$\delta\beta$ (F)-thalassaemia in Sardinia

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SUMMARY A population survey carried out in southern Sardinia on more than 5000 people has shown that $\delta\beta$ (F)-thalassaemia, with a gene frequency of 0.00088, is a rare trait in this population.

We examined the members of three families segregating for both $\delta\beta$ - and β° -thalassaemia and a number of $\delta\beta$ carriers identified during the screening. The doubly heterozygous children suffer from a mild form of Cooley's disease with non- α/α biosynthetic ratios within the range of values observed in β° -thalassaemia homozygotes. Three of them have been transfusion dependent for some time.

The $\delta\beta$ carriers, although in many respects showing the usual picture of $\delta\beta$ -thalassaemia, such as abnormal red cell indices, normal Hb A₂, Hb F heterogeneously distributed in the erythrocytes, and low β/α synthetic ratios, have unusually high levels of Hb F (range 10 to 20%) and particularly low glycine content (range 0.02 to 0.14 residues) in the isolated γ CB3 peptide. These results have led us to the conclusion that the $\delta\beta$ -thalassaemia found in Sardinia is different from the similar kind of $\delta\beta$ defect found in Negroes and in other Mediterranean populations, including continental Italians.

$\delta\beta$ -thalassaemia is a relatively rare form of thalassaemia, characterised by the absence of β and δ globin chain production.¹⁻³ The heterozygotes for this syndrome have thalassaemic red cell indices, normal or reduced Hb A₂ levels, and increased amounts of fetal haemoglobin which is heterogeneously distributed among the red cells.

Globin chain biosynthetic studies have shown that the imbalance in α /non- α chain production is less pronounced than in β -thalassaemia.^{4,5} The rare homozygous subjects present either a clinical picture of intermediate thalassaemia or are apparently in good health, in spite of the typical thalassaemic alteration of red cell morphology. They all have a haemoglobin pattern consisting only of Hb F, indicating, therefore, a total suppression of β and δ chain synthesis.⁶⁻⁸

Globin gene analyses in Sicilian and Greek subjects, homozygous for the trait, have shown a deletion of δ and β structural genes.⁹ An extensive study carried out by Stamatoyannopoulos *et al*¹⁰ has demonstrated that both A γ and G γ loci in cis to the $\delta\beta$ deletion are active in producing fetal haemoglobin. The ratio of glycine to alanine in heterozygotes is, however, not characteristic of the defect but is similar to that present in normal adults. In a few instances only G γ chains have been detected

in the fetal haemoglobin of $\delta\beta$ heterozygotes.^{11,12} In population surveys carried out in Cagliari and in a number of villages of southern Sardinia, we have found that $\delta\beta$ -thalassaemia represents only a small fraction of the total non- α -thalassaemias.

In this paper we report the clinical and haematological characterisation of $\delta\beta$ -thalassaemia in this area and the results of the structural analysis of fetal haemoglobin isolated from $\delta\beta$ -thalassaemia carriers and double heterozygotes for $\delta\beta$ - and β° -thalassaemia.

This study has led us to the conclusion that Sardinian $\delta\beta$ -thalassaemia, particularly because of the remarkable increase of fetal haemoglobin production and the chemical nature of γ chains, represents a different condition from the similar ones observed in other populations, including continental Italians.

Materials and methods

We carried out this investigation on: (1) the members of three families whose probands are $\delta\beta/\beta^\circ$ double heterozygotes. These families comprise 22 carriers of the $\delta\beta$ trait and four double heterozygotes; (2) a group of ten $\delta\beta$ heterozygotes identified during a screening programme for β -thalassaemia syndromes. Besides these, eight high Hb A₂ β -thalassaemia

$\delta\beta(F)$ -thalassaemia in Sardinia

heterozygotes and ten normal subjects were examined.

Routine haematological investigations were carried out with the aid of a Coulter Model S counter. Haemoglobin A₂ was quantified by DEAE cellulose chromatography^{13 14} and Hb F by alkali denaturation.¹⁵ Electrophoresis of the haemolysates

was performed on cellulose acetate and starch gels. The distribution of Hb F in the erythrocytes was examined according to Kleihauer *et al*¹⁶ and by immunofluorescence with a sandwich technique using specific anti- γ chain antibodies obtained in rabbits and FITC conjugated goats' antisera against rabbit IgG (Behringwerke).

