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Clinical and haematological evaluation of β thalassaemia intermedia characterised by unusually low Hb F and increased Hb A_2: β thalassaemia intermedia II

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SUMMARY A total of 15 patients from different families with thalassaemia intermedia was studied. Haematological studies showed that the fetal haemoglobin was only slightly raised, being between 2 and 11·5% of the total haemoglobin. Haemoglobin A_2 was high in all cases. The family study indicated that homozygosity or compound heterozygosity for β thalassaemia was present in five patients, while dominant inheritance was observed in three. In seven patients family studies were not sufficient to predict the genotype. Haematological findings in the parents of the homozygous patients were as severe as those seen in common Hb A_2 β thalassaemia traits. The decrease in MCH and MCV was more severe and the Hb A_2 higher in homozygous patients than in cases of common β thalassaemia major ($p<0·01$, $p<0·01$, and $p<0·001$ respectively). The imbalance in in vitro globin synthesis was more severe in classical β thalassaemia major than in homozygous patients in this study ($p<0·01$). However, the imbalance in α/non-α synthetic ratios showed variation among the homozygous and heterozygous patients in this study (2·1 to 4·0). Haematological severity and Hb F value showed some slight variation among affected persons of the same family in the case of patients with severe β thalassaemia heterozygosity. The $^{G}\gamma/^{A}\gamma$ ratio of haemoglobin F was found to be close to that of the adult level. Haematological studies suggested that clinical and haematological findings were more severe in patients with homozygous β thalassaemia than in patients with heterozygosity for β thalassaemia. The prevalence of thalassaemia intermedia with low Hb F and increased Hb A_2 was found to account for 3% of the Turkish β thalassaemic patients diagnosed before the age of 8 years.

A few patients with β thalassaemia intermedia or β thalassaemia major with unusually low fetal haemoglobin (Hb F) together with raised haemoglobin A_2 (Hb A_2) have been reported from several countries.[1–3] Heterozygosity for a severe β thalassaemia determinant has been shown in some of the cases.[4 5]

Homozygosity for Hb A_2 β^+ thalassaemia or compound heterozygosity for Hb A_2 β^+ thalassaemia and mild β^+ thalassaemia (A_2 normal β^+ thalassaemia or A_2 normal silent β^+ thalassaemia) determinants have been shown in some of these patients.[1–6]

We reviewed clinical and haematological findings of 180 patients with β thalassaemia major or β thalassaemia intermedia of different families. Fifteen of the 180 patients were found to have only slightly raised Hb F ($\leq$11·5%) and Hb A_2. Detailed haematological studies, including in vitro Hb synthesis and determination of the ratio of the γ chain which contains glycine at the 136th position ($^{G}\gamma$) and the one with an alanine residue at the same position ($^{A}\gamma$), were performed on patients and their family members to evaluate: (a) the prevalence of this type of β thalassaemia among thalassaemic patients; (b)

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TABLE 1 *Some of the clinical findings and haematological data of the patients.*

