Thalassaemia of intermediate severity resulting from the interaction between α - and β -thalassaemia

S. MUSUMECI, G. SCHILIRÒ, G. PIZZARELLI, A. FISCHER, AND G. RUSSO

From the Pediatric Clinic, University of Catania, Catania, Italy

SUMMARY A Sicilian family is described in which the α -thalassaemia gene is interacting in several members with β -thalassaemia resulting in a balanced α/β chain production ratio. In one patient, affected by homozygous β -thalassaemia, the presence of α -thalassaemia resulted in a less severe clinical expression of the disease, less marked imbalance in the α /non- α ratio, and a lower level of Hb F. Further studies of haemoglobin synthesis are needed to clarify the complex genetic picture that results from the interaction of different forms of thalassaemia.

The term 'thalassaemia intermedia' is used to describe those forms of thalassaemia of intermediate clinical and haematological severity between thalassaemia major and asymptomatic carriers. These forms are a genetically heterogeneous group which usually result from the interaction between 2 different thalassaemia genes, or else from the combination of β -thalassaemia with genes responsible for structural anomalies of the globin (Weatherall and Clegg, 1972). In particular, it has been observed that the interaction between α - and β -thalassaemia gives a less severe clinical picture than homozygous β -thalassaemia (Kan and Nathan, 1970). In the case reported by Kan and Nathan, one of the parents was a carrier of β -thalassaemia with characteristic alteration of red cell morphology, high Hb A₂, and unbalanced synthesis of α and β chains. The other parent, though presenting the morphological appearance of β -thalassaemia, with increased Hb A₂, had a balanced α/β synthetic ratio. Kan and Nathan concluded that in the propositus the presence of the α -thalassaemia gene could modify the clinical manifestation of the disease, with normal survival and minimal clinical symptoms. The propositus showed, in fact, slight enlargement of the liver and spleen, and his haemoglobin ranged from 9 to 11 g/dl. He seldom needed blood transfusions.

There are 2 forms of α -thalassaemia trait: one without alteration of red cell morphology (α -thalassaemia 2) and only slight imbalance of the α/β ratio,

and the other more severe (α -thalassaemia 1) with altered red cell morphology typical of thalassaemia and greater imbalance of the α/β ratio (Wasi *et al.*, 1969, 1974; Schwartz and Atwater, 1972). It might be expected that the effect of a less severe α -thalassaemia gene on homozygous β -thalassaemia would be smaller than that observed by Kan and Nathan (1970). Here we report a family where the association of α -thalassaemia with β -thalassaemia resulted in normalisation of the α/β ratio in several β -thalassaemia heterozygotes, while one member, a β -thalassaemic homozygote, had a clinical picture of intermediate severity with a moderately imbalanced α /non- α ratio and only slightly increased Hb F.

Case report

The propositus was a 7-year-old boy from Giarre, Catania, who presented with chronic haemolytic anaemia of moderate severity. His mental and physical development was normal; weight, 25 kg and height, 119 cm. The diagnosis of homozygous β -thalassaemia had first been made at the age of 12 months, during admission to hospital because of respiratory distress. His haemoglobin was 6 g/dl, RBC $2.54 \times 10^{12}/\text{l}$, reticulocytes 3.2%, serum iron 150 $\mu\text{g}/100 \text{ ml}$ (26.3 $\mu\text{mol/l}$), Hb F (Singer method) 30%, unconjugated serum bilirubin 1.90 mg/100 ml (32.5 $\mu\text{mol/l}$). Red cell morphology showed anisopoikilocytosis, target cells, and microcytosis. The spleen could just be felt at the costal margin, and the liver 2 cm below the right

costal margin. Since then he has been given small blood transfusions (50 ml monthly during the first year, and thereafter 100 ml every 2 or 3 months), until recent years when the Hb level became stable at around 8 to 10 g/dl with a 200 ml blood transfusion every 3 or 4 months. At age 7, there was marked cutaneous pigmentation, the spleen was palpable at the umbilical line, and the liver was palpable 4 cm below the costal margin.