TABLE 1 Haematological findings, globin synthesis data, and structural analysis of the γ CB3 peptide

Subject	Age	RBC ($\times 10^{12}/l$)	PCV (%)	Hb (g/dl)	MCH (pg)	MCHC (%)	MCV (fl)	Hb A ₂ (%)	Hb F (%)	Hb phenotype (electro- phoresis)	Ratio β/α or γ/α^*	γ CB3	
												GLY	ALA
FAMILY U													
I. 1	38	6.30	44.1	14.8	23.5	33.6	70	5.28	—	A-A ₂	—	—	—
I. 2	38	5.60	45.1	16.3	29.1	36.1	80	2.24	15.0	A-F-A ₂	—	0.10	2.92
II. 1	18	—	—	13.7	—	—	—	5.11	—	A-A ₂	—	—	—
II. 2	17	—	—	14.1	—	—	—	5.13	—	A-A ₂	—	—	—
II. 3	15	3.86	33.4	9.7	24.5	28	83	—	≈ 100	F-A ₂	0.23*	0.66	2.38
II. 4	13	—	—	14.2	—	—	—	3.16	15.0	A-F-A ₂	—	—	—
II. 5	11	—	—	14.6	—	—	—	2.74	—	A-A ₂	—	—	—
II. 6	6	—	—	13.5	—	—	—	2.95	15.0	A-F-A ₂	—	—	—
II. 7	3	—	—	13.1	—	—	—	2.64	—	A-A ₂	—	—	—
II. 8	5 mth	4.53	39.8	12.0	26.4	30.1	88	2.02	5.4	A-F-A ₂	—	—	—
FAMILY F													
I. 1	56	5.46	49.1	16.9	31.4	34.3	89	1.65	—	A-A ₂	—	—	—
I. 2	55	5.24	41.6	14.2	27.5	34.1	79	2.34	12.0	A-F-A ₂	0.67	0.05	2.96
II. 1	38	5.86	41.0	13.5	23.4	32.8	70	5.19	—	A-A ₂	—	—	—
II. 2	31	5.16	41.7	13.7	26.9	32.8	81	2.14	14.4	A-F-A ₂	0.76	0.11	2.92
II. 3	27	5.29	41.9	14.1	27.2	33.8	80	2.35	12.1	A-F-A ₂	0.58	0.12	2.88
II. 4	23	4.21	39.9	14.0	33.8	35.1	95	2.29	—	A-A ₂	—	—	—
III. 1	8	6.44	41.3	14.0	22.0	33.8	64	5.03	—	A-A ₂	—	—	—
III. 2	4	4.31	30.2	9.2	21.8	30.5	70	1.96	98.04	F-A ₂	0.31*	0.58	2.45
FAMILY T													
II. 5	64	6.68	40.3	12.6	18.8	31.2	62	5.51	—	A-A ₂	—	0.72	2.34
II. 6	62	4.69	38.9	13.0	27.7	33.4	85	2.01	—	A-A ₂	—	—	—
III. 1	47	5.46	43.9	15.0	27.8	34.0	80	2.72	15.3	A-F-A ₂	0.81	0.03	2.98
III. 2	38	5.03	41.6	14.3	28.6	34.0	83	2.22	—	A-A ₂	—	—	—
III. 4	42	4.96	40.3	13.6	27.7	33.5	82	1.43	14.9	A-F-A ₂	0.56	0.02	2.96
III. 14	36	5.69	43.8	13.6	23.9	31.0	79	1.84	11.4	A-F-A ₂	0.70	0	3.01
III. 15	34	6.59	42.4	12.0	18.2	28.3	65	6.10	—	A-A ₂	—	0.58	2.43
III. 16	31	5.12	40.4	13.0	25.7	32.1	79	2.10	13.4	A-F-A ₂	—	0.08	2.96
III. 20	27	5.35	40.4	13.5	25.2	33.4	77	2.95	13.8	A-F-A ₂	—	—	—
III. 22	43	5.93	43.9	14.5	24.7	32.8	74	2.61	13.7	A-F-A ₂	0.37	—	—
III. 30	34	5.53	42.1	14.9	26.9	35.3	78	2.70	18.0	A-F-A ₂	—	—	—
III. 31	29	4.65	32.0	12.0	25.8	37.5	70	5.61	—	A-A ₂	—	—	—
III. 33	31	5.04	40.4	13.2	26.5	32.9	79	2.35	15.4	A-F-A ₂	0.59	—	—
IV. 1	18	5.20	40.9	13.9	26.9	34.0	78	2.30	20.7	A-F-A ₂	0.55	0.06	2.98
IV. 2	17	5.27	42.5	14.1	27.0	32.9	81	2.13	18.6	A-F-A ₂	0.48	0.04	2.98
IV. 3	14	5.43	42.2	14.5	27.0	32.2	78	2.33	15.9	A-F-A ₂	0.68	0.08	2.96
IV. 4	18	5.01	42.2	14.4	29.0	33.9	84	2.95	16.9	A-F-A ₂	0.62	0.05	2.93
IV. 5	17	4.94	39.4	12.2	27.0	33.3	80	2.81	14.1	A-F-A ₂	—	0.05	2.95
IV. 6	15	4.77	38.3	12.7	26.9	33.0	80	3.01	12.5	A-F-A ₂	—	0.06	2.98
IV. 8	12	5.52	36.2	10.8	19.5	28.8	68	—	—	—	—	—	—
IV. 9	12	5.41	35.5	11.0	20.3	30.9	68	—	—	—	—	—	—
IV. 10	9	5.48	39.4	13.0	23.7	32.9	74	2.26	9.6	A-F-A ₂	—	0	3.00
IV. 11	6	5.30	38.2	13.0	24.5	34.0	74	2.13	—	A-A ₂	—	—	—
IV. 25	12	4.89	32.7	10.8	22.1	33.0	69	1.00	99.0	F-A ₂	0.35*	0.62	2.41
IV. 28	11	4.27	33.7	11.6	27.2	34.4	79	2.81	—	A-A ₂	—	—	—
IV. 29	10	4.03	29.5	9.2	22.8	31.2	76	—	≈ 100	F-A ₂	0.38*	0.53	2.48
IV. 30	6	4.79	33.0	10.8	22.5	32.7	70	2.15	17.5	A-F-A ₂	—	0.14	2.88
IV. 42	at birth	4.83	42.4	15.0	31.1	35.4	89	—	—	—	—	—	—
TM	23	5.02	36.3	12.4	24.7	34.15	75	2.12	17.8	A-F-A ₂	—	0.12	2.89
ML	30	5.69	40.2	13.8	24.3	34.3	73	2.63	16.1	A-F-A ₂	—	0.08	2.93
AR	27	5.09	38.1	13.2	25.9	34.6	77	2.41	17.1	A-F-A ₂	—	0.10	2.90
PI	26	5.32	44.6	14.6	27.7	32.6	83	2.56	14.7	A-F-A ₂	—	—	—
SF	35	5.19	45.0	14.9	28.9	32.8	87	2.44	13.4	A-F-A ₂	—	—	—
CE	18	5.36	44.3	15.2	28.7	34.1	83	2.73	15.5	A-F-A ₂	—	—	—
GG	20	5.87	45.1	15.1	25.9	33.2	77	2.46	16.9	A-F-A ₂	—	—	—
SV	20	5.66	44.6	15.1	26.8	33.6	79	2.33	18.4	A-F-A ₂	—	—	—
DPN	50	5.80	40.6	13.3	23.2	32.6	70	2.35	9.7	A-F-A ₂	—	—	—
PV	44	5.73	44.8	12.2	21.1	27.2	78	2.41	18.0	A-F-A ₂	—	—	—

