although this finding did not reach statistical significance because of the small numbers of each thalassemia subtype, the increased MCV could make diagnosis of the thalassemia trait or disease more difficult. Genotyping by DNA analysis should help in diagnosis of cases. No additional effect of SAO to the microcytic anemia associated with thalassemia was noted. This data likely indicates that the presence of SAO does not have additional effect on Rbc parameters of thalassemia carriers, and concomitant inheritance of SAO with thalassemia does not produce more severe clinical symptoms. Therefore, screening for the SAO mutation in thalassemic patients is not necessary.

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