Methods

Standard haematological tests were performed, as described by Dacie and Lewis (1968). Hb F was determined by the Singer method (Dacie and Lewis, 1968). Hb electrophoresis was carried out on cellulose acetate strips in glycine buffer at pH 9, and the minor haemoglobin fractions were evaluated after elution from cellulose acetate and their optical densities (415 nm) determined (Di Stefano and Marcellini, 1969). Osmotic fragility was determined by the method of Parpart *et al.* (1947). Globin chain synthesis was measured after incubation of peripheral blood for 2 hours with 10 μ Ci of H^3 leucine in Krebs Ringer bicarbonate buffer, by the method of Bank and Marks (1966). Globin was prepared by cold acid acetone precipitation of total haemolysates. The globin chains were separated by column chromatography on CM 23 cellulose, according to the method of Clegg *et al.* (1966). The α/β ratio and $\alpha/\text{non-}\alpha$ ratio were calculated both as specific activity in the peak tubes and as total counts in pooled fractions.

Results

The family pedigree is shown in the Fig., and the principal haematological data are summarised in the Table. The propositus (III.2) showed alterations of red cell morphology characteristic of a β -thalassaemia homozygote. Osmotic fragility was decreased, Hb F was 13.02%, Hb A₂ was 6.48%, and the $\alpha/\text{non-}\alpha$ ratio was 3.65 as total count, and 2.26 as specific activity ratio. The sister (III.1) and the mother (II.6) showed alterations of erythrocyte morphology typical of the β -thalassaemia trait. Osmotic fragility was decreased, while the level of Hb A₂ was normal. The α/β ratio, expressed both as total count and specific activity, was also within the normal range (Table). The father (II.8)

Fig. Pedigree of family.

Table Haematological data of family

Family member	Age (y)	Hb (g/dl)	RBC ($\times 10^{12}/l$)	MCV (μ^3)	MCH (YY)	MCHC (%)	Osmotic fragility (Parpart <i>et al.</i> , 1947)	Erythrocyte morphology changes (APHT)	Hb A (%)	Hb A ₂ (%)	Hb F (%)	$\alpha/\text{non-}\alpha^*$ (total count)	α/β^* (cpm/mg)	Designated genotype
I.1	67	13.5	4.4	90.0	30.6	33.8	normal	----	98.08	1.47	0.45	0.85 0.66	0.72 0.71	$\alpha A \text{ } \alpha \text{ } \beta A \text{ } \beta A$
I.2	74	12.2	4.6	81.5	26.5	32.6	decreased	$\pm$ ----	95.9	3.56	0.54	1.59 1.19	1.20 1.26	$\alpha A \text{ } \alpha A \text{ } \beta A \text{ } \beta \text{ } \text{th}$
II.1	47	13.0	5	80	20.0	32.5	decreased	$\pm$ $\pm$ $\pm$	96.77	2.57	0.66	—	—	$\alpha A \text{ } \alpha \text{ } \text{th } \beta A \text{ } \beta \text{ } \text{th}$
II.3	42	13.2	4.6	86.2	28.4	33	decreased	$\pm$ $\pm$ $\pm$	95.01	3.97	1.02	—	—	$\alpha A \text{ } \alpha A \text{ } \beta A \text{ } \beta \text{ } \text{th}$
II.5	37	Not studied												
II.6	32	11.6	4.5	80.9	25.3	31.3	decreased	$\pm$ $\pm$ $\pm$	96.61	2.83	0.56	0.97 1.26	1.13 1.14	$\alpha A \text{ } \alpha \text{ } \text{th } \beta A \text{ } \beta \text{ } \text{th}$
II.7	28	5		80	25.4	31.7	decreased	$\pm$ $\pm$ $\pm$	96.53	2.79	0.68	—	—	$\alpha A \text{ } \alpha \text{ } \text{th } \beta A \text{ } \beta \text{ } \text{th}$
II.8	11.7	4.5		81.8	26.0	31.7	decreased	$\pm$ ----	94.4	4.05	1.55	1.89	1.65 1.81	$\alpha A \text{ } \alpha A \text{ } \beta A \text{ } \beta \text{ } \text{th}$
III.1	12	12.5	4.3	84.4	28.5	33.7	decreased	$\pm$ $\pm$ $\pm$	96.38	2.80	0.82	0.89		$\alpha A \text{ } \alpha \text{ } \text{th } \beta A \text{ } \beta \text{ } \text{th}$
III.2	7	6.5	3.4	67.6	19.1	28.3	decreased	$\pm$ $\pm$ $\pm$ $\pm$	80.5	6.48	13.02**	3.65	2.26	$\alpha A \text{ } \alpha \text{ } \text{th } \beta \text{ } \text{th } \beta \text{ } \text{th}$