BIOSYNTHETIC STUDIES

The degree of incorporation of (^3H) leucine into globin chains was determined using peripheral blood, following the method of Kan *et al.*¹⁷ The synthesis of β and γ chains relative to α is expressed as specific activity ratios.

STRUCTURAL STUDIES

Fetal haemoglobin was isolated by alkali denaturation and further purified and concentrated on a column of CM Sephadex, as previously described.¹⁸ Experiments carried out on a sample of pure Hb F with a known G γ /A γ ratio have shown that this

FIG 1 Pedigrees of three families in which $\delta\beta^0$ - and β^0 -thalassaemia are segregating.

ratio is not modified by the alkali denaturation and the concentration step on CM Sephadex. Globin obtained by the acid acetone method was used for the detection and quantification of G γ , A γ , and T γ chains. The γ CB3 fragment containing the position 136 was obtained by incubation of F globin with a hundredfold excess of CNBr for 24 hours in 70% formic acid and then isolated by electrochromatography on paper. The presence and amount of the T γ chain (in which an isoleucine residue is substituted by a threonine in position 75) was determined by peptide mapping of the globin F tryptic digest. Details of both methods and an evaluation of their precision have been reported elsewhere.¹⁹

The traditional *t* test was applied to assess the significance of the differences between the means of a number of haematological parameters. Whenever this test has been employed (as in table 2 and fig 2) the probability ranges are indicated as follows: * = 0.05 > p > 0.01; † = 0.01 > p > 0.001; ‡ = p < 0.001.

