Interaction of α - and $\delta\beta^0$ -thalassaemia: haematological features and globin chain synthesis analysis

R GALANELLO, M FURBETTA, M A MELIS, C ROSATELLI, AND A CAO

From the 2nd Pediatric Clinic, University of Cagliari, Sardinia, Italy

SUMMARY An observation of suspected interaction of $\delta\beta^0$ - and α -thalassaemia, identified through careful examination of the family, is reported. The $\delta\beta^0$ -thalassaemia was of the usual type found in Sardinia, characterised by high Hb F levels and very low levels of glycine in the isolated γCB_3 peptide. The haematological findings in the double $\delta\beta^0$ -/ α -thalassaemia heterozygotes were: normal MCV and Hb A_2 levels, increased Hb F (11.3 to 16.8%) heterogeneously distributed in red cells, and almost balanced α/β globin chain synthesis ratios.

Hereditary persistence of fetal haemoglobin (HPFH) and $\delta\beta^0$ -thalassaemia are closely related groups of genetic disorders of haemoglobin synthesis, characterised by an effective increase of γ chain production, as shown by the high Hb F levels in heterozygotes.¹ Therefore, there are considerable difficulties in the differential diagnosis of these disorders, particularly those involving the G γ types.²⁻¹⁰ In fact, in the heterozygous state there is a large overlap in haematological criteria (normal red cell indices in HPFH versus low MCH and MCV in $\delta\beta^0$ -thalassaemia), globin chain synthesis ratios (balanced versus unbalanced), and haemoglobin F distribution (pancellular versus heterocellular) in both conditions.²⁻¹³ However, this differentiation has very important practical implications in genetic counselling, since β or $\delta\beta^0 \times \delta\beta^0$ heterozygous thalassaemia mating is associated with the risk of producing thalassaemia major or intermedia in the offspring, while HPFH $\times \beta$ or $\delta\beta^0$ mating involves no such risk.

In this paper we report an observation of suspected interaction of $\delta\beta^0$ - and α -thalassaemia which created some difficulties in the counselling of a couple attending our genetic clinic.

Case report

In 1978 a couple presented at our genetic service for counselling. The husband was a high Hb A_2 β -thalassaemia carrier (III.1) and the wife (III.2) had normal MCV and Hb A_2 levels, increased Hb F (>5%) heterogeneously distributed in red cells, and an almost balanced α/β globin chain synthesis ratio. The table shows the haematological features of this couple and some other family members and the figure depicts the family tree.

As can be seen in the figure, the father (II.1) is normal and the mother (II.2) is similar both haematologically and in globin chain synthesis ratio to her daughter (III.2). The grandfather (I.1) had reduced MCV, Hb A_2 levels, and α/β ratio (0.60), as in the α -thalassaemia carrier state (α -thalassaemia-1). The

Received for publication 29 February 1980

TABLE Haematological and globin synthesis data

Subject	Age	Hb	RBC	MCV	MCH	Hb A_2	Hb F	α/β	$\alpha/\beta + \gamma$
I.1	78	14.7	5.06	76	29	1.99	—	0.60	—
I.2	76	11.5	4.66	77	24.6	2.29	11.3	1.68	1.35
II.1	51	14.8	4.80	93	30.8	2.92	0.80	—	—
II.2	49	13.8	5.14	78	26.8	2.14	14.1	1.14	1.01
III.1	35	14.2	6.51	66	21.8	4.48	0.90	—	—
III.2	26	14.0	5.27	80	26.5	2.40	16.8	1.27	1.11
III.3	20	12.7	5.01	72	25.3	2.24	16.3	2.48	2.00

FIGURE Family pedigree.

grandmother (I.2) and the wife's brother (III.3) had classic manifestations of heterozygous $\delta\beta^0$ -thalassaemia with reduced MCV, normal Hb A₂ levels, increased Hb F levels heterogeneously distributed in red cells, and increased α/β ratio. This family shows the $\delta\beta^0$ -thalassaemia type, characterised by unusually high Hb F levels (range 10 to 20%) and a very low glycine content (range 0.02 to 0.14 residues) in the isolated γCB_3 peptide (determination kindly performed by Dr L F Bernini, Leiden, Holland), as seen in all Sardinian $\delta\beta^0$ carriers studied till now.¹³ In II.2 and III.2 the glycine content in the isolated γCB_3 peptide was 0.04 and 0.06, respectively.

