Thalassaemia types and their incidence in Sardinia


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SUMMARY The frequency of thalassaemia syndromes in Sardinia was examined by a population survey. The data indicate that about 12-6% of the Sardinian subjects are carriers of β-thalassaemia, while 6-9% of the population carries an α-thalassaemia gene, with a slight difference between the various provinces. These are among the highest frequencies of thalassaemia genes found in a Caucasian population today.

A survey of hospital inpatients and outpatients showed a newborn incidence of homozygous β-thalassaemia of 1 in 300. The reasons for the difference between the expected and observed incidence figures are discussed. Moreover, 3 subjects with δβ0-thalassaemia trait, 6 carriers of heterocellular persistence of fetal haemoglobin (HPFH), 1 sickle cell trait, and 3 subjects with Hb J Sardegna were found.

Genetic heterogeneity of β-thalassaemia syndromes in this population may generally result from the interaction of α- and β-thalassaemia genes.

The incidence and distribution of thalassaemia syndromes in Sardinia have been previously investigated (Silvestroni and Bianco, 1960; Carcassi, 1963; Siniscalco et al., 1966). However, the osmotic fragility test and the examination of blood smears have usually been the only tests used in these population surveys. Therefore, there is no clear data on the extent of heterogeneity of thalassaemia syndromes in this island.

Recently, in a group of 233 people from the Cagliari province, randomly selected on the basis of Hb A2/Hb A1 ratio, Terrenato (1973) observed 2 groups of thalassaemia carriers: β-thalassaemia carriers with high Hb A2 with a phenotypic percentage of 9-4%, and non-β-thalassaemia carriers with normal Hb A2 and Hb F, with a frequency of 8-2. According to the author, the members of this last group were probably α-thalassaemia carriers.

The present study was undertaken in order to obtain information on the frequency and distribution of thalassaemia carriers in Sardinia.

Materials and methods

SUBJECTS STUDIED
Voluntary testing for thalassaemia was offered to various population groups. All the subjects examined, aged 14 to 45 years, were of Sardinian extraction. A comprehensive programme was sponsored by the Trade Unions. The programme included community education, considerable publicity, confidentiality of the results, and individual counselling for people found to be carriers. Follow-up sessions were provided for families of people who were found to be carriers.

The results of the parents and other relatives of people with β-thalassaemia major were excluded from the calculation of the thalassaemia frequency.

HAEMATOLOGICAL AND HAEMOGLOBIN ANALYTICAL STUDIES
Blood samples were collected and stored in heparin. Haematological data were obtained using the Coulter Counter ZBI and Coulter Haemoglobinometer. Haemoglobin electrophoresis was carried out on cellulose acetate plates (Helena Laboratories, Beaumont, Texas), in Tris-EDTA borate buffer, pH 8-6. The
Hb A₂ was quantified by DE-52 microchromatography (Huisman et al., 1975). The one-tube osmotic fragility test using 0.4% buffered NaCl was performed using the method of Silvestrini and Bianco (1945). Hb F was measured by alkali denaturation (Bekte et al., 1959), and serum iron and iron binding capacity by the method of Lauber (1965). Hb H inclusion bodies were obtained by mixing equal volumes of whole blood and 1% brilliant cresyl blue in citrate saline solution and incubating at 37°C for 20 minutes to 1 hour.

To estimate the incidence of Cooley's anaemia, we examined all hospital records and outpatient registers of the paediatric departments which take care of thalassaemia patients in the Cagliari and Oristano provinces. The frequency at birth of Cooley's anaemia per year was calculated using the yearly number of births in the same provinces. This calculation was limited to the last 2 years because up to that time many patients were referred to hospitals outside the island.

**DIAGNOSTIC SCHEME AND CRITERIA OF DIAGNOSIS**

All subjects had the following examinations: red blood cell count, haemoglobin quantification, packed red cell volume, MCV, MCH, MCHC, one-tube osmotic fragility test, haemoglobin electrophoresis, and quantification of Hb A₂.