Results

Table 1 summarises the haematological findings and the data concerning the globin synthesis studies, as well as the Hb F structure of the members of three families and of ten unrelated subjects heterozygous for $\delta\beta$ -thalassaemia. Fig 1 shows the pedigrees of these families in which, in total, four children are compound heterozygotes for $\delta\beta$ - and β^0 -thalassaemia.

Family studies

FAMILY U

The proband (II.3) was 16 years old. The diagnosis of Cooley's anaemia was made when the patient was 2 years old. At 8 years he started a regular transfusion programme of 200 to 400 ml whole blood every 2 months. He had a splenectomy at 11 years after which his Hb levels were stable around 8 to 9 g/dl and transfusions were only occasionally necessary. At 14 years he had pericarditis with heart failure. Clinical examination at 14 years showed slight bone alteration, cardiomegaly, a liver palpable 6 cm below the costal margin, weight below the 3rd centile, and height between the 3rd and 10th centile. The peripheral blood picture was characterised by hypochromia, anisocytosis, poikilocytosis, and target cells, with 20 normoblasts/100 white cells. Electrophoresis of the haemolysate showed only Hb F and A $_2$. The γ/α biosynthetic ratio was 0.23. The father (I.1) and two sisters (II.1, II.2) were found to be carriers of β -thalassaemia with high Hb A $_2$,

whereas the mother (I.2) and two other sibs (II.4, II.6) with normal Hb A $_2$ and high Hb F levels were classified as $\delta\beta$ heterozygotes.

FAMILY T

The 10-year-old proband (IV.29), after having had severe neonatal jaundice, was in good health until the age of 3 years, when, because of progressive anaemia, he was admitted to hospital where the diagnosis of thalassaemia was made. From the age of 8 years he has been treated with a regular transfusion programme of 500 ml whole blood every 20 to 30 days. After having a splenectomy at 8½ years, the transfusion requirement was reduced to 500 ml every 40 days. His mean annual Hb is 7.4 g/dl and the QT according to Dr B Modell is 2.2.

Clinical examination on admission to hospital showed height and weight below the 3rd centile and slight thalassaemia-like bone modifications. The liver was palpable 7 cm below the costal margin and the heart was slightly enlarged. Osteoporosis was evident. The peripheral blood smear showed hypochromia, anisocytosis, poikilocytosis, and target cells with 30 normoblasts/100 leucocytes. The haemoglobin electrophoretic pattern was characterised by the presence of Hb F and A $_2$ and the biosynthetic ratio γ/α was 0.38. As shown in fig 1 the father (III.15) is a carrier of high Hb A $_2$ β -thalassaemia and the mother (III.16) is heterozygous for the $\delta\beta$ -thalassaemia trait. The diagnosis of $\delta\beta/\beta^0$ -thalassaemia in the double first cousin (IV.25) of the proband was made at the age of 3 years. From 7 to 11 years he has had regular transfusions of 500 ml packed red cells every 2 months. At present he can manage without transfusions with an annual mean Hb of 10 g/dl. At clinical examination he had slight bone alterations and hepatosplenomegaly. The electrophoretic pattern showed Hb F and A $_2$ with a γ/α ratio of 0.35. The mother (III.14) is a $\delta\beta$ -thalassaemia carrier; the father (III.13) refused to be examined, but, considering the genotype of his parents (II.5, II.6) and brother (III.15), he has been classified as a heterozygote for high A $_2$ β -thalassaemia.

FAMILY F

The proband (III.2), at the age of 4 years, was admitted to hospital because the parents had noticed slight anaemia. Clinical examination showed slight bone alterations, spleen and liver 3 and 1 cm respectively below the costal margin, and cardiomegaly. Electrophoresis revealed only Hb F and A $_2$ and incubation of peripheral blood with labelled leucine gave a γ/α ratio of 0.31. Because of the satisfactory Hb levels the patient has not been transfused up to now.

Haematological parameters and globin synthesis in heterozygotes for $\delta\beta$ -thalassaemia

The following criteria were adopted for the diagnosis of the $\delta\beta$ -thalassaemia carrier state: Hb F levels $>5\%$, normal Hb A₂ levels, slight morphological red cell modifications, and fetal haemoglobin heterogeneously distributed among the red cells. Table 2 presents the haematological findings and the globin biosynthetic ratios in the $\delta\beta$ -thalassaemia carriers belonging to the families or found during the screening. A group of β^0 -thalassaemia heterozygotes and normal people are included in the table as controls.