Patients	*Age* and sex*	*Growth retardation (HW)*	*Spleen (cm)*	*Age at splenectomy*	*Transfusion requirement*	*Peripheral† Smear NRBC*	*Hb (g/dl)*	*MCV (fl)*	*MCH (pg)*	*Hb A_2 (%)*	*Hb F (%)*	*In vitro Hb synthesis (α/non-α)*	*γ chain‡*			*Severity of disease§*
													$^{A}\gamma^{T}$	$^{G}\gamma$	$A\gamma^{I}$	
Group A																
Oz	7 M (6)	++	20	7	4	++++20	6·0	69	23	6·0	6·0	4·0	0	44	56	S
Oz	4 F (1·5)	++	7	—	—	++++ 5	5·0	71	18	4·0	10·4	—	—	—	—	S
Bi	19 M (13)	--	13	18	3	++++35	6·0	69	25	5·0	11·5	3·4	40	58	0	S
De	4 M (2·5)	++	8	—	2 per year	++++10	5·8	60	19	5·8	8·0	3·1	25	62	13	S
Le	8 M (6)	++	6	—	2	+++ 2	7·5	78	23	6·0	8·3	3·3	—	20	80	S–M
Ka	8 F (6)	++	6	—	—	+++ 3	6·0	57	19	5·2	2·7	2·7	24	66	10	S–M
Group B																
Us	28 M (26)	--	7	—	—	++++ 0	9·8	78	24	6·4	3·4	3·0	—	35	75	S–M
Ay	29 F (27)	--	8	—	—	++++ 5	11·7	75	23	6·8	2·0	2·0	—	20	80	S–M
Ce	7 M (7)	--	2	—	—	+++ 2	9·1	76	23	5·6	3·9	3·9	—	41	59	M
Group C																
El	19 F (16)	--	7	—	—	+++ 3	7·8	58	18	9·0	7·7	2·79	—	35	65	M
Av	32 M (30)	--	15	30	—	++++13	7·1	78	22	6·0	11·4	2·10	—	36	64	S–M
Al.Ak	6 M (6)	++	7	—	—	+++ 3	8·4	77	23	4·1	10·0	—	—	—	—	M
Kar	34 M (34)	--	?‖	26	1–2 per year	++++12	6·3	68	18	4·0	4·0	2·50	—	—	—	S
Po	28 M (26)	--	13	—	—	++++ 5	10·5	59	18	6·0	5·7	—	—	—	—	M
Ya	40 F (40)	--	12	40	—	+++ 0	7·5	—	—	4·7	4·1	3.50	—	20	80	M
Og	6 F (6)	++	13	—	—	++++ 5	5·4	68	18	6·0	6·2	—	—	—	—	S

*Age of patients at time of study. Figures in parentheses refer to age of diagnosis.
†RBC morphology. +++ indicates mild abnormalities, ++++ indicates severe abnormalities, NRBC: normoblasts per 100 WBC.
‡$^{A}\gamma^{T}$:Hb F variant of $^{A}\gamma$ which contains threonine at position 75 instead of isoleucine.
§S: severe, M: mild, S–M: severe-mild (see the text for criteria for severity of the disease).
?‖: splenectomy was performed before the first visit.

the mode of inheritance of the β thalassaemia-like condition; and (c) the haematological differences between these patients and those with β thalassaemia major, and the differences between standard Hb A_2 β thalassaemia heterozygotes and the parents of the patients of this study.

Patients

In the Haematology Units of Hacettepe University Hospital, during the period 1976 to 1982, 180 patients from different families aged between 4 months and 40 years were diagnosed as having β thalassaemia major (160 patients) or β thalassaemia intermedia (25 patients). All the 160 patients with β thalassaemia major and 13 of the 25 patients with β thalassaemia intermedia were under the age of 8 years when the diagnosis was made. Fifteen of them from unrelated families were found to have raised Hb A_2 and only slightly raised Hb F, which was less than 11·5% of the total haemoglobin in all cases. These 15 patients and their families are the subjects of this study. The clinical severity of the disease was recorded as (a) severe, (b) mild to severe, and (c) mild, according to the following parameters:

(1) Hb level $\leq$7 g/dl.

(2) The degree of abnormality in the red cell morphology (the designation ++++ indicates severe changes).

(3) The presence of nucleated red blood cells in the peripheral smear.

(4) A blood transfusion needed before the age of 10 years.

(5) Splenomegaly of $\leq$5 cm below the costal margin.

Patients with four of the above parameters were classified as severe cases, with three parameters as mild to severe, and as mild if less than three of the above parameters were present (table 1).

