Association of heterocellular HPFH, β^+ -thalassaemia, and $\delta\beta^0$ -thalassaemia: haematological and molecular aspects

L CIANETTI*, A CARE*, N M SPOSI*, A GIAMPAOLO*, M CALANDRINI*, M PETRINI*, A MASSA*, M MARINUCCI*, F MAVILIO*†, M CECCANTI‡, AND G F SASSO‡

From *the Department of Haematology, Istituto Superiore di Sanità; †the Institute of Experimental Medicine, Consiglio Nazionale delle Ricerche; and ‡the Cattedra di Patologia Speciale Medica II, University La Sapienza, Rome, Italy.

SUMMARY An Italian family in which heterocellular hereditary persistence of fetal haemoglobin (HPFH) interacts with both β^+ - and $\delta\beta$ -thalassaemia is described. The index case was an 8 year old girl who was presumed to inherit both heterocellular HPFH and β^+ -thalassaemia from her mother and $\delta\beta$ -thalassaemia from her father. She was healthy and never needed blood transfusions. The possible contribution of heterocellular HPFH to the less severe expression of the compound $\delta\beta/\beta^+$ -thalassaemia heterozygosity is discussed. By DNA analysis the specific $\delta\beta$ -thalassaemia defect on the $\gamma\delta\beta$ globin gene region has been established. In addition, a previously unreported association of a polymorphic restriction site haplotype with a β^+ -thalassaemia mutation has been observed.

Hereditary persistence of fetal haemoglobin (HPFH) is a group of non-thalassaemic disorders of human haemoglobin synthesis characterised by Hb F production in adult life.^{1,2} Two main types of HPFH are commonly recognised, namely (1) pancellular HPFH, in which Hb F synthesis is increased in the whole RBC population, and (2) heterocellular HPFH. In the latter condition the increased Hb F synthesis is restricted to an expanded F cell pool.

The molecular defects underlying the former condition are, in most cases, extensive deletions in the $\gamma\delta\beta$ globin gene region.^{1,2} However, very little is known at the present about the molecular basis of the heterocellular forms of HPFH. In these forms no gross structural abnormality has been detected by gene mapping studies on the $\gamma\delta\beta$ globin gene region.³⁻⁵

The heterocellular HPFH determinant(s) would act on the erythropoietic bone marrow by enhancing the propensity of the erythroid progenitors to generate erythroblasts with the potential for γ chain synthesis, that is, the precursors of F cells, thus increasing the Hb F level in peripheral blood. It is generally recognised that high Hb F levels in adult life may greatly ameliorate the clinical expression of

a number of inherited disorders of haemoglobin synthesis,¹ like sickle cell anaemia. In particular, the interaction of heterocellular HPFH with β -thalassaemia has been reported to be responsible for an almost complete suppression of the clinical symptoms in homozygous β -thalassaemia.⁵⁻⁸

In the present report we describe a Sicilian family with a previously undescribed interaction of heterocellular HPFH with both β^+ - and $\delta\beta$ -thalassaemia. The index case, who presumably carried a heterocellular HPFH/ β^+ -thalassaemia gene complex associated with a $\delta\beta$ -thalassaemia determinant, was apparently healthy.

Material and methods

Standard haematological methods were used. Hb A₂ level was estimated by an elution method after cellulose acetate electrophoresis of haemolysates.⁷ Hb F level was determined by alkali denaturation (F_{AD})⁹ or by DEAE-cellulose (DE-52) column chromatography.¹⁰ The intracellular distribution of Hb F was evaluated on peripheral blood smears by indirect immunofluorescence using anti- γ chain antibodies.¹¹ Determination of $^G\gamma/^G\gamma + ^A\gamma$ globin chain ratios was carried out by densitometric tracing of globin chain electrophoresis on Triton-urea

Received for publication 7 October 1983.

Accepted for publication 28 December 1983.

polyacrilamide gel slabs.¹² Chain biosynthesis ratios were determined on fractionated reticulocytes as previously described.⁷

High molecular weight DNA was prepared from peripheral blood leucocytes by standard techniques¹³ and digested with several restriction endonucleases according to the supplier's specifications. DNA fragments were separated by 0.8 to 1.0% agarose gel electrophoresis and transferred to nitrocellulose filters according to Southern.¹⁴ Filter hybridisation, washing, and autoradiography were carried out as previously described.¹⁵ Plasmid JW 102, JW 151, and JW 101 containing human β , γ , and α cDNA sequences¹⁶ were ³²P labelled by nick translation to a specific activity of 2 to 6 $\times 10^8$ dpm/ μ g and used as hybridisation probes.