Mean red cell count, PCV, Hb, and MCHC values are in the normal range although, particularly in males, the means of both Hb levels and red cell count of the $\delta\beta$ carriers are significantly different from those of normal controls and β^0 -thalassaemia heterozygotes. The average values of MCH and MCV are definitely low and intermediate between those of β^0 carriers and the control population; their distribution, however, overlaps with both of them. Haemoglobin A₂ values range from 1.43 to 3.16%, but, in spite of the large overlap with normal values, the mean in $\delta\beta$ carriers is significantly lower than that of the control sample. The prominent feature of our $\delta\beta$ -thalassaemia heterozygotes, as in carriers of the same defect in other populations, is the very high levels of Hb F: an average of 15.08% with a range of 9.6 to 20.7. The distribution of fetal haemoglobin in red cells is considered a very important criterion in the differential diagnosis between $\delta\beta$ -thalassaemia and the pancellular forms of HPFH. In the $\delta\beta$ heterozygotes studied during this investigation, the Hb F distribution in erythrocytes is clearly heterogeneous when evaluated with the method of Kleihauer. This heterogeneity, although present, is much less pronounced when blood smears are examined by immunofluorescence.

This observation agrees with the suggestion that the difference in intercellular distribution of Hb F in the two conditions is more apparent than real and depends mainly on the percentages of Hb F in peripheral blood and on the sensitivity of the method of detection.²⁰ Also shown in table 2 are the β/α biosynthetic ratios of $\delta\beta$ -thalassaemia carriers compared with those of β^0 -thalassaemia carriers and normal subjects belonging to the same Sardinian population. $\delta\beta$ -thalassaemia heterozygotes have a β/α ratio of 0.61 ± 0.12 with a range of 0.37 to 0.81. These values are not significantly different from the β/α ratios found in β^0 -thalassaemia. Because of the increased synthesis of fetal haemoglobin, however, the imbalance between α and non- α chains is less pronounced in $\delta\beta$ -thalassaemia; in nine carriers, in fact, the average $\beta^+\gamma/\alpha$ ratio is 0.72 ± 0.17 with a range of 0.44 to 1.06.

Clinical and haematological findings in $\delta\beta/\beta^0$ double heterozygotes

The relevant haematological data are given in table 1 together with the chemical analysis of Hb F. In one patient (family T, IV.29) the haematological examination was carried out 30 days after a transfusion. Another one (family F, III.2) has never been transfused and the other two patients (family T, IV.25 and family U, II.3) received the last transfusion one year ago. The complete suppression of haemoglobin A synthesis in the four patients allows us to classify the β -thalassaemia segregating in the three pedigrees as β^0 (non-Hb A producing).

The clinical picture exhibited by the patients cannot be defined as intermediate thalassaemia because of its severity, but rather as a relatively mild Cooley's anaemia. The mean γ/α biosynthetic ratio of 0.32 (range 0.23 to 0.38) is within the limits observed in our β^0 -thalassaemia homozygotes. This pronounced imbalance of globin chain synthesis

TABLE 2 Red cell indices and β/δ biosynthetic ratios in Sardinian $\delta\beta$ -thalassaemia heterozygotes. Comparison with β^0 -thalassaemia heterozygotes and normal controls

	Normal controls			$\delta\beta$ -thalassaemia heterozygotes			β^0 -thalassaemia heterozygotes		
	n	Mean $\pm$ SD	Range	n	Mean $\pm$ SD	Range	n	Mean $\pm$ SD	Range
RBC ($\times 10^{12}/l$)	177 M	5.18 $\pm$ 0.40	3.65-6.69 *	15 M	5.39 $\pm$ 0.35	4.77-5.93 ‡	43 M	6.12 $\pm$ 0.53	4.99-7.38
	195 F	4.69 $\pm$ 0.43	3.88-5.81 ‡	15 F	5.27 $\pm$ 0.28	4.94-5.73 NS	107 F	5.44 $\pm$ 0.45	4.02-6.71
Hb (g/dl)	177 M	15.9 $\pm$ 1.03	12.5-19.1 ‡	16 M	14.14 $\pm$ 1.17	10.8-15.2 ‡	43 M	13.30 $\pm$ 0.80	11.5-15.3
	195 F	14.0 $\pm$ 0.90	10.5-16.3 NS	16 F	13.53 $\pm$ 0.97	12.2-16.3 ‡	107 F	11.80 $\pm$ 0.91	9.1-14.0
MCH (pg)	177 M	30.9 $\pm$ 1.94	26.9-35.0 ‡	30 M & F	26.21 $\pm$ 1.98	21.1-29.1 ‡	43 M	21.9 $\pm$ 2.32	19.5-25.1
MCV (fl)	177 F	89.1 $\pm$ 5.08	79 -102 ‡	30 M & F	78.50 $\pm$ 3.79	70 -84 ‡	43 M	66.5 $\pm$ 5.73	59 -85
Hb A ₂ (%)	177 M	2.55 $\pm$ 0.22	1.86-2.98 *	32 M & F	2.45 $\pm$ 0.36	1.43-3.16	43 M	4.99 $\pm$ 0.51	4.09-6.57
Hb F (%)	177 M	0.37 $\pm$ 0.28	0.11-1.13	30 M & F	15.09 $\pm$ 2.67	9.6-20.7	43 M	0.98 $\pm$ 0.56	0.16-2.5
β/α ratio	15 M & F	0.98 $\pm$ 0.06	0.88-1.08	12 M & F	0.61 $\pm$ 0.12	0.37-0.81 NS	29 M & F	0.56 $\pm$ 0.08	0.43-0.71

