Thalassaemia intermedia in a family with \(\beta^0\)-thalassaemia and Hb Hasharon

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SUMMARY A Brazilian family of Italian descent is described in which the \(\beta\)-thalassaemia gene is interacting with an \(\alpha\) chain variant Hb Hasharon (\(\alpha\) 47 Asp→His). One patient who was affected by homozygous \(\beta^0\)-thalassaemia and heterozygous \(\alpha\)\textsuperscript{Hasharon} displayed the clinical picture of thalassaemia intermedia. Her haemolysate contained 8.6% Hb F\textsubscript{Hasharon} (\(\alpha_2\)\textsuperscript{Hasharon}\(\beta_2\)) and 1.1% Hb A\textsubscript{2}, the remaining haemoglobin being Hb F. Hb A was not detected. Globin chain synthesis in reticulocytes showed non-\(\alpha\)/total \(\alpha\) ratios of 0.29, 0.39, and 0.73 respectively for the patient, the mother, and the father, who is heterozygous for both the \(\beta^0\)-thalassaemia and Hb Hasharon genes. The possible contribution of Hb Hasharon heterozygosity to the less severe expression of homozygous \(\beta^0\)-thalassaemia is discussed.

With regard to clinical expression, the \(\beta\)-thalassaemias can usually be classified into the most severe forms of Cooley's anaemia or thalassaemia major, or the symptomless heterozygous carrier state.\(^1\) The term \(\beta\)-thalassaemia intermedia, although ill defined, is used to describe patients who have symptoms intermediate to these two groups. Anaemia is moderate, transfusions are rarely if ever required, bone alterations are mild or absent, and somatic and sexual development is normal. This clinical picture can be produced by a variety of genotypes and may also result from the combination of \(\beta\)-thalassaemia trait and environmental factors.\(^1\)\(^-\)\(^7\)

In this report we describe a patient with thalassaemia intermedia belonging to a Brazilian family of Italian origin. In this case the syndrome results from the association of homozygous \(\beta^0\)-thalassaemia with Hb Hasharon.

Material and methods

The proband is a 6-year-old girl who has been moderately anaemic and slightly jaundiced since the age of 6 months. She has not been transfused and has been treated with folic acid. Her growth has been normal (weight 24.8 kg). She has a mildly abnormal facies and the spleen and the liver are palpable 12 cm and 5 cm below the costal margin respectively. The family is of Italian descent and the parents and sister are asymptomatic.

Red blood cells and haemoglobin concentrations were determined electronically (Microcellcounter CC-108 and Hemoglobin Counter Hb-100, TOA) and the PCV was measured using capillary tubes. Haemoglobin electrophoresis was performed on cellulose acetate with Tris-EDTA-boric acid buffer at pH 8.9 and on agar gel with citrate buffer at pH 6.1.\(^8\) Hb Hasharon, Hb F\textsubscript{Hasharon}, and Hb A\textsubscript{2} were measured spectrophotometrically after elution from cellulose acetate strips following electrophoresis.\(^8\) Fetal haemoglobin (Hb F) was determined by alkali denaturation.\(^9\)

The abnormal haemoglobin was isolated from the father's haemolysate by chromatography on DEAE cellulose (DE-52, Whatman) using glycine (15 g/l) NaCl (0.01-0.035 mol/l) buffers, as described by Abraham et al.\(^10\) It eluted before Hb A, was concentrated under vaccum, and the globin prepared by acetone/HCl precipitation. The tryptic fingerprint analysis\(^11\) was performed at the MRC Molecular Haematology Unit, Oxford.

The measurement of globin synthesis was carried out by incubating peripheral blood reticulocytes with \(^3\)H-leucine for 1 hour.\(^12\) Whole cell lysates were converted to globin immediately after the completion of the labelling experiment and the chains were separated by chromatography on CM cellulose.\(^13\) Radioactivity was measured by liquid scintillation counting and the non-\(\alpha\)/\(\alpha\) ratio was calculated on the basis of the total counts in each chain.

Results

The haematological data and the results of the
TABLE Haematological data, haemoglobin composition, and globin chain synthesis in a family with \( \beta^+ \)-thalassaemia and Hb Hasharon.

<table>
<thead>
<tr>
<th></th>
<th>Hb (g/dl)</th>
<th>RBC (×10^{12}/l)</th>
<th>HTC</th>
<th>Hb A2* (%)</th>
<th>Hb F (%)</th>
<th>Electrophoresis</th>
<th>Non-( \alpha )/( \alpha ) ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>8.3</td>
<td>3.1</td>
<td>0.20</td>
<td>1.1</td>
<td>66.0</td>
<td>( \dagger )Hb F + Hb F\text{Hash}</td>
<td>0.29</td>
</tr>
<tr>
<td>Father</td>
<td>14.8</td>
<td>5.7</td>
<td>0.46</td>
<td>4.0</td>
<td>2.1</td>
<td>( \dagger )Hb A + Hb Hash + Hb A2 + Hb A2\text{Hash}</td>
<td>0.71</td>
</tr>
<tr>
<td>Mother</td>
<td>10.1</td>
<td>5.0</td>
<td>0.33</td>
<td>5.6</td>
<td>4.1</td>
<td>Hb A + Hb A2</td>
<td>0.39</td>
</tr>
<tr>
<td>Sister</td>
<td>10.1</td>
<td>5.0</td>
<td>0.32</td>
<td>3.9</td>
<td>3.8</td>
<td>Hb A + Hb Hash + Hb A2 + Hb A2\text{Hash}</td>
<td>—</td>
</tr>
</tbody>
</table>