Methods

Routine haematological studies including red cell fragility tests were performed by standard methods. Serum iron, serum total iron binding capacity, and serum bilirubin levels were determined in all cases.[7] Starch gel electrophoresis was performed at pH 9·0. Hb A_2 was quantified by microcolumn chromatography and Hb F was measured by Betke's alkali denaturation method.[8 9]

Distribution of Hb F among the red cells was examined by the Kleihauer technique.[10] The $^{G}\gamma^{A}$ ratio of the Hb F was determined by high pressure liquid chromatography (HPLC) in some and by acid urea triton X-100 acrylamid gel electrophoresis (AUTAGE) in all of the subjects.[11 12] Since δ chains move with $^{G}\gamma$ in the latter system, Hb A_2 was removed from the haemolysate by column chromatography before application of the sample to the gel in order to determine the $^{G}\gamma$:$^{A}\gamma$ ratio. In vitro haemoglobin synthesis analyses were performed by previously published methods.[13]

Results

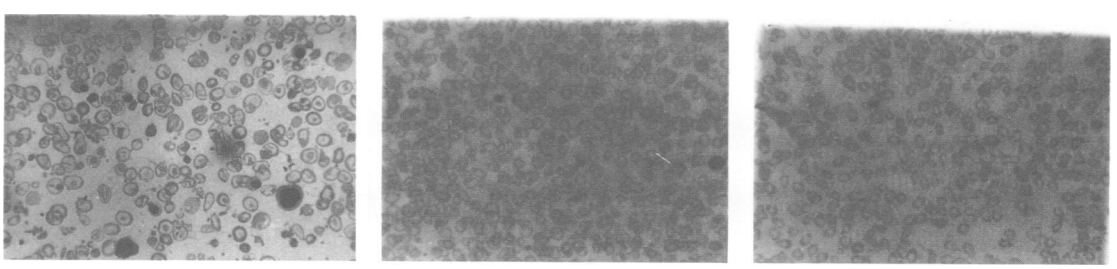
The patients were divided into three groups according to the mode of genetic inheritance of the disease.

Group A. This group comprised six patients from five different families with homozygous β thalassaemia or compound heterozygosity for two different β thalassaemia determinants.

Group B. This group comprised three patients from three different families with severe heterozygous β thalassaemia.

Group C. This group comprised seven patients; the family studies of these seven were not sufficient to predict the genotype.

The clinical findings of the patients are summarised in table 1. The peripheral smears of some of the patients are shown in fig 1. The haematological



(a) *(b)* *(c)*

FIG 1 *Peripheral smear from (a) a patient with homozygous β thalassaemia, (b) a severe heterozygote, and (c) one of the patients with unidentified genetic transmission.*

data of the patients and their parents are shown in tables 1 and 2 and figs 2, 3, 4, and 5. The serum saturation of iron was normal or increased in all the persons examined.

The distribution of Hb F among the red cells was heterogeneous in all the subjects and the percentage of red cells containing Hb F was usually two to three times higher than the percentage Hb F value found by alkali denaturation.

Statistical analysis of some of the haematological values of the patients and their parents are shown in table 3.

Discussion

The five patients from four different families in group A are homozygous for Hb A_2 β^+ thalassaemia, which is indicated by the haematological abnormalities comparable with Hb A_2 β thalassaemia heterozygosity in both parents (fig 2a, b, table 2).

In family Ka the haematological findings of the father are within normal limits except for a slight imbalance in the α/non-α ratio (table 2). This indicates that he is most likely to be a heterozygote