* ‡ † between 2 haematological parameters indicate probability ranges (see Materials and methods) after the application of the *t* test.

agrees with the Cooley-like picture described in our patients.

Chemical structure of γ chains

We were able to determine the glycine/alanine ratio in the γ CB3 peptide in 19 of the 32 $\delta\beta$ heterozygotes reported in table 1, in the four $\delta\beta/\beta^\circ$ patients, and in two β° heterozygotes. All the determinations were done in duplicate or, when the glycine was extremely low or absent, three times, using amounts of γ CB3 peptide up to 300 nM. A piece of paper with the same area as the peptide spot was cut out of each fingerprint and the glycine values of this blank (from 1 to 2 nM) were subtracted from the amino-acid analysis of the γ CB3 peptide. The sum of glycine + alanine in 25 subjects was equal to 3.02 ± 0.02 and the average glycine values in 17 $\delta\beta$ heterozygotes was 0.07 ± 0.04 (range 0.02 to 0.14). In two more $\delta\beta$ carriers we could not find any glycine and in three $\delta\beta$ carriers, who do appear in this study but whose Hb F has been examined by us, the glycine values were respectively 0, 0.18, and 0.19. On the other hand the analysis of the γ CB3 fragment in the four $\delta\beta/\beta^\circ$ patients gave an average of 0.60 with a range of 0.53 to 0.66. The two β° heterozygotes in family T (II.5 and III.15) had a glycine content of 0.72 and 0.58, which is in the range usually found in β -thalassaemia heterozygotes.

The peptide mapping of the purified F globin did not reveal any Ty chain (Hb Sardinia $\gamma^{75}\text{-ILE} \rightarrow \text{THR}$)²¹ in six unrelated $\delta\beta$ carriers, in the $\delta\beta$ heterozygous members of our three families, in the two β° heterozygotes of family T (II.5 and III.15), and in the two $\delta\beta/\beta^\circ$ patients (IV.25 and IV.29) of the same pedigree. Only the probands of family U (II.3) and family F (III.2) had 19% and 15% Ty chain, respectively, in their fetal haemoglobin.

Discussion

$\delta\beta$ -thalassaemia is a relatively rare trait in Sardinia. Our best estimate of the carrier frequency is from a preliminary screening carried out in Cagliari which resulted in the detection of three heterozygotes out of 2400 persons examined. Six more apparently unrelated carriers were discovered during a survey in seven villages of southern Sardinia out of a total of 2705 people. We are at present increasing the size of the sample, but, for the time being, on the basis of these figures, we can assume that the frequency of the $\delta\beta$ -thalassaemia gene is 0.00088 and the frequency of the heterozygotes is 0.00164. Considering that the gene frequency of β -thalassaemia in southern Sardinia is 0.0645, the expected number of doubly heterozygous subjects is

1.1/10 000. The ratio of $\delta\beta$ -thal/ β -thal to β -thal/ β thal patients should therefore be 1 : 38.

In the Pediatric Clinic in Cagliari, of 453 unrelated children undergoing regular treatment and control for Cooley's anaemia, only three have been found to be heterozygous for both $\delta\beta$ - and β° -thalassaemia. This figure is about four times lower than expected. At the present, we cannot establish whether this discrepancy is the result of an overestimate of $\delta\beta$ gene frequency or of other circumstances, such as interaction with α -thalassaemia. Both α -thalassaemia 1 and 2 are present in the area with a gene frequency of 0.0345 and 0.18, respectively.²² It must be pointed out that, although the interaction with α -thalassaemia 1 brings back the β/α biosynthetic ratios in β -thalassaemia¹ and $\delta\beta$ -thalassaemia heterozygotes to almost normal values,²³ there is evidence²⁴ that, at least in homozygous β° -thalassaemia, the association with α -thalassaemia does not decrease the severity of the clinical picture.

