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Clinical and haematological evaluation of β thalassaemia intermedia with increased Hb F and Hb A_2 in heterozygotes: β thalassaemia intermedia I

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SUMMARY Family studies were performed in 10 patients from seven different families with homozygous β° thalassaemia intermedia and in three patients with homozygous β^+ or compound heterozygous β^+ and β° thalassaemia intermedia. In nine of the 10 families at least one of the parents was found to have raised Hb A_2 and Hb F. In the heterozygotes with increased Hb A_2 and Hb F, the means of Hb F and MCV were significantly higher than those observed in regular Hb A_2 thalassaemia heterozygotes. However, the severity of imbalance in in vitro haemoglobin synthesis was similar in these two groups. The imbalance in the α/non-α synthetic ratio was heterogeneous in the patients, being 2·1 and 4·0. Segregation of the raised Hb F from the Hb A_2 β thalassaemia determinant was found to be possible in only one of the 36 heterozygotes. This may exclude the possibility of the presence of an additional determinant responsible for the activation of the γ chain. The $^{G}\gamma/^{A}\gamma$ ratio of Hb F was that of the fetal type ($^{G}\gamma$ was between 50 and 71% of the total γ chain). The $^{A}\gamma^{T}$ variant of γ chain was not detected in cis of the β° thalassaemia determinant characterised by increased Hb F and Hb A_2. A retrospective study of 180 patients with β thalassaemia and their parents indicated that the combined rise in Hb A_2 and Hb F was more common in the heterozygous parents (11 out of 30 parents) of the patients with β° thalassaemia than it was in the parents of patients with β^+ thalassaemia (three out of 140 parents). The presence of increased Hb A_2 and Hb F in the heterozygote may in some cases determine the relative mildness of the disease.

β thalassaemia intermedia results from various genetic combinations such as (a) homozygosity for mild β thalassaemia of the African type, silent β thalassaemia (A_2 normal β thalassaemia type 1), and δβ thalassaemia; (b) compound heterozygosity for one of the above β thalassaemia determinants and a severe β thalassaemia determinant (β^+ or β°); (c) compound heterozygosity for one of the severe β thalassaemia determinants and a structural variant of the β chain of haemoglobin with a reduced synthesis rate;[1–4] (d) α thalassaemia coinciding with homozygous β thalassaemia; and (e) the interaction of a severe β thalassaemia determinant with a condition of increased α chain production due to the presence of an excess of α chain genes on one chromosome 16.[5 6]

A mild form of β° thalassaemia determinant characterised by increased haemoglobin F (Hb F) and haemoglobin A_2 (Hb A_2) in heterozygotes from a Dutch family has been reported by Schokker *et al.*[7] Since then there have been several other reports from various countries of similar conditions.[7–10] Family studies in connection with some of these subjects with increased Hb F and Hb A_2 indicated the presence of compound heterozygosity for β thalassaemia and heterocellular hereditary persistence of fetal haemoglobin (HPFH) determinants.[11 12]

A clinical and haematological review of our 180 thalassaemic patients and their parents disclosed the presence of 13 patients in 10 different families with thalassaemia intermedia with increased Hb F. Patients from seven families had β° thalassaemia and those from three had β^+ thalassaemia (homozygous β^+ thalassaemia or compound heterozygosity for β^+/β° thalassaemia could not be distinguished). Increased Hb F and Hb A_2 were present in one or both parents in all but one of these 10 families.

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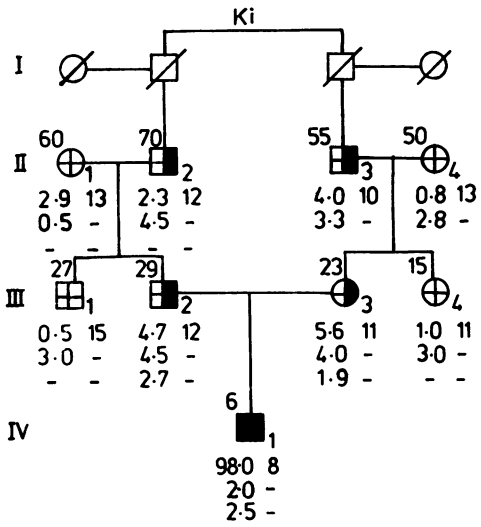