During a later counselling session, the couple was informed that there was a 25% risk of thalassaemia intermedia ($\delta\beta^0/\beta^0$ -thalassaemia genetic compound) in their offspring. The predicted mildness of the disease was stressed. The couple decided on a pregnancy monitored by antenatal diagnosis.

Methods

Red blood cell indices from venous blood collected in EDTA were estimated with Coulter Counter

model S. Haemoglobin electrophoresis was carried out on cellulose acetate plate (Titan III, Helena Laboratories, Beaumont, Texas). Hb A₂ was quantified by DE-52 microchromatography¹⁴ and Hb F by alkali denaturation.¹⁵ The distribution of Hb F in erythrocytes was shown using the acid elution technique of Kleihauer *et al.*¹⁶ Biosynthetic ratios were determined from peripheral blood according to Kan *et al.*¹⁷ and expressed as specific activity. Hb F structural studies were performed as previously described.¹⁸

Discussion

The haematological features (table) (normal red cell indices, increased Hb F heterogeneously distributed in red cells, and balanced globin chain synthesis ratio) found in one member of this couple (III.2) are consistent with a diagnosis of heterocellular HPFH or $\delta\beta^0$ -thalassaemia carrier state. Information for differentiating between these two conditions was obtained by the study of the family tree.

The father (II.1) had normal results and the mother (II.2) showed haematological characteristics similar to those of her daughter (III.2). However, the grandfather (I.1) had haematological features compatible with the α -thalassaemia carrier state (α -thalassaemia-1) while the grandmother (I.2) and the wife's brother (III.3) had phenotypic manifestations consistent with a diagnosis of $\delta\beta^0$ -thalassaemia (low MCV and MCH, increased Hb F heterogeneously distributed in red cells, and increased α/β globin chain synthesis ratio).

Therefore, it can be assumed that the wife (III.2) and her mother (II.2) had inherited the $\delta\beta^0$ -thalassaemia gene from the grandmother (I.2) together with the α -thalassaemia-1 gene from the grandfather (I.1). The presence of $\delta\beta^0$ - and α -thalassaemia-1 genes determined an almost balanced globin chain synthesis ratio. It should be pointed out that Sardinian $\delta\beta^0$ -thalassaemia carriers have always been found to have unbalanced α/β chain synthesis ratios,¹³ at variance with the findings of Kinney *et al.*¹¹ who reported α/β ratios overlapping those of normal subjects in a group of Negro and Caucasian $\delta\beta^0$ -thalassaemia carriers.

The above interpretation is likely to be correct, but DNA restriction enzyme analysis should be carried out for confirmation.

The normal MCV in the wife and her mother is compatible with the $\delta\beta^0$ -thalassaemia carrier state, since in the Sardinian population a large overlap between MCV and MCH values of $\delta\beta^0$ -thalassaemia carriers and those of normal subjects has been found.¹³

In conclusion this study exemplifies the difficulties

involved in the differential diagnosis of HPFH and $\delta\beta^0$ -thalassaemia, and in particular the necessity of carrying out family studies for the identification of the $\alpha/\delta\beta$ interaction.