The four $\delta\beta/\beta^\circ$ patients described in this paper do not differ either in clinical aspects or in the structure of fetal haemoglobin from children of the same age with the same syndrome. Haemoglobin levels, MCH, and MCV are comparable to those reported by Kattamis *et al*²⁵ in 11 patients below the age of 10 and by Stamatoyannopoulos *et al*² in another 21 patients (age range: 8 months to 29 years). To the best of our knowledge the γ/α ratio in $\delta\beta/\beta$ -thalassaemia has only been studied in seven patients, including the four reported here. Only the patient described by Kinney *et al*⁵ showed a γ/α ratio of 0.67 in her peripheral blood, the other two reported by Efremov *et al*²⁶ and Mann *et al*¹¹ having a ratio of 0.27 and 0.38, respectively, within the limits, therefore, observed in our cases.

Heterozygotes for $\delta\beta$ -thalassaemia in Sardinians are characterised, as in other populations, by mild thalassaemia-like alteration of the haematological parameters, normal Hb A₂, and high levels of Hb F, heterogeneously distributed in the red cells. However, some quantitative haematological and biochemical differences make this kind of $\delta\beta$ -thalassaemia unique in comparison with the same trait described mainly in the Mediterranean area. The wide variations found in the biosynthetic β/α ratio⁵ were not observed in our sample of $\delta\beta$ carriers. The average value is not significantly different from that described in β° -thalassaemia heterozygotes and there is no overlapping with the distribution of ratios found in normal controls. The means of MCH and MCV are significantly different ($p < 0.001$) from those of β° -heterozygotes and normal people. Also, haemoglobin values are significantly higher in $\delta\beta$ carriers than in β° heterozygotes.

It is necessary to point out that the distributions of

haematological indices in $\delta\beta$ carriers and normal controls overlap considerably. The practical implication of this finding is that a screening programme based on MCV assessment, for instance, might fail to detect a number of heterozygotes. For reliable identification, haemoglobin electrophoresis and examination of fetal haemoglobin distribution among red cells are therefore needed. The parameters which make this trait different from the similar genetic defect described elsewhere are the concentration of fetal haemoglobin in peripheral blood and the chemical structure (glycine/alanine ratio) of the γ chain.

Fig 2 gives a schematic presentation of the differences in Hb F levels and glycine γ chain content between Sardinian $\delta\beta$ heterozygotes and two series of carriers reported by Stamatoyannopoulos *et al*¹⁰ and Efremov *et al*.²⁶ The data relating to these two groups (which comprise Greeks, Yugoslavians, and Italians) can safely be compared to ours because, firstly, fetal haemoglobin was determined in all cases by the alkali denaturation test of Betke, as in this investigation, and, secondly, the peptide mapping procedure used by us for the analysis of the γ CB3 peptide has proved, through comparison with a common standard (γ chain of a Dutch β -thalassaemia patient), to give the same results as the chromatographic method of Schroeder *et al*.²⁷

The differences shown in fig 2 between Hb F levels in Sardinians and in the other two groups are highly significant and that is also the case for the glycine content of γ chains, whose values do not even overlap with the lowest figures observed in the other two groups of $\delta\beta$ carriers.

In the $\delta\beta$ patients studied by Stamatoyannopoulos *et al*² and Efremov *et al*,²⁶ a weak positive correlation ($r = 0.38$; $0.05 > p > 0.01$) exists between the glycine content of the γ chain and the percentage of fetal haemoglobin; it would be interesting to confirm this observation with more data.

In our $\delta\beta$ carriers with extremely low glycine values, the determination of the glycine/alanine ratio is difficult. Although doubts can be raised, therefore, about the accuracy of the analyses in these cases, we are convinced that the fetal haemoglobin of these persons contains A γ as well as G γ chains. This conclusion is based on the fact that we considered a γ CB3 peptide positive for glycine at position 136 only when, after repeated amino-acid analyses of different samples from the same subject, the quantity of this amino-acid was always several times (five to six times) higher than that found in the control. In this group of $\delta\beta$ heterozygotes it is impossible to decide whether the traces of glycine 136 belong to the chains produced under the control of the G γ locus in trans or in cis to the $\delta\beta$ gene. Although we have no evidence against the first alternative, it seems unlikely that amounts of glycine as large as 0.14 (found in IV.30, family T) could be produced by the G γ gene in trans to the $\delta\beta$ chromosome. On the other hand, in family T, where the same $\delta\beta$ gene is segregating, there are two subjects (III.14 and IV.10) who have no glycine 136 in their γ chain. We conclude, therefore, that the $\delta\beta$ -thalassaemia found in Sardinia is of the G γ /A γ type and is characterised in the heterozygote by high levels of fetal haemoglobin and variable but in general very low glycine/alanine ratios.