The diagnosis of β-thalassaemia carrier was made on the basis of reduced MCV, and reduced osmotic fragility, associated with high Hb A₁ levels (Fig.). Estimation of Hb A₁ levels with DE-52 microchromatography in our laboratory in samples from normal subjects gave values of between 1.71 to 3.20 (mean 2.50 ± 0.43), and in β-thalassaemia heterozygous values of between 4.23 to 6.80 (mean 5.35 ± 0.55) (Galanello et al., 1977). Hb A₂ levels were determined in all subjects, since in our experience and in accordance with the data of other authors (Pootrakul et al., 1973; Pearson et al., 1974; Mazza et al., 1976), the osmotic fragility test and MCV assessment may give false negative results. In fact, in these subjects with a diagnosis of β-thalassaemia trait, based on microcytosis and raised Hb A₂ levels, the one-tube osmotic fragility test gave false negative results in 3.6% of the cases. On the other hand, in the mass screening of our population this test gave false positive results in 6.5% of cases. These were probably carriers of α-thalassaemia.

In our laboratory, MCV gave a high number of false negative results too. Taking 79 fl in males and 77 fl in females as minimum normal values (mean ± SD), 3.0% of male and 3.8% of female β-thalassaemia carriers had normal values (unpublished results).

All subjects with low MCV and normal or reduced Hb A₂ levels had iron studies performed. Subjects showing Hb F by electrophoresis had quantification of this haemoglobin.

As a preliminary to this survey, a series of experiments was carried out to ascertain the lowest level of Hb F that can be distinguished by our method. Serial dilution of a haemolysate containing about 10° Hb F was made with a normal adult haemolysate, containing 0.6% Hb F, of the same concentration, and the mixture was analysed by cellulose acetate electrophoresis as described in the Methods section. It was found that Hb F was easily identified when its concentration was about 2% of total haemoglobin. Therefore, the

![Fig. Scheme for mass screening for thalassaemia syndromes.](http://jmg.bmj.com/)
it is probable that, with this method, we have missed some \( \delta^{+} \)-thalassaemia heterozygotes or heterocellular HPFH carriers who had low levels of Hb F.

Cases with microcytosis, low or normal Hb A\(_2\) levels, Hb F levels higher than 5%, heterogeneously distributed, were considered to have the \( \delta^{+} \)-thalassaemia trait.

Subjects with microcytosis, normal Hb F, normal or reduced Hb A\(_2\), and normal serum iron were diagnosed as having the \( \alpha \)-thalassaemia trait when the Hb H inclusion bodies test was positive and/or a first degree relative showed the same haematological findings. The presence of rare cells with Hb H inclusion bodies was observed in 78% of subjects with the above characteristics.

In a group of 20 randomly selected subjects showing these thalassaemia-like red cell indices, globin chain synthesis studies gave an \( \alpha/\beta \) ratio of 0.66 ± 0.10 (unpublished results). In these subjects identified as \( \alpha \)-thalassaemia carriers on the basis of \( \alpha/\beta \) ratio, the percentage of positive Hb H inclusion body tests was found to be similar.

Nevertheless, it is possible that some cases categorised as having \( \alpha \)-thalassaemia were in part carriers of \( \beta \)-thalassaemia with normal levels of haemoglobin A\(_1\) and F (\( \delta^{+} \)-thalassaemia).

**Results**

**THALASSAEMIA CARRIER FREQUENCY AND DISTRIBUTION**

The results are summarised in the Table. In the Cagliari and Oristano provinces (Southern Sardinia), 12.9% of the 2400 people tested were found to have the \( \beta \)-thalassaemia trait and 6.6% the \( \alpha \)-thalassaemia trait.

Three subjects with the \( \delta^{+} \)-thalassaemia trait, one with the sickle cell trait, and two carriers of Hb J Sardegna were observed.

In addition, 6 people with normal haematological findings, normal osmotic fragility, and Hb A\(_2\) levels ranging from 2.17 to 2.87% had Hb F levels ranging from 1.5 to 5.2%, heterogeneously distributed.

