Erythroid marrow activity and functional anemia in patients with the rare interaction of a single functional α-globin and β-globin gene

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Background and Objectives. The degree of globin chain imbalance and tissue hypoxia are important determinants of clinical severity in thalassemia syndromes. Thus phenotypic expression may be modified by interaction of α- and β-thalassemia defects, level and type of hemoglobin synthesized and oxygen release to the tissues. We evaluated hematologic, erythroid marrow activity and functional anemia in patients with the rare interaction of a single α-globin gene and heterozygous β-thalassemia (HbH/β-thal trait).

Design and Methods. In 7 patients characterized by DNA analysis to have HbH disease genotypes with β-thalassemia trait, we assessed hematologic findings, serum transferrin receptor (sTfR), serum erythropoietin (Epo), red cell 2,3-disphosphoglycerate (2,3-DPG) and whole blood oxygen releasing capability.

Results. Patients with HbH/β-thal trait had moderate anemia, marked hypochromasia and microcytosis, normal or raised HbA2, and no electrophoretically chromatographically detectable HbH. Epo and sTfR levels were significantly higher than in β-thalassemia heterozygotes, but lower than in patients with HbH disease; 2,3-DPG levels were highest in HbH/β-thal trait. Oxygen binding studies and simulations showed reduced oxygen affinity (P50) in HbH/β-thal trait, resulting in increased oxygen release (O2R).

Interpretation and Conclusions. Hematologic findings and bone marrow activity in patients with HbH/β-thal trait were consistent with the modified globin chain imbalance and hemoglobin synthesis expected from interaction of HbH disease with heterozygous β-thalassemia, although this rare complex genotype may elude diagnosis based on hematology alone. Significantly higher red cell 2,3-DPG levels were an unexpected finding, and the consequent increase in oxygen release capability resulted in a compensated functional anemia relative to hemoglobin levels.

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Key words: HbH disease; heterozygous β-thalassemia; complex genotype; bone marrow activity; functional anemia

The α-thalassemia and β-thalassemia syndromes are characterized by reduced production of α-globin or β-globin chains, respectively and are simultaneously prevalent in many populations of the Mediterranean basin and S.E. Asia. A major factor influencing their phenotypic expression is the degree of globin chain imbalance which underlies ineffective erythropoiesis, and thus interactions of α- and β-thalassemia mutations may modify classical phenotypes of α- or β-thalassemia. Another important determinant of clinical severity is the degree of tissue hypoxia, which is influenced by the levels and type of hemoglobin and the oxygen release to the tissues.

Co-inheritance of heterozygous α-thalassemia in β-thalassemia heterozygotes or homozygotes has been well documented. However, interaction of heterozygous β-thalassemia with a single α-globin gene (HbH disease) is much less common and only a few patients with this rarer genotype have been reported. Although the hemoglobin production in these patients is directed from a single α- and a single β-globin gene, they have only moderately reduced hemoglobin levels and no clinical manifestations. This study set out to evaluate bone marrow activity and functional anemia in 7 patients with HbH disease genotypes and interacting β-thalassemia trait (HbH/β-thal trait), through measurements of serum transferrin receptor (sTfR), serum erythropoietin (Epo), red cell 2,3-disphosphoglycerate (2,3-DPG), whole blood oxygen releasing capability, and assessment of hematologic findings. Diagnostic pitfalls associated with this rare genotype are also presented.

Subjects. This study included seven patients from five families: one adult female, one infant and five children between 4-14 years old. In addition 55 β-thalassemia heterozygotes and 21 normal (non-thalassemic) individuals were studied for comparison.
Results

This study included seven patients from five families. Five of the patients were children between 4-14 years old (Table 1) and patient VI was an adult female. Patient VII was an infant, diagnosed at birth with subsequent follow-up for 7 months; his hematologic findings are presented separately (see below).

α- and β-globin gene mutations in the seven patients are summarized in Table 1. Five common Mediterranean α-thalassemia mutations were characterized, interacting to produce three different HbH disease genotypes, and each case carried one of four different β-thalassemia mutations. The α/ non-α globin biosynthesis ratios, measured in patients III, IV, V and VII, ranged between 0.5-0.7 after 1 hour of incubation (normal range 0.9-1.2).

Excluding Patient VII, mean hemoglobin levels in the children were 98.4±4.5g/L, with MCV 56.4±4.1fL, MCH 15.8±1.6 pg and MCHC 282.0±7.8g/L (Table 1). The hematologic findings in the single adult patient (VI) were within the same range (Table 1). HbA2 levels were raised (4.0-4.8%) in four patients and within the normal range (2.1%) in two. HbF levels were below 1% in all cases. HbH was not detected by wce-HPLC in any patient but traces of Hb Bart’s were detected in patients VI and VII who had 14% HbF at 7 months, which is associated with an increase of oxygen availability (O2A) to the tissues (Table 2).