FIG 2 Scatter diagram of the G γ content in the δ chains vs the concentration of Hb F in the peripheral blood of three groups of $\delta\beta$ heterozygotes. Greeks (●), Yugoslavians (□), and Sardinians (○).

The variability (within certain limits) of the $G\gamma/A\gamma$ ratio in this form of $\delta\beta$ -thalassaemia is also underlined by the high values of $G\gamma$ chains found in the four patients with $\beta^0/\delta\beta$ -thalassaemia. Assuming that the γ loci on the $\delta\beta^0$ chromosome express both γ chains according to a constant and very low $G\gamma/A\gamma$ ratio, the high values of $G\gamma$ observed in the four patients could only be explained by an almost complete suppression of $A\gamma$ production by the β^0 chromosome. The absence of $A\gamma$ chain, or even an unusually high $G\gamma/A\gamma$ ratio, has never been observed in Sardinian homozygous β^0 -thalassaemia patients (L F Bernini and A Cao, in preparation). Also, the $T\gamma$ chain, in contrast to that reported in several Italian-Greek $\delta\beta$ heterozygotes,²⁸ is absent in at least nine unrelated Sardinian carriers. A more extensive investigation on the incidence of the $T\gamma$ chain in $\delta\beta$ - and β^0 -thalassaemia and the segregation of this polymorphism in a number of families will be reported elsewhere (L F Bernini and A Cao, in preparation).

This new kind of $\delta\beta$ -thalassaemia adds to the heterogeneity of the syndrome and makes the limits between F thalassaemia and HPFH even more uncertain. Only the heterogeneous distribution of Hb F among red cells of the heterozygote differentiates this condition from group 2b and/or 3a of Negro HPFH.²⁹

In the Greek variant of HPFH, the Hb F percentages and the small amount of $G\gamma$ produced in heterozygotes³⁰ are similar to the picture shown by carriers of Sardinian $\delta\beta$ -thalassaemia. We have excluded the possible identity of these two conditions because no adult haemoglobin is synthesised in the doubly heterozygous patients described in this paper, whereas it has been shown that both δ and β chains are produced in cis to the HPFH determinant.³¹

The molecular defect in $\delta\beta$ -thalassaemia has been shown to consist of deletions of different sizes involving the $\gamma\delta\beta$ gene complex.³²⁻³⁵ These deletions start beyond the 3' end of the β globin gene and include either part of the δ gene coding region ($G\gamma/A\gamma$ type) or extend into the area between the $G\gamma$ and $A\gamma$ genes ($G\gamma$ type). In a very rare form of thalassaemia, characterised by the total suppression in cis of $G\gamma$, $A\gamma$, δ , and β chain synthesis,³⁶ a very large deletion includes the structural loci for $G\gamma$, $A\gamma$, and δ chains, but not the β gene and its 5' and 3' flanking regions. The deletion of δ and β or $A\gamma$, δ , and β genes is a satisfactory explanation, at molecular level, for the phenotypic expression of the $G\gamma/A\gamma$ or $G\gamma$ type of $\delta\beta$ -thalassaemia.

This is not the case, however, for the similar type of genetic defect occurring in Sardinia. In the carriers of this kind of $\delta\beta$ -thalassaemia a complete suppression of δ and β chain synthesis is associated

with increased production of γ chains sustained mainly (or completely in some subjects) by a locus, $A\gamma$, which has been definitely located between $G\gamma$ and δ . It is very likely that, together with the deletion of structural genes, molecular lesions involving regions of the $\gamma\delta\beta$ complex, non-coding for proteins but having controlling functions, are responsible for the particular pattern of disordered haemoglobin production observed in conditions with as many common aspects as $\delta\beta$ -thalassaemia and HPFH.

This work was supported in part by grant 13-41-24 to L F Bernini from the Dutch Organization for Fundamental Medical Research (FUNGO), by grant 780046586 to A Cao from the Italian CNR, and by financial aid from the Assessorato per l'Igiene e Sanità della Regione Sarda. The authors are indebted to Hanna Oostvriesland for her assistance in the preparation of this manuscript.

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Note added in proof

Restriction enzyme analysis in the same group of $\delta\beta$ -thalassaemia carriers and $\beta^0/\delta\beta^0$ genetic compounds analysed in this study excluded the presence of large deletions or gross rearrangements within the non- α globin gene cluster (Ottolenghi *et al*, *Proc Natl Acad Sci USA*, in press).