TABLE 2 *Haematological data of the parents and sibs of the patients.*

Family	*Hb (g/dl)*	*PCV (l/l)*	*RBC (10^{12}/l)*	*MCV (fl)*	*MCH (pg)*	*Hb A_2 (%)*	*Hb F_{AD} (%)*	*α/non-α*	*γ chain*		
									$A_\gamma T$	*G_γ*	*$A_\gamma I$*
Group A											
Oz											
Father	14·80	0·40	5·63	71	25	4·0	0·9	2·10	—	—	—
Mother	11·80	0·34	5·29	65	22	3·9	1·0	1·85	—	—	—
De											
Father	12·39	0·39	5·09	69	24	4·3	0·6	1·81	—	—	—
Mother	10·80	0·35	5·18	67	20	4·2	0·7	2·12	19	24	57
Le											
Father	15·35	0·47	5·40	87	28	5·0	0·9	1·95	—	—	—
Mother	12·39	0·36	4·30	83	28	4·7	0·9	1·30	—	30	70
Sib	12·00	0·36	4·80	86	25	3·3	1·0				
Ka											
Father	13·50	0·43	5·77	80	23	2·0	0·7	1·35	—	35	65
Mother	11·80	0·32	4·79	68	21	5·7	0·7	2·10	—	—	—
Sib	12·39	0·39	5·80	72	21	3·7	0·7				
Bi											
Father	13·00	0·42	6·00	72	22	3·9	1·6	1·38	—	—	—
Mother	9·50	0·31	4·28	78	23	4·0	0·8	1·38	—	—	—
Group B											
Us											
Mother	12·60	0·38	5·60	72	20	3·6	0·7	1·80	—	15	85
Wife	12·63	0·38	4·30	86	33	2·0	0·7	1·00			
Brother	14·23	0·43	—	—	—	4·0	0·6	—			
Daughter	10·10	0·31	4·98	70	21	4·0	1·0	2·50			
Ce											
Father	15·55	0·47	5·32	88	29	2·5	0·6	1·10			
Mother	11·25	0·35	6·28	73	18	5·2	2·6	2·30	—	40	60
Sib	11·51	0·34	6·38	71	16	5·6	2·6	2·30			
Ay											
Daughter	11·10	0·41	4·66	89	24	2·4	0·9	1·10			
Group C											
El											
I.1	—	—	—	—	—	3·0	0·5				
I.2	11·9	0·35	5·07	79	23	4·0	0·5				
Ak											
I.1	11·50	0·34	5·98	69	19	4·6	3·0				
I.2	—	—	—	—	—	3·0	0·5				
Og											
I.1	8·15	0·29	3·98	66	20	7·0	5·0				
I.2	12·79	0·39	4·60	88	27	3·5	0·5				
Ka											
III.2	—	—	—	—	—	3·7	2·0				
III.3	—	—	—	—	—	3·8	2·5				

