δβ-thalassaemia in southern Italy: evidence for a single mutational event

A CARÉ*, N M SPOSI*, A GIAMPAOLO*, T IMPROTA*, M CALANDRINI*, M PETRINI*, M MARINUCCI*, A TAGARELLI†, AND C BRANCATI†

From *the Department of Hematology, Istituto Superiore di Sanità, Roma, Italy; and †Istituto per lo Studio delle Malattie Ereditarie e Carenziali, CNR e Centro di Studi per le Microcitemie, Cosenza, Italy.

SUMMARY Haematological and molecular studies on 32 heterozygotes for GγAγδβ+α-thalassaemia from 15 unrelated families from southern Italy are reported. The haematological features of GγAγδβ+α-thalassaemia carriers are compared with those of β-thalassaemia and Hb Lepore heterozygotes. Striking similarity exists between the phenotypic expression of β-thalassaemia and Lepore mutations. Globin gene mapping studies indicated that the molecular lesion underlying δβ-thalassaemia is a large deletion starting from the large intervening sequence of the δ gene and extending downstream from the β gene. The possibility that δβ-thalassaemia haplotypes in southern Italy originated from a single mutational event is discussed.

δβ-thalassaemias are characterised by absence of adult (δ and β) globin gene expression and persistent fetal (γ) globin synthesis in heterozygotes, directed by one (GγAγδβ-thalassaemia) or both (GγAγδβ-thalassaemia) γ globin genes.1 Gene mapping studies showed that in most Gγδβ- and GγAγδβ-thalassaemias, the molecular defects involved large deletions of all or part of the Aγ δ and β globin genes.2 3 At least one form of δβ-thalassaemia was not associated with gene deletion.4

We report the haematological and molecular characterisation of a number of δβ-thalassaemia carriers from southern Italy.

Materials and methods

Thirty-two adult δβ-thalassaemia carriers from 15 unrelated families originating from Calabria, southern Italy, were studied. Haematological data, including Coulter Counter S analysis, were obtained by routine methods. Hb A2 was determined by elution following cellulose acetate electrophoresis. Hb F (FAD) was evaluated by alkali denaturation.5

The Gγ/Aγ ratio of Hb F was determined by Triton-urea polyacrylamide gel electrophoresis of globin chains as previously described.6 Hb F was first purified to >80% by alkali denaturation and then dialysed against Tris 0-01 mol/l HCl buffer pH 7-4.

High molecular weight DNA was obtained from WBC by standard techniques, digested with restriction endonucleases, electrophoresed on 0-8% agarose gel, and transferred to nitrocellulose filters as previously described.7 Filters were hybridised to plasmids JW 102 and JW 151 containing human β and γ cDNA sequences,8 washed under stringent conditions, and autoradiographed.

Results

Haematological studies

δβ-thalassaemia heterozygotes were clinically asymptomatic. The percentages of Hb F and Hb A2 averaged 10·4±0·35 and 2·7±0·09, respectively (mean ± SE). The other RBC and Hb parameters showed fairly low variability, and are listed in the table together with those obtained from two groups of β-thalassaemia and Hb Lepore carriers comparable for number, age, and sex distribution. The Gγ/Gγ+ Aγ ratio averaged 0·231±0·41, ranging from 0·142 to 0·317.

Restriction endonuclease analysis

The restriction endonuclease pattern obtained from genomic DNA of δβ-thalassaemia carriers was
Comparable with that reported for GyAγδβ-thalassaemia subjects. In particular, Eco RI yielded the four normal β-like fragments of 5·2, 3·2, 2·2, and 1·8 kb plus an additional 3·0 kb fragment (figs 1 and 2) extending from the Eco RI site upstream from the δ gene to the first site downstream from the deletion. Bam HI yielded only the normal fragments (5′ δ 15·0, 3′ δ 4·4, 5′ β 1·8, 3′ β 9·3 kb) indicating that the Bam HI site within the δ gene is not removed by the deletion (figs 1 and 2). Hpa I yielded, in addition to the normal fragments of the unaffected chromosome, a new fragment of 2·3 kb extending from site 5′ to the δ gene to a new site downstream from the deletion. This indicated that the Hpa I site located in the large intervening sequence (IVS-2) of the δ gene was missing (figs 1 and 2). Bgl II and Pst I yielded two abnormal bands of 4·0 and 9·7 kb respectively, as previously described (results not shown). These results are consistent with a large DNA deletion starting somewhere between the Bam HI and the Hpa I site within the δ gene IVS-2 and extending several kb downstream from the β gene (see fig 2).

### Table Comparison of RBC and haemoglobin parameters in δβ-thalassaemia, β-thalassaemia, and Hb Lepore carriers.

<table>
<thead>
<tr>
<th></th>
<th>δβ-thalassaemia (n = 32)</th>
<th>β-thalassaemia (n = 32)</th>
<th>Hb Lepore (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10¹²/l)</td>
<td>6·025 ± 0·412</td>
<td>5·926 ± 0·028</td>
<td>5·735 ± 0·033</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12·859 ± 0·204</td>
<td>11·573 ± 0·264</td>
<td>12·272 ± 0·263</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>68·656 ± 0·414</td>
<td>62·781 ± 0·938</td>
<td>69·875 ± 1·426</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>21·344 ± 0·236</td>
<td>19·881 ± 0·345</td>
<td>21·525 ± 0·396</td>
</tr>
<tr>
<td>Hb A (%)</td>
<td>2·600 ± 0·090</td>
<td>5·186 ± 0·018</td>
<td>2·334 ± 0·021</td>
</tr>
<tr>
<td>Hb F (%)</td>
<td>10·363 ± 0·348</td>
<td>1·231 ± 0·042</td>
<td>2·825 ± 0·049</td>
</tr>
<tr>
<td>β chains (pg/cell)</td>
<td>9·231 ± 0·003</td>
<td>9·384 ± 0·117</td>
<td>9·235 ± 0·138</td>
</tr>
<tr>
<td>γ chains (pg/cell)</td>
<td>1·156 ± 0·132</td>
<td>0·133 ± 0·092</td>
<td>0·300 ± 0·049</td>
</tr>
<tr>
<td>δ chains (pg/cell/cis)</td>
<td>0·282 ± 0·032</td>
<td>0·261 ± 0·012</td>
<td>0·250 ± 0·011</td>
</tr>
<tr>
<td>δβ chains (pg/cell)</td>
<td>—</td>
<td>—</td>
<td>1·324 ± 0·038</td>
</tr>
</tbody>
</table>