A, anisocytosis; P, poikilocytosis; H, hypochromia; T, target cells
*Data obtained in different chromatographies.
**This patient had periodic blood transfusions.

was a carrier of β -thalassaemia with typical red cell morphology, increased Hb A₂ (4.05), and an α/β ratio of 1.78, characteristic of the Sicilian β -thalassaemia trait. It was possible to study 3 of the relatives of the mother's family. II.1 and II.7 showed the same changes of red cell morphology as II.6, and their Hb A₂ levels were normal, while II.3 had altered red cell morphology and increased Hb A₂. In all subjects the Hb F levels were normal. The maternal grandparents I.1 and I.2 were also studied. I.1 was a carrier of α -thalassaemia, as judged by the normal red cell morphology and by a slightly unbalanced α/β ratio similar to that found in the so-called 'silent gene' for α -thalassaemia (Schwartz and Atwater, 1972; Russo *et al.*, 1973). I.2 was a typical carrier of β -thalassaemia with high Hb A₂ (3.56) and an unbalanced α/β ratio.

Discussion

The association of α - and β -thalassaemia in the same patient has been determined by genetic and globin chain synthesis studies (Fessas, 1961; Bernini *et al.*, 1962; Pearson, 1966; Wasi *et al.*, 1969; Kan and Nathan, 1970; Knox-Macaulay *et al.*, 1972). The coexistence of these 2 different forms of thalassaemia results in attenuation of the clinical effects. The compound heterozygotes for both genes are well and show altered erythrocyte morphology, but a balanced α/β ratio (Kan and Nathan, 1970; Knox-Macaulay *et al.*, 1972). The subject who is homozygous for β -thalassaemia, and who also carries an α -thalassaemia gene, shows a less severe clinical picture than that of Cooley's disease, and a less marked imbalance of globin chain synthesis (Kan and Nathan, 1970). Finally, in the heterozygote for α -thalassaemia 1 and α -thalassaemia 2, that is, in the condition which is usually expressed as Hb H disease, the coexistence of a β -thalassaemia gene results in a mild clinical picture (Kan and Nathan, 1970). In the family presented here, the study of erythrocyte morphology, haemoglobin analysis, and globin chain synthesis, combined with the examination of the pedigree, allowed us to diagnose the heterozygous state for both α - and β -thalassaemia in several subjects, and the coexistence of homozygous β -thalassaemia with heterozygous α -thalassaemia in the proband.

However, there are several differences between the data of this family and that reported by others who have described the association of α - and β -thalassaemia (Kan and Nathan, 1970; Knox-Macaulay *et al.*, 1972). A difference has been observed regarding the Hb A₂ level, which in our family was normal, while in that of Kan and Nathan and Knox-Macaulay *et al.* was increased. We have observed another patient with interaction between α - and β -thalassaemia and with high Hb A₂ (Musumeci *et al.*, 1978). It is possible that

the decreased availability of the α chains due to the α -thalassaemia gene, through a reduced α/β ratio, impairs the synthesis of Hb A₂ (Pornpatkul *et al.*, 1969), and that some δ chains remain free, rather than combining with α chains as Hb A₂. If the β chain synthesis is also depressed to the same degree, the ratio A/A₂, and therefore the percentage of Hb A₂, would be normal (Table), while decreased Hb A₂, both in percentage and in absolute values, would indicate a preferential affinity of α chains for β chains rather than for δ chains. Another difference between our case and that of Kan and Nathan (1970) is in the level of Hb F, which is lower in our patient (30% against 41%). This difference is difficult to explain, but at least 3 factors may interfere with the Hb F synthesis in such a condition: (1) The type of interacting α -thalassaemia gene which results in different lowering of Hb F formation; (2) The depressed β chain synthesis, which could stimulate the γ chain synthesis as a compensatory mechanism; and (3) The affinity of α chains for β chains and γ chains, respectively. The preferential affinity of α chains for β chains in respect to γ chains (Lehman, 1961; Huens and Beaven, 1962) decreases the availability of α chains for Hb F synthesis and hence the Hb F percentage might be reduced.

