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**β-thalassaemia types in southern Sardinia**

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**Summary** In this study the prevalence of the different β-thalassaemia types in southern Sardinia was investigated by cellulose acetate and agar gel electrophoresis or globin chain synthesis analysis on column chromatography or both in (1) all the patients (347) presenting with thalassaemia major or intermedia at our haematology service from 1976 to 1979, and (2) a group of 82 patients with transfusion-dependent thalassaemia major randomly chosen from 236 under our care. Apart from six subjects with δβ°/β°-thalassaemia genotype and eight with β°/β+ or less probably β+/β+-thalassaemia, all thalassaemia major and intermedia patients studied were β°-thalassaemia homozygotes. Globin chain synthesis on peripheral blood cells from these patients, performed at different intervals from blood transfusion, showed no incorporation of radioactive leucine into β-globin peak, the same as before the transfusion. No correlation between α/γ ratios and clinical severity or hypersplenism was found. Globin chain synthesis analysis carried out at birth in three infants later found to have homozygous β°-thalassaemia demonstrated imbalanced or borderline α/γ ratios.

The β-thalassaemias (thal) are a heterogeneous group of genetic disorders which can be divided into β°-, β+-, and δβ-thal according to the severity of the defective synthesis of the β-globin chains, which is completely suppressed in β° and partially suppressed in β+-thal, and the eventual association of defective δ-globin chain synthesis. At molecular level each β-thal type can be further subdivided into different subtypes.4 The world distribution of the above mentioned β-thal types is uneven: in the Ferrara region of Italy and in Cyprus β°- and β+-thal, respectively, are found almost exclusively, while in Sicily, Greece, and Thailand both types are found in variable proportions.5–9

The purpose of this study is twofold: (1) to establish the prevalence of the different β-thal types in southern Sardinia and (2) to see whether peripheral blood cells from Sardinian β°-thal homozygotes synthesise any β-chains after blood transfusion, as has been seen in homozygous Ferrara β°-thal.10,11

**Subjects and methods**

The subjects studied were: (1) all patients presenting with thalassaemia major or intermedia at our haematology centre from 1976 to 1979, and (2) a group of 82 patients with thalassaemia major randomly chosen from 236 under continuous transfusion treatment at our centre.

In the first group diagnosis of β-thal type was based on cellulose acetate, pH 8·6, and agar gel electrophoresis, pH 6·0, plus genotype assessment in the parents. In addition, 61 of this group also had globin chain synthesis analysis on carboxymethyl-cellulose columns.

In the second group diagnosis was based on globin chain synthesis analysis carried out on peripheral blood taken just before transfusion and examination of the parents.

The genotype of the parents was assessed in each case by the following examinations: red cell indices, haemoglobin electrophoresis, Hb A2 quantification, and, when necessary, Hb F quantification, Hb F erythrocyte distribution, and globin chain synthesis analysis.

Three subjects, offspring of couples at risk, had globin chain synthesis analysis on cord blood.

Blood samples were collected in EDTA for haematological studies and in heparin for globin chain synthesis analysis. Haematological parameters were performed using Coulter Counter model S. Hb A2 was quantified by DE–52 microchromatography and Hb F by alkali denaturation. Distribution of Hb F in the erythrocytes was assessed according to Kleihauer et al.14 Haemoglobin electrophoresis was carried out on cellulose acetate plates (Titan
III, Helena Laboratories, Beaumont, Texas) using TEB buffer, pH 8.6, and on agar citrate plates, pH 6.0. Globin chain synthesis analysis was done using peripheral blood and following the method of Kan et al.16

Results

Fig 1 shows the haemoglobin electrophoretic pattern on cellulose acetate (A) and agar gel (B) of representative homozygous $\beta^0$-thal patients with no Hb A. It should be noted that preliminary experiments with both electrophoretic methods showed Hb A even when the concentration was less than 0.5%.

Fig 2 shows representative radiochromatograms of a $\beta^0$-thal homozygote (A) at presentation and (B)

15 days after blood transfusion: in neither is there $\beta$-chain synthesis.

The number of patients from Cagliari, Oristano, and Nuoro provinces are representative of the distribution of thalassaemia types in these areas, since they are 64, 69, and 25%, respectively, of living patients with thalassaemia major or intermedia, ascertained in a survey of all the admissions at in- and outpatient services throughout these parts of the island. However, the number of patients from Sassari province is too low, approximately 3.3% of the total, to draw any meaningful conclusion.