Results

The haematological and haemoglobin findings and the results of the in vitro haemoglobin synthesis of the family members are listed in the table.

The index case (II.1, fig 1) was an 8 year old girl who showed moderate pallor. The spleen and liver were palpable 4 cm and 2 cm below the costal margin respectively. Her growth was normal and she never received blood transfusions. Her parents and sister were asymptomatic.

She showed abnormal RBC indices and morphology. The Hb A₂ level was in the normal range (2.82% of the total Hb), and Hb F was largely prevailing in peripheral blood (82.1%), whereas Hb A accounted for 15.1%. All RBCs were F cells, and the mean Hb F/F cell content was 19.1 pg. The proband showed an unbalanced α /non- α chain synthesis ratio (1.72). The γ /non- α chain synthesis ratio (0.76) was roughly of the same order of magnitude as the Hb F level observed in the red cell haemolysate.

Both parents (I.1 and I.2) showed abnormal RBC indices and morphology. In particular, her mother (I.2) was shown to be a β -thalassaemia carrier. In addition, she showed an increased Hb F level (3.8%), consistently higher than that usually observed in β -thalassaemia carriers (1.23% $\pm$ 0.04%, n=32, in our laboratory). Hb F was heterogeneously distributed in RBCs with a high F cell count (15.3%).

These findings strongly suggested the presence of a heterocellular HPFH determinant coexistent with β -thalassaemia in this subject. The father (I.1) had a normal Hb A₂ level (2.66%), increased Hb F percentage (7.7%), and a thalassaemia like α / β chain synthesis ratio (1.93). By indirect immunofluorescence assay nearly all his RBCs appeared to be F cells. These findings indicated that he was a $\delta\beta$ -thalassaemia carrier.

A second daughter (II.2) showed slightly decreased MCV and MCH values, but normal Hb A₂, Hb F, and serum iron levels.

The presence of the $\Delta\gamma^1$ allele was ruled out in all family members by peptide mapping carried out on

FIG 1 Family pedigree.

TABLE Haematological and globin chain synthesis data.

Subject	Age (yr)	Hb (g/dl)	RBC ($\times 10^{12}/l$)	Hct (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	G γ (%)	non- α / α	β / α	γ / α	Hb F/F cell (pg)
I.1	40	15.0	6.52	44.0	67.5	23.0	34.1	29.0	0.56	0.52	0.04	...
I.2	38	12.4	5.13	36.7	71.5	24.2	33.8	24.4	...	0.65	—	6.0
II.1	8	10.8	4.62	30.3	65.5	23.4	35.6	44.8	0.58	0.14	0.44	19.2
II.2	6	12.6	5.48	38.5	70.3	23.0	32.7	40.7	0.93	0.91	0.02	10.9

FIG 2 Electrophoretic patterns (top) of Eco RI fragments from a normal subject (A) and I.1 (B). DNA $\gamma\delta\beta$ globin gene maps (bottom) with the studied restriction sites in normal (β^+) and $\delta\beta^0$ -thalassaemia chromosomes. In both cases the expected Eco RI fragments are indicated below the map. B=Bam HI, H=Hind III, E=Eco RI, A=Ava II. Asterisks above a letter mean polymorphic site.

purified γ chains. $G\gamma/G\gamma + A\gamma$ ratio values are listed in the table.

The structure of the $\gamma\delta\beta$ globin gene region was studied by restriction endonuclease mapping on genomic DNA obtained from peripheral WBCs from all family members. The restriction patterns obtained by Eco RI, Pst I, and Bgl II digestion of DNA from subject I.1 and II.1 indicated that a deletion, starting from the δ IVS-2, typical of the so-called southern Italian $\delta\beta^0$ -thalassaemia,^{17,18} occurred heterozygously in both of them (fig 2). However, normal restriction patterns were observed in the $\gamma\delta\beta$ region in subjects I.2 and II.2.

In all subjects the chromosomal linkage of the four polymorphic sites studied (Hind III $G\gamma$, Hind III $A\gamma$, Ava II β , and Bam HI β) was established (fig 1). In particular, subject II.2 was shown to have received the 'normal' chromosomes from her parents. The β^+ -thalassaemia chromosome in both subjects I.2 and II.1 carried a Hind III $G\gamma$ —, Hind III $A\gamma$ —, Ava II β^+ , Bam HI β — haplotype. The Bam HI map on the α globin gene region ruled out the presence of gross deletions or rearrangements in all subjects.