References

- ¹ Weatherall DJ, Clegg JB. *The thalassaemia syndromes*. 2nd ed. Oxford: Blackwell Scientific Publications, 1972.
- ² Mann JR, MacNeish AS, Bannister D, Clegg JB, Wood WG, Weatherall DJ. $\delta\beta$ -thalassaemia in a Chinese family. *Br J Haematol* 1972;**23**:393–402.
- ³ Sukumaran PK, Huisman THJ, Schroeder WA, et al. A homozygote for Hb G γ type of foetal haemoglobin in India: a study of two Indian and four Negro families. *Br J Haematol* 1972;**23**:403–17.
- ⁴ Huisman THJ, Miller A, Schroeder WA. A G γ type of hereditary persistence of fetal hemoglobin with β -chain production in cis. *Am J Hum Genet* 1975;**27**:765–77.
- ⁵ Huisman THJ, Miller A, Cook L, Gordon S, Schroeder WA. The molecular heterogeneity of some types of hereditary persistence of fetal hemoglobin (HPFH). In: Aksoy E, ed. *International Symposium on Abnormal Haemoglobins and Thalassaemia*. Ankara: TBTA, 1975: 85–110.
- ⁶ Altay C, Schroeder WA, Huisman THJ. The G γ - $\delta\beta$ -thalassaemia and G γ - β^0 -HPFH condition in combination with β -thalassaemia and Hb S. *Am J Haematol* 1977; **3**: 1–14.
- ⁷ Wood WG, Clegg JB, Weatherall DJ. G γ - $\delta\beta$ -thalassaemia and G γ HPFH (Hb Kenya type): a comparison of two new cases. *J Med Genet* 1977;**14**:237–44.
- ⁸ Wood WG, Clegg JB, Weatherall DJ. Hereditary persistence of fetal haemoglobin (HPFH) and $\delta\beta$ -thalassaemia. *Br J Haematol* 1979;**43**:509–20.
- ⁹ Clegg JB, Metaxatou-Mavromati A, Kattamis C, Sofroniadou K, Wood WG, Weatherall DJ. Occurrence of G γ Hb F in Greek HPFH: analysis of heterozygotes and compound heterozygotes with β -thalassaemia. *Br J Haematol* 1979;**43**:521–36.
- ¹⁰ Amin AB, Pandya NL, Diwin PP, et al. A comparison of the homozygous states for G γ and G γ A γ $\delta\beta$ -thalassaemia. *Br J Haematol* 1979;**43**:537–48.
- ¹¹ Kinney TR, Friedman SH, Cifuentes E, Kim HC, Schwartz E. Variations in globin synthesis in delta-beta-thalassaemia. *Br J Haematol* 1978;**38**:15–22.
- ¹² Higgs DR, Clegg JB, Wood WG, Weatherall DJ. G γ β^+ type of hereditary persistence of fetal haemoglobin in association with Hb C. *J Med Genet* 1979;**16**:288–95.
- ¹³ Cao A, Melis MA, Galanello R, et al. $\delta\beta$ (F) thalassaemia in Sardinia. *Blood* (in press).
- ¹⁴ Huisman THJ, Schroeder WA, Brodie AN, Mayson SM, Jackway J. Microchromatography of hemoglobins. III. A simplified procedure for the determination of hemoglobin A₂. *J Lab Clin Med* 1975;**86**:700–2.
- ¹⁵ Betke J, Marti HR, Schlicht I. Estimation of small percentages of fetal hemoglobin. *Am J Hematol* 1959;**3**: 1–14.
- ¹⁶ Kleihauer E, Braun H, Betke K. Demonstration von fetalem haemoglobin in den erythrocyten eines blutansstrichs. *Klin Wochenschr* 1957;**35**:635–6.
- ¹⁷ Kan YW, Schwartz E, Nathan DG. Globin synthesis in the alpha thalassaemia syndromes. *J Clin Invest* 1968;**47**: 2515–22.
- ¹⁸ Ricco G, Mazza U, Tori RM, et al. Significance of a new type of human fetal hemoglobin carrying a replacement isoleucine-threonine at position 75 (E19) of the γ -chain. *Hum Genet* 1976;**32**:305–13.

Requests for reprints to Professor Antonio Cao, Clinica Pediatrica II, C/Post 251, Via Porcell 1, 09100 Cagliari, Sardinia, Italy.