The Table also shows the distribution of the different traits in the other two provinces in the centre and north of the island (Nuoro and Sassari). In these provinces, the incidence figures for \( \beta \)-thalassaemia did not differ from those observed in Southern Sardinia. However, it is worth pointing out that there was a higher incidence of the \( \alpha \)-thalassaemia trait in the Nuoro province, where the screening programme even revealed a patient with Hb H disease.

**Hospital Survey**

Patients with Cooley’s anaemia, 51 born in 1975 and 53 born in 1976, were admitted or examined as outpatients in the paediatric haematological centres of the Cagliari province at least once.

In these 2 years the number of live births in the Cagliari and Oristano provinces was 16 083 and 15 163, respectively. The newborn incidence of Cooley’s anaemia in the Cagliari and Oristano provinces is, therefore, 1 in 300.

**Discussion**

The \( \beta \)-thalassaemia carrier frequency found in our study gives a gene frequency of 0.065, which is in accordance with the figure of 0.05 observed by Terrenato (1973).

This frequency is much lower than previous estimates (Silvestroni and Bianco, 1960; Carcassi, 1963; Siniscalco et al., 1966). With this frequency, the couples at risk are 1 in 60 and the expected incidence at birth of homozygous \( \beta \)-thalassaemia is 1 in 250.

Even with this figure, Sardinia shows one of the highest frequencies of \( \beta \)-thalassaemia among Caucasian populations.

The difference between the expected and observed figures of the incidence of Cooley’s anaemia in the Cagliari and Oristano provinces probably depends on 2 factors. Firstly, the sampling method was not strictly random, because people with a family history of anaemia may have been more likely to participate in the screening programme. This fact tends to introduce a bias which produces a rise in the true incidence.

**Table**  Incidence of thalassaemia syndromes and hereditary haemoglobinopathies in Sardinia

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<thead>
<tr>
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<th>Cagliari and Oristano provinces</th>
<th>Nuoro province</th>
<th>Sassari province</th>
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</thead>
<tbody>
<tr>
<td>Subjects examined</td>
<td>Abnormal subjects</td>
<td>%</td>
<td>Subjects examined</td>
</tr>
<tr>
<td>( \beta )-thalassaemia heterozygotes</td>
<td>310</td>
<td>12.9</td>
<td>54</td>
</tr>
<tr>
<td>( \delta^{+} )-thalassaemia heterozygotes</td>
<td>160</td>
<td>6.7</td>
<td>39</td>
</tr>
<tr>
<td>Heterocellular HPFH heterozygotes</td>
<td>3</td>
<td>0.12</td>
<td>1</td>
</tr>
<tr>
<td>Hb J Sardegna carriers</td>
<td>1</td>
<td>0.04</td>
<td>1</td>
</tr>
<tr>
<td>Sickle cell trait</td>
<td>1</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Hb H disease</td>
<td>1</td>
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</table>
Secondly, the direct calculation of the frequency of Cooley's anaemia, though reasonably accurate, is subject to considerable underestimation. In fact, some patients are unlikely to be ascertained by this kind of survey, because in our country some parents are against treatment of this disease in their children. Moreover, it is possible that in some patients, born in the last 2 years, the disease has not yet been clearly expressed at the phenotypic level. This fact would cause a reduction in the number of patients observed.

In this survey, 3 subjects with the $\delta^b$-thalassaemia trait were found. The data showed that the thalassaemia mutation at $\beta$ locus or $\delta^b$ loci is heterogeneous in Sardinia. In fact, 2 have been ascertained and there are probably more.

However, the classical $\beta$-thalassaemia with high Hb A$_2$ is by far the most prevalent, as has been shown in this work.

In accordance with this, all the parents but 2 (who were $\delta^b$-thalassaemia carriers) out of 350 Southern Sardinian patients affected by $\beta$-thalassaemia syndromes turned out to be classic $\beta$-thalassaemia carriers.

Further evidence of the prevalence of the $\beta$-thalassaemia trait with high Hb A$_2$ can be derived from the observation that 107 out of 350 patients, randomly selected for globin chain synthesis studies, were found to be homozygous $\beta^b$-thalassaemia carriers (unpublished results).