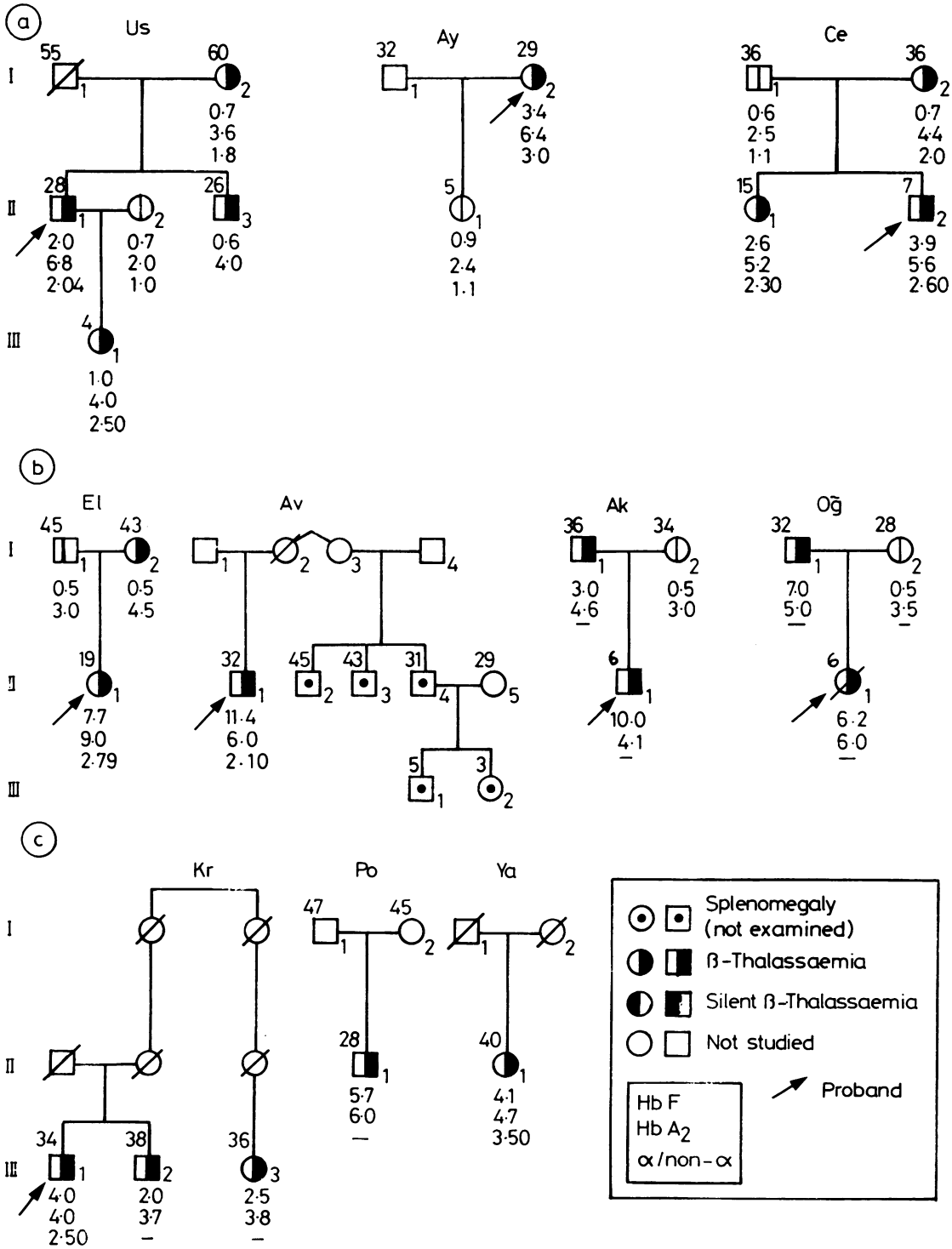


FIG 2 *Pedigree of the patients. (a) Group B heterozygotes. (b, c) Group C patients with insufficient family study.*

TABLE 3 *Statistical analysis of some of the haematological data.*

Groups	*Hb (g/dl)*	*PCV (l/l)*	*RBC (10^{12}/l)*	*MCV (fl)*	*MCH (pg)*	*Hb A_2 (%)*	*Hb F_{AD} (%)*	*In vitro Hb synthesis (α/non-α)*
A Homozygous patients	6·03±0·82 (6)	0·21±0·03 (6)	2·95±0·68 (6)	67±7 (6)	21±2·8 (6)	5·33±0·28 (6)	8·28±2·36 (6)	3·3±0·47 (5)
B β thalassaemia major	6·09±2·02 (96)	0·19±0·05 (96)	2·6±0·85 (96)	76±10 (96)	25±5 (96)	3·01±1·43 (96)	52·00±20 (96)	4·0±0·2 (10)
C Parents of homozygous patients	12·57±1·69 (10)	0·38±0·06 (10)	5·17±0·58 (10)	74±7·5 (10)	23·6±2·72 (10)	4·17±0·96 (10)	0·86±0·3 (10)	1·73±0·34 (10)
D Common carrier	12·13±1·41 (137)	0·37±0·04 (137)	5·22±0·87 (137)	71±7 (137)	23±3·11 (137)	5·09±0·95 (137)	1·08±1·0 (137)	2·00±0·2 (10)
Homozygous patients vs thalassaemia major	p>0·50	p>0·10	p>0·50	p<0·01	p<0·01	p<0·001	p<0·001	p<0·02
Homozygous patients vs their parents	p<0·001	p<0·01	p<0·001	p>0·1	p>0·5	p>0·5	p<0·001	p<0·001
Parents of homozygotes vs patients/common carriers	p>0·10	p>0·5	p>0·5	p>0·10	p>0·10	p<0·01	p>0·10	p<0·05

for Hb A_2 normal type 1 β^+ thalassaemia. Thus the proband (Se Ka) is a compound heterozygote for Hb A_2 β thalassaemia and silent β^+ thalassaemia.