*Including Hb A2 + Hb A2 Hasharon
†Hb F Hasharon = 8.6%; Hb Hasharon = 12.1%
‡Non-\( \alpha \)/\( \alpha \) (\( \alpha \) + \( \alpha \) Hash) ratio

FIG 1 Electrophoretic patterns of haemoglobins on cellulose acetate. (1) Father, Hb A and Hb Hasharon (Hb A2 is split). (2) Patient, Hb F and Hb F Hasharon. (3) Mother, Hb A and Hb A2.

Globin chain synthesis measurements revealed the absence of \( \beta \) chain synthesis in the patient and the presence of \( \gamma \) and \( \alpha \) chains and, in addition, a small peak of abnormal \( \alpha \) chains (\( \alpha' \)) that eluted after the normal \( \alpha \) peak (Fig 2). Therefore, the patient was a \( \beta^+ \)-thalassaemia homozygote and also a heterozygous carrier of the \( \alpha \)-Hasharon gene. The non-\( \alpha \)/total \( \alpha \) ratio was 0.29 for the patient, 0.73 for the father, and 0.39 for the mother. The abnormal \( \alpha \) chains corresponded to 9.8% and 9.5% of the total \( \alpha \) chains of the father and the patient, respectively, which agrees with the percentage of Hb Hasharon (12.1%) and Hb F Hasharon (8.6%) detected by other methods.

FIG 2 CM-cellulose chromatography of globin chains from the patient (top) and her father (bottom). The first peak is \( \beta^+ \) for the father and \( \gamma \) for the patient. In both cases the normal \( \alpha \) chains are followed by a small peak of abnormal \( \alpha \) chains.
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Discussion

The $\beta$-thalassaemia gene is present in the heterozygous state in about 0.8% of the population in our region, the north-east of the state of São Paulo. It is frequently found in the homozygous state or in combination with Hb S. Homozygosity is usually associated with the more severe course of Cooley's anaemia but the clinical picture of thalassaemia intermedia is not infrequently observed.

In the family described here the syndrome affects a girl who is a $\beta^a$-thalassaemia homozygote, since neither $\beta$ chain synthesis nor Hb A could be detected in the peripheral blood. In addition, she is a heterozygous carrier of an $\alpha$ chain variant Hb Hasharon ($\alpha_{H,\alpha'=2}$) which could reduce the severity of the $\beta$-thalassaemia. The abnormal $\alpha$ chains bind to $\gamma$ chains because of the unavailability of $\beta$ chains and give rise to a new fetal-like haemoglobin consisting of $\alpha_{Hasharon}^\gamma$, showing an electrophoretic mobility slower than normal Hb F. The small amount of Hb Hasharon detected in heterozygotes when a $\beta$-thalassaemia gene is also present has been reported previously and is thought to be the result of the latent instability in vivo of the abnormal $\alpha$ chains. In situations of relative deficiency of non-$\alpha$ chains, $\alpha^A$ globin would bind to $\beta$ or $\gamma$ chains more effectively than $\alpha_{Hasharon}$. Since the abnormal $\alpha$ chains are removed from the cytoplasm at a faster rate than normal $\alpha$ globin, this could reduce the $\alpha$ chain excess and therefore contribute to the milder clinical expression observed.

A second explanation is based on the observation of Pich et al and Giglioni et al who have demonstrated that the $\alpha_{Hasharon}$ gene observed in the Po river delta region of northern Italy is linked to an $\alpha$-thalassaemia gene, while in Ashkenazi Jewish carriers it is probably linked to a normal $\alpha$ gene on chromosome 16. The association of an $\alpha$-thalassaemia defect would also explain the mildness of the $\beta^a$-thalassaemia in this patient. This hypothesis is compatible with the Italian ancestry of this family and with the $\beta/(\alpha+\alpha')$ ratio of 0.73 for the father, which is in the upper limit of the range for simple $\beta$-thalassaemia heterozygotes in our laboratory (0.55±0.16, mean±SD). However, this could be tested only by restriction endonuclease analysis of the DNA from the patient and her parents.

However, a definite explanation at the molecular level cannot be presented. Because the severity of the $\beta$-thalassaemia is reduced, it seems clear that the association of these two mutations provides an advantage over simple $\beta^a$-thalassaemia homozygosity.

Giglioni et al proposed that the $\alpha_{Hasharon}$ gene reached high frequencies in northern Italy because it is a neutral mutant that was selected by virtue of its linkage to an $\alpha$-thalassaemia gene that confers selective advantage to the carriers in a malaria area. However, since the $\beta^a$-thalassaemia gene is also highly prevalent in that region, the effect of the $\alpha_{Hasharon}/\alpha$-thalassaemia gene complex in reducing the severity of $\beta^a$-thalassaemia could be an additional factor in explaining the frequency of the $\alpha_{Hasharon}$ gene.

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References


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