Since the present survey shows a high incidence of $\alpha$-thalassaemia in our population, the heterogeneity of $\beta$-thalassaemia syndromes may depend on the concurrent inheritance of an $\alpha$-thalassaemia gene which can modify the degree of chain imbalance and reduce the clinical severity of the disease (Kan and Nathan, 1970; Bate and Humphries, 1977). Less frequently, the interaction between homozygous $\beta$-thalassaemia and heterocellular HPFH gene could result in the same effect, as suggested by Wood et al. (1977).

Because of the high number of false negative and false positive results, the one-tube osmotic fragility test and MCV assessment cannot be considered reliable for genetic screening for $\beta$-thalassaemia syndromes in our island. Thus, we think the diagnostic scheme proposed by us in the Methods section is necessary to avoid missing carriers.

In the present survey, a patient with Hb H disease was observed and a high frequency of an $\alpha$-thalassaemia trait was found. It must be taken into account that this incidence figure could be slightly lower, as it is possible with our approach to have diagnosed some $\beta$-thalassaemia carriers with normal levels of haemoglobin A$_2$ and F as $\alpha$-thalassaemia carriers. In the last 3 years, 24 patients with Hb H disease were observed in our haematological service, which takes care of the population of Southern Sardinia. In general, on the basis of the haematological data and the $\alpha/\beta$ ratio, the genetics of Hb H disease in Sardinia follow a pattern similar to that observed in Orientals: one parent showing thalassaemia-like red cell indices associated with a low $\alpha/\beta$ ratio, and the other haematologically normal with a slight $\alpha/\beta$ chain imbalance (Galanello et al., 1978). A newborn screening survey of 229 subjects for the presence of Hb Bart's in the same population revealed a detectable amount, >1% in about 12.6%. The distribution of Hb Bart's appears to be trimodal: 7.0% of the subjects had 1 to 2% of Hb Bart's, 5-6% had about 3 to 5% of Hb Bart's, and 4 subjects had about 25% of Hb Bart's. However, in the same population Hb Bart's hydrops fetalis syndrome has never been observed (unpublished results).

Further investigations, particularly family studies and follow-up of newborn babies showing Hb Bart's higher than 1% at birth, and cDNA/DNA hybridisation experiments, are needed to determine the molecular pathology of $\alpha$-thalassaemia in Sardinia.

The gene frequency of $\alpha$-thalassaemia was not calculated because the problem of the number of $\alpha$ loci has not yet been clarified, at least in Sardinia.

In this screening, a subject with sickle cell trait was observed. One of his grandparents was of Turkish extraction. This confirms previous findings on the absence of the sickle cell trait in the Sardinian population (Siniscalco et al., 1966).

It is well known that the frequency of the Hb S gene is usually inversely correlated with that of $\beta$-thalassaemia in the populations which show a high frequency of these genes (Barnicot et al., 1963).

Hb J Sardegna (Tangheroni et al., 1968) has been described on numerous occasions in Sardinia (Macciotta et al., 1969; Tangheroni et al., 1969). In this survey, 3 subjects with this haemoglobin variant were found, giving a carrier frequency of 1 in 1000.

Four unrelated subjects with Hb F levels ranging from 1.5 to 2.5%, with no haematological abnormalities, were observed. This could be a low estimate because our diagnostic scheme can miss some of these carriers.

Since Hb F was heterogeneously distributed among the red blood cells, this condition may be a form of heterocellular HPFH, as recently defined by Boyce et al. (1977). It appears similar to the Swiss type (Marti, 1963; Knox-Macaulay et al., 1973), with Hb F being heterogeneously distributed among the red cells in the 1 to 2% range.

The Hb F levels in the 1.5 to 5.2% range is the main difference between the type of HPFH described here and that recently reported in a British family, which showed higher Hb F levels segregating in groups with mean values of 19.8% in homozygous and 8.9% in heterozygous, subjects (Weatherall et al., 1975).
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Family studies, biosynthetic experiments, and Hb F structural analysis, to characterise this haematological abnormality, are in progress.

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References


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