In conclusion, in our family the interaction between the (silent) α -thalassaemia gene and homozygous β -thalassaemia causes a less severe clinical picture of β -thalassaemia, which is between the classical picture of Cooley's disease and that described by Kan and Nathan (1970), where the more pronounced action of the α -thalassaemia gene resulted in a milder clinical expression of the disease.

References

- Bank, A., and Marks, P. (1966). Excess alpha chain synthesis relative to beta chain synthesis in thalassaemia major and minor. *Nature*, **212**, 1198-1200.
- Bernini, L., Colucci, C. F., De Michele, D., Piomelli, S., and Siniscalco, M. (1962). A possible case of alpha-beta thalassaemia. *Acta Genetica et Statistica Medica*, **12**, 202-208.
- Clegg, J. B., Naughton, M. A., and Weatherall, D. J. (1966). Abnormal human haemoglobins: separation and characterization of the alpha and beta chains by chromatography and the determination of two new variants, Hb Chesapeake and Hb J Bangkok. *Journal of Molecular Biology*, **19**, 91-108.
- Dacie, J. V., and Lewis, S. M. (1968). *Practical Haematology*, 4th ed. Churchill, London.
- Di Stefano, G., and Marcellini, M. (1969). La determinazione dell'Hb A₂ mediante elettroforesi su acetato di cellulosa. *Rivista Pediatrica Siciliana*, **24**, 36-45.
- Fessas, P. (1961). Beta-chain thalassaemias. In *Haemoglobin Colloquium*, Wien, p. 90. Ed. by H. Lehman and K. Betke Thieme, Stuttgart.
- Huens, E. R., and Beaven, G. H. (1962). The reaction of haemoglobin alpha⁺ with Hb H and Hb Bart's. *Biochemical Journal*, **83**, 40.
- Kan, Y. W., and Nathan, D. G. (1970). Mild thalassemia: the result of interaction of alpha and beta thalassemia genes. *Journal of Clinical Investigation*, **49**, 635-642.

- Knox-Macaulay, H. H., Weatherall, D. J., Clegg, J. B., Bradley, J., and Brown, M. J. (1972). The clinical and biosynthetic characterization of alpha beta thalassaemia. *British Journal of Haematology*, **22**, 497-512.
- Lehman, H. (1961). Haemoglobins and haemoglobinopathies. A review of the recent advances. In *Haemoglobin Colloquium*, Wein, p. 1. Ed. by H. Lehman and K. Betke. Thieme, Stuttgart.
- Musumeci, S., Schilirò, G., Pizzarelli, G., Tentori, L., Marinucci, M., Bruni, E., and Russo, G. (1978). Interaction between alpha, beta thalassaemia and Hb G St Jose in a Sicilian family. (In press.)
- Parpart, A. K., Lorenz, P. B., Parpart, E. R., Gregg, J. R., and Chase, H. M. (1947). The osmotic resistance (fragility) of human red cells. *Journal of Clinical Investigation*, **26**, 636.
- Pearson, H. A. (1966). Alpha-beta thalassemia disease in a Negro family. *New England Journal of Medicine*, **275**, 176-181.
- Pornpatkul, M., Wasi, P., and Na-Nakorn, S. (1969). Haematological parameters in obligatory alpha thalassaemia traits. *Journal of the Medical Association of Thailand*, **52**, 801-809.
- Russo, G., Schilirò, G., Musumeci, S., D'Agata, A., and Pizzarelli, G. (1973). Haemoglobin synthesis in alpha thalassaemia. *Atti Congresso sulle Microcitemie*, Cosenza.
- Schwartz, E., and Atwater, J. (1972). Alpha thalassemia in the American negro. *Journal of Clinical Investigation*, **51**, 412-418.
- Wasi, P., Na-Nakorn, S., and Pootrakul, S. (1974). The alpha thalassaemias. *Clinics in Haematology*, **3**, 383-410.
- Wasi, P., Na-Nakorn, S., Pootrakul, S., Sookanek, M., Disthasongchan, P., Pornpatkul, M., and Panich, V. (1969). Alpha and beta thalassemia in Thailand. *Annals of the New York Academy of Sciences*, **165**, 60-82.
- Weatherall, D. J., and Clegg, J. B. (1972). *The Thalassaemia Syndromes*, 2nd ed. Blackwell, Oxford.

Requests for reprints to Dr S. Musumeci, Pediatric Clinic of the University, Viale A. Doria, 95125 Catania, Italy.