In the southern and middle part of the island (Cagliari, Oristano, and Nuoro provinces) (fig 3),
Apart from six subjects with $\delta^0/\beta^0$-thal genotype and eight with $\beta^+/\beta^+$ or less probably $\beta^+/\beta^0$-thal, all patients were $\beta^0$-thal homozygotes. It is worth noting that, except for one, all $\beta^+$-thal patients had at least one non-Sardinian parent, from Sicily or Calabria. The same was seen in five subjects with Hb S/$\beta^+$-thal genotype where, apart from one case, the parent carrier of the sickle cell trait was of non-Sardinian extraction (table 1).

The $\alpha/\gamma$ globin chain synthesis ratios of homozygous $\beta^0$-thal patients showed a high variability with no statistically significant difference in the mean values between transfusion dependent (thal major) (2.37 ± 1.12) and non-transfusion dependent patients (thal intermedia) (1.95 ± 1.40).

There was no statistically significant difference in the mean $\alpha/\gamma$ ratios between transfusion dependent (thal major) splenectomised and non-splenectomised subjects. Similarly, $\alpha/\gamma$ ratios of splenectomised non-transfusion dependent that intermedia patients did not differ from the ratios found in the other groups (table 2).

In our Service splenectomy is usually carried out in that major children over 5 years of age when there is an increasing blood transfusion requirement (TQ > 1.5 according to Modell) and in that intermedia children when there is a documented reduced erythrocyte life span.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Genotypes of $\beta$-thalassaemia major and intermedia in Sardinia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
<td>Cagliari</td>
</tr>
<tr>
<td>At presentation</td>
<td>$\beta^0/\beta^0$</td>
</tr>
<tr>
<td>By electrophoresis</td>
<td>233</td>
</tr>
<tr>
<td>By electrophoresis and globin chain synthesis</td>
<td>36</td>
</tr>
<tr>
<td>During transfusion treatment by globin chain synthesis*</td>
<td>62</td>
</tr>
<tr>
<td>Total</td>
<td>331</td>
</tr>
</tbody>
</table>

*Subjects randomly chosen from 236 patients on regular transfusion programme at our centre.
†Genotype assessment determined by examination of the parents.
‡All but one have the parent carrying the sickle trait of Sicilian origin.
§All of these subjects, of which 3 were sibs, have one parent of non-Sardinian extraction.
†Three of these were sibs.

Globin chain synthesis analysis carried out at birth in three infants, subsequently found to have homozygous $\beta^0$-thal, showed unbalanced $\alpha/\gamma$ ratios in two (1.26 and 1.79) and a borderline value in one (1.12).

Globin chain synthesis analysis, performed in 103 subjects at different intervals from blood transfusion, showed no incorporation of radioactive leucine into the $\beta$-globin peak, the same as before the transfusion.

**Discussion**

This study clearly shows that in southern Sardinia $\beta$-thal is almost exclusively of the $\beta^0$-thal type, which most likely arose as a single mutational event. This is not surprising since Sardinia has been free from external colonisation for a long period of time.

In fact, Greek colonisation was localised in the north-east of the island while Romans and Carthaginians settled only along the southern coast. Moreover, other populations, such as Vandals, Goths, Saracens, Pisans, and Spanish did not stay in the island long enough or in a sufficient number to modify significantly the genetic structure of the autochthonous population. According to the hypothesis of a single $\beta^0$-thal mutation, a recent restriction endonuclease analysis of the $\beta$-globin gene in Sardinians disclosed a constant association of the $\beta^0$-thal genes with the normal 9-3 kilobase Bam HI site, while 1/3 of the normal $\beta$-globin genotypes are associated with the variant Bam HI site with 22.0 Kb fragment.17

The data concerning northern Sardinia are not sufficient to come to any conclusion. Nevertheless, the relatively high amount of $\beta^+$-thal in this part of the island, where the only Greek colonisation took place, must be noted.
This study also shows a constant absence of \( \beta \)-globin chain synthesis in Sardinian \( \beta^+ \)-thal erythroid cells after blood transfusion, unlike \( \beta^0 \)-thal homozygotes in Ferrara.\(^{10,11}\) Accordingly, hybridisation experiments in Sardinian homozygous \( \beta^2 \)-thal showed \( \beta \)-globin mRNA levels to be uniformly low, with a \( \beta/\alpha \) mRNA ratio of 0.05 (unpublished results).

The third interesting conclusion from our study is that the degree of \( \alpha/\gamma \) globin chain synthesis imbalance in \( \beta^2 \)-thal homozygotes did not predict the severity of the clinical course, unlike previous findings.\(^{18,19}\)

Finally, the last finding deserving comment is the variable imbalance found at birth in infants subsequently found to have homozygous \( \beta^2 \)-thal. This variability is probably the result of individual difference in gestation time or maturity or both with variation in turning off of \( \gamma \)-chain synthesis.

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