<table>
<thead>
<tr>
<th>Patient α-genotype</th>
<th>β-genotype</th>
<th>Hb</th>
<th>MCV</th>
<th>MCH</th>
<th>MCHC</th>
<th>RDW</th>
<th>HbA2</th>
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<tr>
<td>I</td>
<td>(α)2ε-</td>
<td>I-6/N</td>
<td>102</td>
<td>58.0</td>
<td>16.4</td>
<td>283</td>
<td>20</td>
</tr>
<tr>
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<td>(α)2ε-</td>
<td>I-6/N</td>
<td>102</td>
<td>62.0</td>
<td>18.2</td>
<td>291</td>
<td>22</td>
</tr>
<tr>
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<td>I-2/N</td>
<td>99</td>
<td>51.4</td>
<td>14.0</td>
<td>272</td>
<td>19.2</td>
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<tr>
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<td>-α/αε-</td>
<td>I-2/N</td>
<td>98</td>
<td>53.7</td>
<td>15.4</td>
<td>287</td>
<td>17.5</td>
</tr>
<tr>
<td>V</td>
<td>-α/αε-</td>
<td>I-110/N</td>
<td>91</td>
<td>56.9</td>
<td>14.8</td>
<td>287</td>
<td>18.5</td>
</tr>
<tr>
<td>VI</td>
<td>α-αε/</td>
<td>I-110/N</td>
<td>98</td>
<td>58.1</td>
<td>16.7</td>
<td>287</td>
<td>18.1</td>
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<td>VII (1 m)</td>
<td>(α)2ε-ε-</td>
<td>cd39/N</td>
<td>82</td>
<td>90</td>
<td>29.8</td>
<td>300</td>
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<td></td>
<td>101</td>
<td>50.0</td>
<td>17.9</td>
<td>285</td>
<td>26.6</td>
</tr>
</tbody>
</table>

*α-thalassemia mutations: I-6 = IVS I-6 T>C; I-1 = IVS I-1 G>A; I-110 = IVS I-110G>A; cd39 = c393C>T. °Traces of Hb Bart’s detected by wce-HPLC. *Single adult patient. †HbA2 was not detected (ND) at 1 month of age, wce-HPLC detected HbF 7.8% at 1 month, falling to 14% by 7 months) and Hb Bart’s (5% at 1 month, falling to 2.5% by 7 months).

Blood cell indices were measured using an automatic continuous method (Abbott Laboratories, IL, USA). Oxygen equilibrium curves (OEC) were recorded using an automatic continuous method (Hemox-Analyzer) as previously described. The P50 values were calculated from the OECs. Oxygen transport and release simulations were performed using the Siggaard-Andersen’s Oxygen Status Algorithm. Red cell 2,3-disphosphoglycerate (2,3-DPG) levels were measured enzymatically (Boehringer Mannheim, Germany). In vitro globin chain synthesis was analyzed in peripheral blood reticulocytes from 4 patients at incubation times of 1 hour, according to standard methods. α- and β-globin gene mutations were characterized according to previously described methods.

Statistical comparison of data was carried out using the Student’s t-test.

III, IV and V. Red cell morphology showed HbH inclusions in occasional red blood cells in all patients. Reticulocyte counts were normal or slightly raised (1.0-4.5%) and the reticulocyte production index or RPI, as calculated using reticulocyte count × Hct/45, was significantly lower and 2,3-DPG levels significantly higher than in patients with HbH disease (Table 1). Oxyhemoglobin binding studies and simulations showed a decrease in the oxygen affinity in all patients (excluding patient VII, who had 14% HbF at 7 months), which is associated with an increase of oxygen availability (O2A) to the tissues (Table 2).
hemoglobin component HbH. This condition is usually characterized by the detection of abnormal hemoglobin tetramers, which are insufficient to form electrophoretically or chromatographically detectable tetramers of HbH.