**Fig. 1** δ and β globin gene fragments in Bam HI (left), Eco RI (middle), and Hpa I (right) digests of DNA from a normal subject and from δβ-thalassaemia heterozygotes. Sizes are in kb.

**Fig. 2** Restriction endonuclease map of the γδβ globin gene region from normal human DNA (top) and δβ-thalassaemia DNA (bottom). B = Bam HI, E = Eco RI, H = Hind III, Hp = Hpa I. Asterisks indicate polymorphic sites.
δβ-thalassaemia in southern Italy: evidence for a single mutational event

In order to characterise the γδβ chromosome associated with the δβ deletion, DNA was also digested with the Hind III and Hind II endonucleases, which recognise polymorphic sequences within the IVS-2 of both γ genes and 5' to the ε globin gene, respectively. In more than half of the cases unequivocal evidence was obtained that both γ genes on the δβ-thalassaemia chromosome lack the polymorphic Hind III site (that is, they were homozygotes for the absence of both sites). In the other cases the Hind III pattern was always compatible with this assumption, Gγ and Aγ Hind III polymorphic sites being absent on at least one chromosome. The ε Hind II polymorphic site was present in both chromosomes in most cases, the remainder being heterozygotes.

Discussion

δβ-thalassaemia has been observed in many ethnic groups. The blood picture in heterozygotes is characterised by thalassaemia-like abnormal RBC indices and morphology and slightly reduced Hb levels. We have compared this picture to that of two other conditions (heterozygous β-thalassaemia and Hb Lepore) showing similar features of globin chain imbalance and RBC parameters, despite the substantially different underlying molecular lesion. Both β-thalassaemia and Hb Lepore carriers appear to be significantly less hypochromic and microcytic than δβ-thalassaemia heterozygotes (p < 10⁻³, table). This can be ascribed to the significant chain overproduction observed in the former (1.16 ± 0.13 pg/cell vs 0.14 ± 0.03 pg/cell in β-thalassaemia carriers), and to the δβ chain output (1.31 ± 0.05 pg/cell) in the latter. The mean β chain synthesis in δβ-thalassaemia accounts for 9.23 ± 0.03 pg/cell, which is assumed to represent the product of the single normal β gene in trans to the deletion. The overproduction with respect to a β gene in normal subjects (7.47 ± 0.06 pg/cell/cis) indicates the capability of the β gene to respond to a compensatory stimulus in this condition (about 1/4). The same extent of compensation was also observed in both thalassaemia and Hb Lepore heterozygotes (β chain = 9.38 ± 0.12 and 9.24 ± 0.14 pg/cell, respectively). The single functional δ gene in thalassaemia showed increased activity when compared to half of the δ chain production in normal subjects (0.28 ± 0.03 pg/cell vs 0.21 ± 0.01 pg/cell/cis, p < 10⁻⁵). This overproduction (of about 1/3) is slightly higher than that observed in both Hb Lepore (0.25 ± 0.01 pg/cell) and δ-thalassaemia trait (0.26 ± 0.01 pg/cell/cis) (p < 0.05 in both cases).

The percentage Aγ chain in southern Italian δβ-thalassaemia (14.2 to 31.7) was significantly higher than that reported for Sardinian non-deletion δβ-thalassaemia (0.05 to 0.10) and lower than that observed in Spanish δβ-thalassaemia (0.30 to 0.35), in which the deletion extends ∼3 kb upstream from the δ gene. Since in all the three syndromes it has been proven or assumed that γ chain synthesis is directed by the γ genes in cis to the deletion, these data suggest a direct correlation between Gγ gene activation and the extent of deletion in the 5' region of the adult globin gene domain.

Restriction endonuclease mapping indicates that in all the southern Italian δβ-thalassaemia carriers the molecular defect involves the same deletion, starting within the δ gene and including the whole β gene and its 3' region. Furthermore, more than half of the δβ chromosomes, and possibly all of them, were homogeneous as regards the polymorphic sites within the γ and ε genes.

These data support the conclusion that δβ-thalassaemia in southern Italy originated from a single mutational event. The relatively high frequency observed in well-defined regions (for example, 0.04% in Calabria) is most likely the result of selection by malaria, which is thought to be the major cause of the high incidence of β-thalassaemia in these areas.

This work was supported by CNR grant No 82.02429.04.

References


Correspondence and requests for reprints to Dr M Marinucci, Department of Hematology, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma, Italy.
Delta beta-thalassaemia in southern Italy: evidence for a single mutational event.

A Carè, N M Sposi, A Giampaolo, T Improta, M Calandrini, M Petrini, M Marinucci, A Tagarelli and C Brancati

doi: 10.1136/jmg.21.2.117

Updated information and services can be found at:
http://jmg.bmj.com/content/21/2/117

_Email alerting service_

_These include:_

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

_Notes_

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/