The presence of increased α chain production rather than α chain deficiency was suggested in persons with 'silent' β thalassaemia and, later, the presence of three α globin genes instead of two on one of the homologous chromosomes 16 was described by Higgs *et al.*[14] Some of the subjects with type 1 β thalassaemia from Greece and Turkey did not show any abnormalities in the α, β, or γ gene regions of the haemoglobin, indicating that the presence of an increased number of α chain genes is

FIG 3 *Distribution of Hb in patients and their parents or offspring.*

FIG 4 *Distribution of Hb A_2 level in patients and their parents or offspring.*

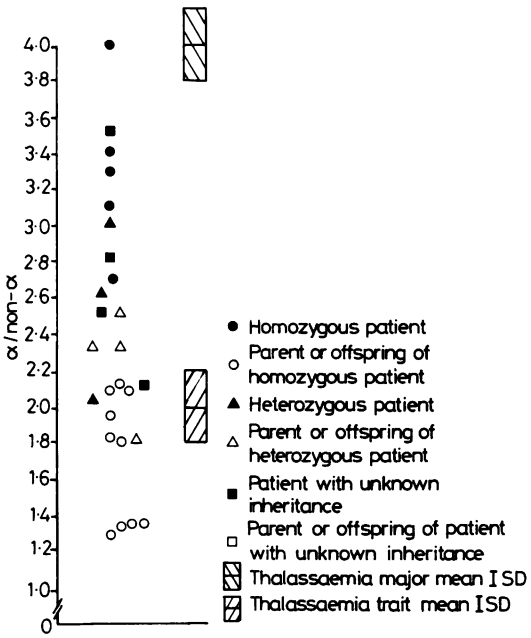


FIG 5 *α/non-α ratio of in vitro globin synthesis obtained from peripheral reticulocytes and normoblasts.*

not common in cases with a silent β thalassaemia-like picture.[15] In vitro haemoglobin synthesis analysis did not suggest the coexistence of α thalassaemia in the heterozygotes of group A (table 1). This is in agreement with the fact that α thalassaemia is not common in Turkey and thalassaemia intermedia resulting from the coexistence of α thalassaemia with homozygous β thalassaemia is likely to be relatively uncommon.

Statistical analysis of group A indicated that the mean value of Hb A_2 was higher, and the imbalance in α/non-α ratio more severe, in classical β thalassaemia traits than in those obtained from the parents of this study (table 3). However, when the father of Ka family with normal Hb A_2 silent β thalassaemia was excluded from the calculations the differences became insignificant. This indicates that if there is a difference between the β thalassaemia determinants, this is not reflected in the haematological parameters of the heterozygotes.

The decrease in MCV and MCH values was more severe and Hb A_2 values were higher in our patients in group A than in those patients with classical β thalassaemia major (table 3, fig 4). These changes may be related to the presence of fewer cells of fetal origin. Similar observations have been made by Tamagnini *et al.*[16] In group B (families Us, Ay, and Çe), dominant inheritance of β thalassaemia determinant (severe β thalassaemia heterozygosity) was observed (fig 2a, table 2).

Clinical and haematological expression of the disease was more severe in the proband in the Us family than in the mother (I.2), the brother (II.3), and his offspring (fig 2a, table 2). In the Ce family, the Hb F value was higher in II.1 and II.2 than in the mother (fig 2a). Splenomegaly was absent in the affected family members other than the probands of these two families. Diversity in clinical expression and Hb F level between the generations has been previously reported in cases with severe heterozygosity for β thalassaemia.[4]

In the Ay family the possibility of the proband being homozygous for β thalassaemia is excluded by the absence of any thalassaemic features in the offspring (fig 2a, table 2).

Increased α chain synthesis was shown in some of the patients with similar conditions. However, according to Weatherall *et al,*[1] the presence of hypochromia in affected subjects of group B of this study may exclude this possibility.