The patients in this study were characterized as having HbH disease genotypes interacting with β-thalassemia trait (Table 1). Three HbH genotypes were characterized (-α/αβ)3, 4, -α/αβHbH, and homozygous αβαβ, all commonly encountered in Greek HbH disease patients.13,14 The interacting β-thalassemia mutations included the four most common mutations in the Greek HbH disease population.13

Hematologic findings in the patients of this study (Table 1) were comparable to those previously reported for this rare genotype interaction;4-6 they were distinguished from typical HbH disease by the absence of HbH, and from simple heterozygous β-thalassemia by the markedly reduced MCV and MCH values.14,16 HbH disease is usually characterized by detection of the abnormal hemoglobin component HbH (HbH) in the peripheral blood. As indicated from the α/α′ globin biosynthesis ratios we observed in HbH/β-thal trait, the interaction of a β-thalassemia mutation reduces the β chain excess to levels found in heterozygous α-thalassemia, which are insufficient to form electrophoretically or chromatographically detectable tetramers of HbH.

Compared to simple β-thalassemia heterozygotes, patients with HbH/β-thal trait had lower levels of hemoglobin and more pronounced microcytosis and hypochromasia.27 HbA2 levels were within the range typically found in β-thalassemia heterozygotes in four patients, in accordance with previous reports for patients with HbH/β-thal trait,4,6 although two patients (I and II) had normal HbA2 levels. This is probably because they carried the mild β+-thalassaemia donor splice-site mutation IVSI-6 C>T, which is often associated with normal/borderline HbA2 levels in the simple heterozygous state.12,13 Overall, the hematologic findings in patients with HbH/β-thalassemia trait were almost indistinguishable from those found in iron-deficient β-thalassemia heterozygotes,20 and thus this complex genotype may confound diagnosis based on hematology alone, especially when HbA2 levels are normal or borderline.

The raised sTfR levels in HbH/β-thal trait, along with relatively low RPI values (<2) indicate erythropoietic, due mainly to ineffective erythropoiesis.21 A major factor underlying ineffective erythropoiesis is the relative globin chain imbalance, and accordingly the sTfR levels in the patients of the study were between those found in simple β-thalassemia heterozygotes and HbH disease patients (Figure 1), although the difference with the HbH disease patients was not statistically significant, probably due to the wide range of sTfR values in the latter group.12,14 However, the degree of globin chain imbalance is apparently not the only determinant of ineffective erythropoiesis, since sTfR levels in HbH/β-thal trait are much higher than in α-thalassemia heterozygotes, despite comparable α/α′ chain synthesis ratios.23 Presumably the markedly reduced overall synthesis of α- and β-globin chains in HbH/β-thal trait exacerbates the red cell abnormalities and thus leads to
more pronounced dyserythropoiesis.

Epo levels in patients with HbH/β-thal trait (Table 2) were higher than in simple β-thalassemia heterozygotes, but lower than those we have previously observed in HbH disease patients. In the absence of conditions such as inflammation and abnormal kidney function, Epo production is closely related to the level of oxygen supply to the tissues, which is in turn influenced by red blood cell mass (hemoglobin levels) and the presence of certain hemoglobin variants. Hemoglobin levels in patients with HbH/β-thal trait were much lower than in simple β-thalassemia heterozygotes, explaining the relatively higher Epo levels in the former. The absence of HbH in HbH/β-thal trait probably accounts for the relatively lower Epo level relative to that found in HbH disease patients, despite comparable hemoglobin levels; we have previously demonstrated a negative influence of HbH on tissue oxygenation, since HbH is unable to deliver oxygen to the tissues. Finally red cell 2,3-DPG levels were higher in patients with HbH/β-thal trait than in either HbH disease patients or β-thalassemia heterozygotes, leading to reduced oxygen affinity and thus increased oxygen release to the tissues (Table 2). The higher levels of 2,3-DPG may be due to the severe microcytosis in HbH/β-thal trait, a mechanism which has been suggested to occur in simple β-thalassemia heterozygotes. The compensated oxygen delivery in HbH/β-thal trait is also implied by the lower observed (24.5±3.4 IU/L) Epo levels relative to those predicted (32.5 IU/L) for the level of hemoglobin.

Overall the hematologic findings and bone marrow activity in HbH/β-thalassemia trait are consistent with the modified globin chain imbalance and hemoglobin synthesis caused by the complex genotype interaction, such that these features are better than in HbH disease but worse than in heterozygous β-thalassemia. Despite the synthesis of globin chains by only a single α- and single β-globin gene, these patients' functional anemia is compensated relative to their hemoglobin levels.

Contributions and Acknowledgments
All authors made a valuable contribution towards the study and article, and the order of the authors’ names reflects their relative contribution. JT-S, IP, and CV were involved in all laboratory analyses, scientific overview and writing of the paper; CS was involved in the laboratory analysis of normal and β-thal heterozygote controls; AS and EK played a role in the clinical and hematologic evaluation of patients.

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Potential implications for clinical practice
The hematologic and molecular data in the study may be useful for better patient management and family counselling.

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