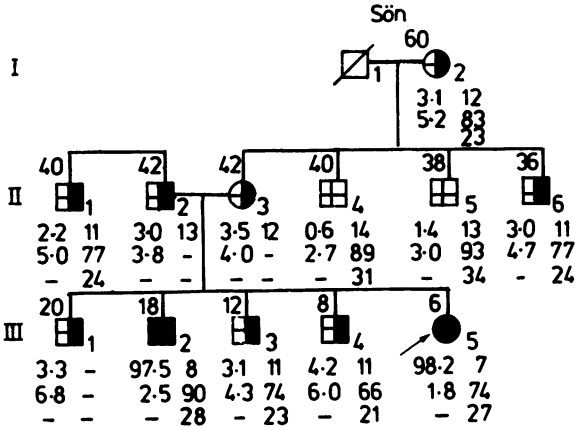

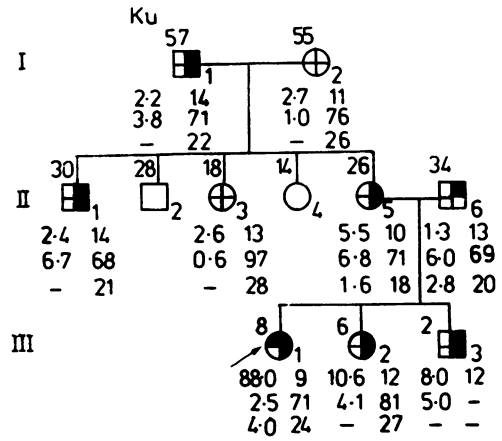

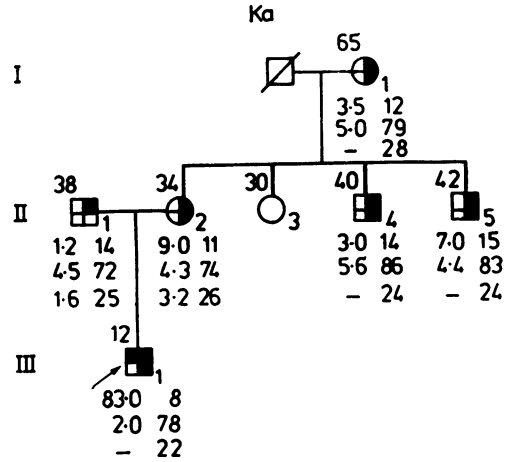

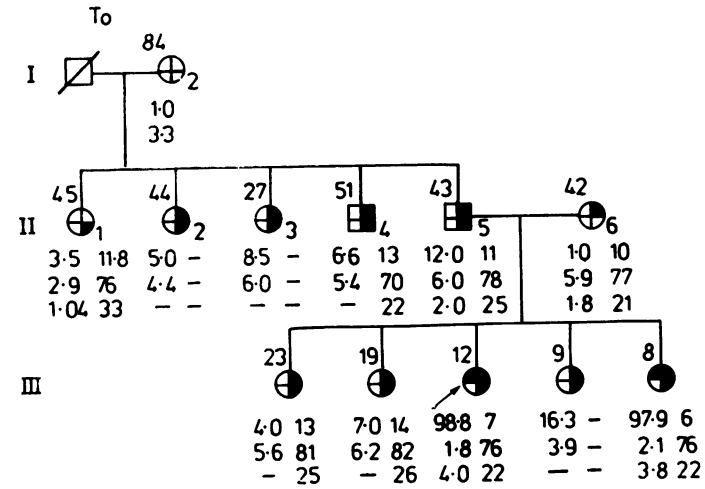

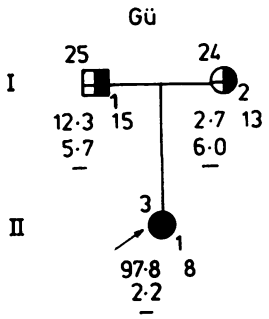

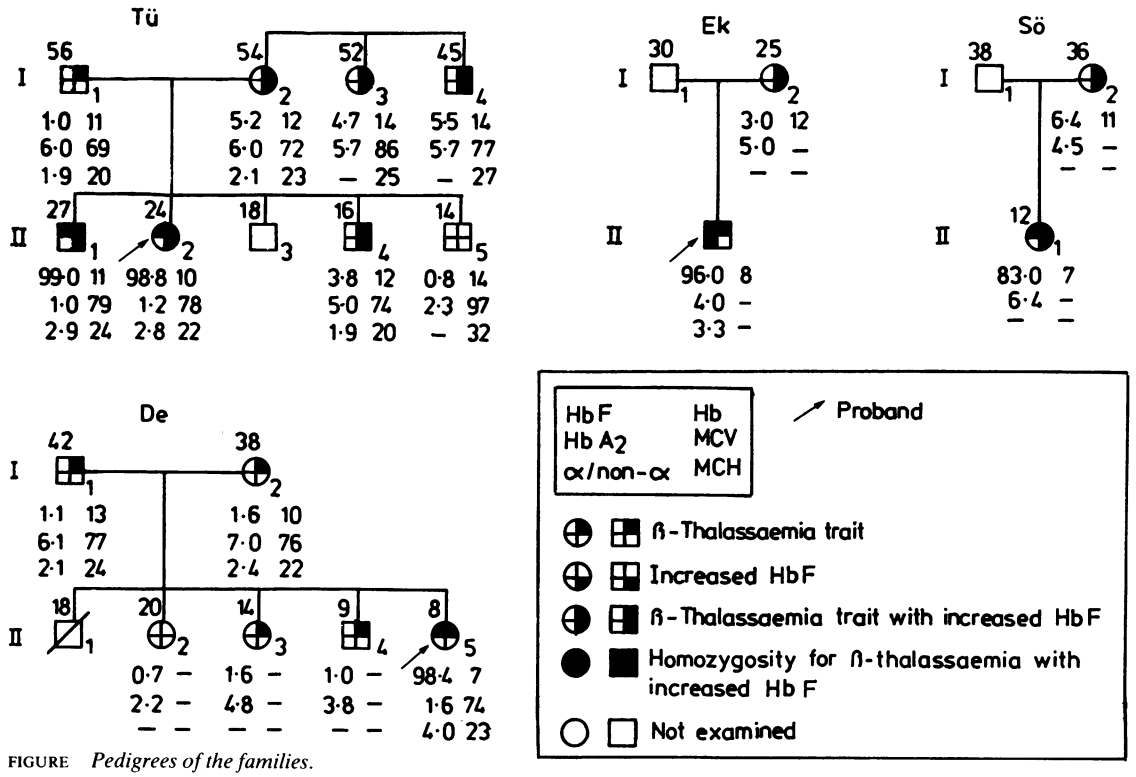

FIGURE *Pedigrees of the families.*

Detailed family studies were made in order to evaluate whether increased Hb F and Hb A_2 are the haematological expression of a single β thalassaemia determinant or compound heterozygosity for β thalassaemia and heterocellular HPFH. We concluded that in most cases an additional determinant for HPFH was not present.

Patients

During the period 1977 to 1982 thalassaemia intermedia with increased Hb F (Hb F ≥50) was diagnosed in 13 patients from 10 families attending our clinic. These families are the subjects of this study. In eight families both parents, and in the remaining two only the mothers, were available for study. In four families (Tu, To, Ki, and De) there were consanguineous marriages.

Methods

Routine haematological studies were done by standard methods.[13] Starch gel electrophoresis of the haemolysate was performed at pH 9·0.[14] The Hb A_2 level was measured by microcolumn chromatography.[14] The Hb F was determined by Betke's alkali denaturation and the distribution of Hb F among the red cells was examined by Kleihauer's technique.[15 16]

The $^{G}\gamma/^{A}\gamma$ ratio was measured and the frequency of $^{A}\gamma^{T}$ was determined by high pressure liquid chromatography (HPLC) and acid urea triton X-100 acrylamid gel electrophoresis (AUTAGE) in 11 persons from the five families.[17 18] Hb A_2 was removed from the haemolysate of the heterozygotes by column chromatography to eliminate δ chain contamination of the $^{G}\gamma$ region before the application of the sample to the gel.

In vitro haemoglobin synthesis analysis was performed by previously established methods.[19]

Results

The study of 180 patients with homozygous β thalassaemia and their parents showed that 158 patients had β^+ thalassaemia and 22 patients had β° thalassaemia.

A combined rise in Hb A_2 and Hb F was found in only three out of 140 parents of the 158 patients with β^+ thalassaemia, but in 11 out of 30 parents of 22 patients with β° thalassaemia. Ten of the patients

TABLE 1 *Clinical evaluation of the patients and some of the haematological findings of the patients and parents.*