Discussion

A number of cases of compound heterozygosity for β^+ - and $\delta\beta^0$ -thalassaemia have been reported.¹⁻¹⁹ The clinical expression of this condition is very hetero-

geneous, ranging from severe to moderate anaemia. In most cases the disorder is milder than in homozygous β -thalassaemia and patients show the haematological and clinical picture of thalassaemia intermedia. Although the reasons for this wide clinical variability are not quite clear, some genetic determinants, which are known to reduce the overall globin chain imbalance in red cells, could be regarded as responsible for some, at least, of the milder conditions observed in this compound heterozygosity. α -thalassaemia and heterocellular HPFH have repeatedly been reported, when found in association with homozygous β -thalassaemia,¹⁻⁵⁻⁸ to produce clinical pictures much milder than Cooley's disease (that is, moderate anaemia without need of transfusion).

In the present family, α -thalassaemia can be ruled out on the grounds of globin chain synthesis ratios and genomic DNA mapping. However, heterocellular HPFH is reasonably associated in cis with a β -thalassaemia determinant in one of the patient's parents (I.2). Indeed, analysis of haplotype segregation indicated that the maternal chromosome inherited by the non-thalassaemic daughter (II.2) does not carry HPFH and β -thalassaemia (normal Hb A₂ and Hb F levels, normal F cell count). Evidence has been provided that the heterocellular HPFH determinant is, at least in most cases, linked to the $\gamma\delta\beta$ globin gene cluster.⁴⁻⁶⁻⁸ Therefore, both

HPFH and β -thalassaemia determinants could be associated in cis in I.2. Alternatively, the normal Hb F and F cell count observed in subject II.2 may be accounted for assuming that (1) HPFH and β -thalassaemia determinants lie in trans in I.2 and (2) a recombination event between these determinants is responsible for the normal chromosome in II.2. Nevertheless, a number of families have been reported in which heterocellular HPFH determinant is linked in cis to a β -thalassaemia mutation, this situation being not uncommon in southern Italy.^{5,7} Thus, it may reasonably be suggested that both determinants are inherited together by the patient (II.1), although it is not possible to be positive, since the expression of $\delta\beta$ -thalassaemia trait would hide the haematological expression of HPFH (that is, increased Hb F level and F cell percentage). If this is the case, the almost complete suppression of any severe clinical manifestation in II.1 could be attributable, in the absence of α -thalassaemia, to the presence of heterocellular HPFH determinant in the β^+ -/ $\delta\beta$ -thalassaemia condition.

In fact, the determinant for heterocellular HPFH seems to act first by enhancing both the average F cell production in bone marrow and the level of the physiological response of the erythropoietic compartment to an erythropoietic stimulus. However, when the erythropoiesis is more stressed, it would also enhance the cellular γ chain synthesis in the erythroid precursors. The amount of Hb F produced in this way would be capable in homozygous β -thalassaemia or in other related thalassaemic conditions of reducing the globin chain synthesis imbalance in red cells and to reach haemoglobin (chiefly Hb F) levels only slightly below normal in peripheral blood. This would be obtained in the absence of severe bone marrow expansion or dramatic increase of the ineffective erythropoiesis.⁵

As far as $G\gamma/G\gamma + A\gamma$ ratios are concerned, a 0.290 ratio observed in the patient's father (I.1) is compatible with the $G\gamma/G\gamma + A\gamma$ ratio range found in southern Italian $\delta\beta$ -thalassaemia (0.142 to 0.317, unpublished data), but in the absence of $A\gamma^T$ chains it is not possible to conclude that the considerable γ chain output observed in patient II.1 is sustained by both chromosomes and to what degree.

It is worthy of note that in both members I.2 and II.1 the β -thalassaemic chromosome carries the *Hind* III $G\gamma^-$, *Hind* III $A\gamma^-$, *Ava* II β^+ , *Bam* HI β^- haplotype. This pattern has been reported in association with a G→A substitution in IVS-1 or IVS-2 5' splice junction, thus giving a β^0 -thalassaemia mutation.^{20,21} In subject II.1 the β -thalassaemia is inherited in trans to a $\delta\beta$ gene deletion, and yet there is some expression of Hb A (15.1%). Therefore, the

β -thalassaemia mutation appears to be a β^+ mutation. This β^+ -thalassaemic haplotype has never been described before. It could have arisen either by an independent mutation on the same haplotype or by crossing over within the $\gamma\delta\beta$ gene region, involving a different β^+ -thalassaemic haplotype.

This work was supported in part by CNR grant (contribute No 82.02429.04).

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Correspondence and requests for reprints to Dr Luciano Cianetti, Laboratorio di Ematologia, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma, Italy.