The presence of a heterocellular HPFH-like determinant in cis or in trans of the β thalassaemia determinant was excluded because Hb F values have been reported to be higher in those with compound heterozygosity for β thalassaemia and HPFH than the value obtained from our patients. Secondly, imbalance in in vitro haemoglobin synthesis is expected to be milder in compound heterozygotes for β thalassaemia and heterocellular HPFH than was the case with our patients.[17]

In group C, family studies were not sufficient to predict the genotype of seven patients. In the El family (fig 2b, table 2) haematological studies indicated that the mother was the carrier for typical Hb A_2 β thalassaemia. Haematological study of the father excluded the possibility of his being a carrier of classical Hb A_2 β thalassaemia.

The presence of many persons with splenomegaly in the Av family, and members with the haematological findings similar to those of the patients in the Kr, Ak, and Og families, may suggest the presence of severe heterozygous β thalassaemia in these families.

Haematological and clinical findings seem to be more severe in patients with homozygosity for a β thalassaemia determinant than in those with heterozygosity for a severe β thalassaemia determinant (table 3, figs 3 and 5).

The possibility of the presence of a silent β chain mutant like Hb Knossos was excluded by the absence of an abnormal β chain in AUTAGE in all patients.[18]

The mild β^+ thalassaemia determinant of the patients in group A is different from that of the African type β^+ thalassaemia.[19] This is indicated by the presence of a severe thalassaemic picture together with very low Hb F levels observed in our patients.

Statistical analysis has shown that the mean imbalance in chain synthesis was found to be significantly higher in patients with β thalassaemia major than homozygous patients observed in this study. However, in some of the patients, the imbalance in chain synthesis was as severe as in those with β thalassaemia major (table 1). The cause of the variation in severity of the imbalance in the α/non-α ratio among the patients needs to be explained and the question of the absence of reactivation of the α chain, even in the presence of severe imbalance in the α/non-α synthetic ratio, remains to be solved.

The $^{G}\gamma/^{A}\gamma$ ratio of Hb F was found to be at the adult level in eight out of the 11 patients. This finding suggests that the fetal type of reactivation of γ chain synthesis, which is usually observed in β thalassaemia, is absent in our patients.

The coexistence of a γ thalassaemia might be possible. Examination of the patients with this genotype during the newborn period and cloning and mapping studies of the γ and β regions would help to verify this hypothesis.

The frequency of $^{A}\gamma^{T}$ was calculated as 70% in the patients and the heterozygotes; this is the expected figure for those with β thalassaemia from the Mediterranean basin.[20]

Recent studies have shown that β thalassaemia with low Hb F levels has a common haplotype and a single base mutation position 6 of the first intervening sequence of the β genes.[16 21] The similarities of the clinical and haematological findings, including increased MCH, in our heterozygotes and those with the Portuguese type of β^+ thalassaemia indicate that our patients in group A may have a similar molecular abnormality and that this type of thalassaemia intermedia is probably not confined to Portugal but exists in other Mediterranean countries, as suggested by Traenges *et al.*[22]

Mapping and cloning studies may also be valuable in the differentiation of patients with β thalassaemia intermedia with various genetic inheritances.

The total number of patients with β thalassaemia intermedia characterised by slightly increased Hb F and raised Hb A_2 appears quite high owing to the selective survival of this type of patient over patients with β thalassaemia major. The prevalence of this type of β thalassaemia intermedia is around 3% when only those patients with β thalassaemia major and β thalassaemia intermedia who were diagnosed before the age of 8 years are taken into consideration.