Families	*Age* and sex*	*Growth retardation (HW)*	*Bone changes (on x-ray)*	*Splenomegaly (cm)*	*Age at splenectomy (y)*	*Transfusion requirement*		*F cell (%)*	*γ chain*			*Genotype‡*
						Before (splenectomy)	*After*		$^{A}\gamma^{T}$	$^{G}\gamma$	$A\gamma^{I}$	
1. Tü												
Proband	27 M (24)	−−	Severe	20	24	None	None	100	−†	70	30	$A_2F\beta^{\circ}/A_2\beta^{\circ}$
Sib	25 F (22)	−−	Severe	25	23	Total 3	None	100				$A_2F\beta^{\circ}/A_2\beta^{\circ}$
Mother								18	−	50	50	$A_2F\beta^{\circ}/\beta$
Father								3	0†	41	59	$A_2\beta^{\circ}/\beta$
2. To												
Proband	12 F (6)	++	Severe	10	8	Total 4	Rare	100	0	73	27	$A_2F\beta^{\circ}/A_2\beta^{\circ}$
Sib	8 F (4)	++	Severe	18	6	None	—	100	0	70	30	$A_2F\beta^{\circ}/A_2\beta^{\circ}$
Mother								3	0	46	54	$A_2\beta^{\circ}/\beta$
Father								30	0	71	29	$A_2F\beta^{\circ}/\beta$ or $A_2F\beta^{\circ}$/HPFH β^{+}
3. Sö												
Proband	6 F (6)	++	Mild	6	—	None	—	100	0	78	22	$A_2F\beta^{\circ}/A_2F\beta^{\circ}$
Sib	18 M (18)	++	Mild	(−)	12	None	None	100	—	60	40	$A_2F\beta^{\circ}/A_2F\beta^{\circ}$
Mother								11	0	71	29	$A_2F\beta^{\circ}/\beta$
Father								8	0	55	45	$A_2F\beta^{\circ}/\beta$
4. Ki												
Proband	6 M (4)	++	Mild	8	5	Total 4	None					$A_2F\beta^{\circ}/A_2F\beta^{\circ}$
Mother									—	40	60	$A_2F\beta^{\circ}/\beta$
Father									—	44	56	$A_2F\beta^{\circ}/\beta$
5. Gül												
Proband	3 M (2)	++	Mild	3	—	None	—	100				$A_2F\beta^{\circ}/A_2F\beta^{\circ}$
Mother								8				$A_2F\beta^{\circ}/\beta$
Father								25				$A_2F\beta^{\circ}/\beta$
6. Ek												
Proband	5 M (4)	++	Mild	10	—	Total 3	—	100	0	56	44	$A_2F\beta^{\circ}/A_2\beta^{\circ}$ or $A_2F\beta^{\circ}/A_2F\beta^{\circ}$
Mother								12	12	56	32	$A_2F\beta^{\circ}/\beta$
7. De												
Proband	8 F (6)	++	Mild	6	—	None	—	100	—	70	30	$A_2\beta^{\circ}/A_2\beta^{\circ}$
Mother								7				$A_2\beta^{\circ}/\beta$
Father								3				$A_2\beta^{\circ}/\beta$
8. Ka												
Proband	12 M (8)	−−	Mild	6	—	None	—	90	—	60	40	$A_2F\beta^{\circ}/A_2\beta^{+}$ or $A_2F\beta^{+}/A_2\beta^{+}$ or $A_2F\beta^{+}/A_2\beta^{\circ}$
Mother								17				$A_2F\beta^{\circ}/\beta$ or $A_2F\beta^{+}/\beta$
Father								3				$A_2\beta^{+}/\beta$ or $A_2\beta^{\circ}/\beta$
9. Ku												
Proband	8 F (6)	−−	Severe	14	6	None	—	95	—	65	35	$A_2F\beta^{\circ}/A_2\beta^{+}$ or $A_2F\beta^{+}/A_2\beta^{+}$ or $A_2F\beta^{+}/A_2\beta^{\circ}$
Mother								15	—	58	42	$A_2F\beta^{\circ}/\beta$ or $A_2F\beta^{+}/\beta$
Father								5				$A_2\beta^{\circ}/\beta$ or $A_2\beta^{+}/\beta$

TABLE 1—*continued.*

10. Sög										
Proband	11 F (11)	--	Mild	4	—	None	—	93	0	68	32	$A_2F\beta^{\circ}/A_2\beta^{+}$ or $A_2F\beta^{+}/A_2\beta^{+}$ or $A_2F\beta^{+}/A_2\beta^{\circ}$
Mother								20				$A_2F\beta^{-}/\beta$ or $A_2F\beta^{+}/\beta$

*Age of patients at time of study. Figure in parentheses indicates age at time of diagnosis.
†(—) γ chain analysis made by AUTAGE and the presence of $^{A}\gamma^{T}$ was not known. (0) γ chain analysis was performed by HPLC and $^{A}\gamma^{T}$ was not present.
‡β: normal gene, $A_2F\beta^{\circ}$:β° thalassaemia with raised Hb F and Hb A_2 present in the heterozygotes. $A_2\beta^{\circ}$: common β° thalassaemia with raised Hb A_2 in heterozygotes. $HPFH\beta^{+}$: β chain production is present in cis of HPFH.