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References

1. Weatherall DJ, Clegg JB. *The thalassaemia syndromes*. 3rd ed. Oxford: Blackwell Scientific Publications, 1981.
2. Aksoy M, Erdem S. The thalassaemia syndromes. V. Cooley's anaemia with low level of fetal haemoglobin. A genetic study in four families. *Acta Haematol* 1965;**34**:291–300.
3. Zago MA, Costa FF, Bottura C. β^+-thalassaemia intermedia with low Hb F. *Klin Wochenschr* 1983;**61**:95–8.
4. Friedman SH, Özsoylu Ş, Luddy R, Schwartz E. Heterozygous beta thalassaemia of unusual severity. *Br J Haematol* 1976;**32**:65–77.
5. Stamatoyannopoulos C, Woodson R, Papayonnopoulou T, Heywood D, Kurachi S. Inclusion body β-thalassaemia trait. A form of β-thalassaemia producing clinical manifestations in simple heterozygotes. *N Engl J Med* 1974;**290**:939.
6. Aksoy M, Bermek E, Almis G, Kutlar A. β-thalassaemia intermedia homozygous for normal A_2 β-thalassaemia. Study in four families. *Acta Haematol* 1982;**67**:57–61.
7. Wintrobe MW, Lee RC, Boggs DB, Bithell TC, Athens JW, Foerster J. *Clinical hematology*. 7th ed. Philadelphia: Lea and Febiger, 1974.
8. Huisman THJ, Jonxis JHP. *The haemoglobinopathies: techniques of identification*. New York: Marcel Dekker, 1977.
9. Betke K, Marti HR, Schlicht J. Estimation of small percentages of foetal hemoglobin. *Nature* 1959;**184**:1877–8.
10. Kleihauer E, Braun H, Betke K. Demonstration von fetalem hemoglobin in der erythrozyten eines blut ausstriches. *Klin Wochenschr* 1957;**35**:637–8.
11. Huisman THJ, Altay C, Webber B, *et al.* Quantitation of three types of chain of Hb F by high pressure liquid chromatography: application of this method to Hb F patients with sickle cell anemia or the S-HPFH condition. *Blood* 1981;**57**:75–82.
12. Efremov GD, Markovska B, Stonovski N, Petkov G, Nikolov N. The use of globin chain electrophoresis in polyacrylamid gels for the quantitation of the $^{G}\gamma$ to $^{A}\gamma$ ratio in fetal hemoglobin. *Hemoglobin* 1981;**5**:637–6.
13. Weatherall DJ, Clegg JB, Naughton MA. Globin synthesis in thalassaemia: an in-vitro study. *Nature* 1965;**208**:1061–5.
14. Higgs DR, Old JM, Pressley L, Clegg JB, Weatherall DJ. A novel α globin gene arrangement in man. *Nature* 1980;**284**:632–5.
15. Kanavakis E, Metaxotou-Mavromati A, Kattamis C, Aksoy M, Weatherall DJ, Wood WG. Globin gene mapping in normal Hb A_2 types of β-thalassaemia. *Br J Haematol* 1982;**51**:59–64.
16. Tamagnini GP, Lopes MC, Castanheira ME, Wainscoat JS, Wood WG. β^+ thalassaemia—Portuguese type: clinical, haematological and molecular studies of a newly defined form of thalassaemia. *Br J Haematol* 1983;**54**:189–200.
17. Soummer AM, Testa U, Dujardin P, *et al.* Genetic regulation of γ gene expression: study of the interaction of β-thalassaemia with heterocellular HPFH. *Hum Genet* 1981;**57**:371–5.
18. Fessas P, Loukopoulos D, Loutradi-Anognostou A, Komis K. Silent β-thalassaemia caused by a "silent" β-chain mutant. The pathogenesis of a syndrome of thalassaemia intermedia. *Br J Haematol* 1982;**51**:577–83.
19. Willcox MC, Weatherall DJ, Clegg JB. Homozygous β thalassaemia in Liberia. *J Med Genet* 1975;**12**:165–73.

[20] Huisman THJ, Schroeder WA, Reese A, *et al.* The Tγ chain of human fetal hemoglobin at birth and in several abnormal hematologic conditions. *Pediatr Res* 1977;**11**:1102–5.

[21] Wainscoat JS, Old JM, Weatherall DJ, Orkin SH. The molecular basis for the clinical diversity of β thalassaemia in Cypriots. *Lancet* 1983;**i**:1235–7.

[22] Traenges C, Sortino G, Cacciola R, Lombardo T, Cacciola E, Labie D. Haplotype VI associated mild β⁺-thalassaemia in Sicily: Mediterranean type? *Lancet* 1983;**ii**:509.

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