TABLE 2 *Haematological findings and some statistical analysis of the patients and carriers.*

Groups	*Hb (g/dl)*	*PCV (l/l)*	*RBC (10^{12}/l)*	*MCV (fl)*	*MCH (pg)*	*A_2 (%)*	*F (%)*	*α/non-α*
A Present patients	8·09±1·32 (13)*	0·26±0·03 (13)	3·40±0·74 (9)	75±5 (9)	23±2·3 (9)	2·16±0·82 (13)	95±0·03 (13)	3·49±0·62 (9)
B β thalassaemia major patients known to our institution†	6·09±2·02 (96)	0·19±0·05 (96)	2·60±0·85 (96)	76±10 (96)	25±5 (96)	3·01±1·43 (96)	52±20 (96)	4·0±0·2 (10)
C Heterozygotes with increased Hb F and Hb A_2 in this study	12·20±1·43 (32)	0·38±0·06 (32)	5·17±0·70 (21)	76±5·78 (21)	23±2·48 (21)	5·10±0·90 (36)	5·43±3·28 (36)	2·16±0·61 (5)
D A_2 β thalassemia heterozygotes in this study	11·73±1·56 (6)	0·38±0·03 (6)	5·27±0·55 (6)	73±3·83 (6)	22±2·10 (6)	5·92±0·80 (6)	1·20±0·23 (6)	2·10±0·44 (6)
E Classical β thalassaemia heterozygotes	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)	1·30±0·23 (137)	2·00±0·10 (10)
Group A vs group B	p<0·01	p<0·001	p<0·01	p>0·5	p<0·05	p<0·01	p<0·001	p<0·01
Group C vs group D	p>0·5	p>0·5	p>0·5	p>0·5	p>0·5	p<0·05	p<0·001	p>0·5
Group C vs group E	p>0·5	p>0·5	p>0·5	p<0·001	p>0·5	p>0·5	p<0·001	p>0·5
Group D vs group E	p>0·5	p>0·5	p>0·5	p>0·5	p>0·5	p<0·02	p>0·5	p>0·5

*Figures in parentheses indicate the number of patients.
†In some of the patients the haematological values were obtained after blood transfusion.

with β° thalassaemia proved to have thalassaemia intermedia and three of the patients with β^{+} thalassaemia with raised Hb F had thalassaemia intermedia. The clinical and haematological data on these families with thalassaemia intermedia are given in table 1 and in the figure.

The group was to some extent heterogeneous and the families were divided into three groups as follows.

(1) In the De family (family 7) neither parent carried increased Hb F or Hb A_2.

(2) In the To family (family 2) segregation of the high Hb F from the high Hb A_2 was present. In addition, this family showed the highest haemoglobin F levels in the heterozygotes. One of the parents carried a common Hb A_2 β° thalassaemia determinant.

(3) (a) Raised Hb F and Hb A_2 was present in both parents in three of the eight remaining families (families 3, 4, and 5) and in all these families there was β° thalassaemia. (b) Of the three families only one parent had raised Hb F and Hb A_2; the other parent in each case had the common Hb A_2 β° thalassaemia and two had Hb A_2 β^{+} thalassaemia. (c) In two families only one parent was available for study; in both, this one parent was found to have raised Hb A_2 and Hb F.

The proposed genotypes of these families are given in table 1. Statistical analyses of the patients and of their parents are given in table 2.

Discussion

Mild β° thalassaemia with raised Hb A_2 and Hb F in heterozygotes was first reported in a Dutch family by Schokker *et al.*[7] Since then there have been several reports concerning similar conditions from various countries.[8–10] In some of them the presence of heterocellular HPFH determinants in addition to homozygosity for β° thalassaemia was reported.[9 10]

Raised Hb A_2 and Hb F were present in at least one of the parents in nine out of the 10 families used in this study of β thalassaemia intermedia, suggesting that the clinical and haematological mildness of β° or β^{+} thalassaemia in our patients is closely related to the genetic mechanism which causes an increase in Hb F synthesis (table 1, figure). However, the group is to some extent heterogeneous. In family De (family 7), where neither parent has increased Hb F plus A_2, this indicates that β° thalassaemia intermedia determinants are not always characterised by increased Hb F in the heterozygote.

Extensive examination of the members of the To family disclosed only one person (II.1, figure) in whom raised Hb F did not coincide with raised Hb A_2. The Swiss type HPFH determinant may also be present in addition to the β° thalassaemia determinant characterised by increased Hb A_2 and Hb F. This assumption may also explain the range of Hb F levels among persons in the second generation of the family. Subject II.5 with high Hb F could be a compound heterozygote for the Swiss type HPFH and a β° thalassaemia determinant of increased Hb A_2 and Hb F, while II.2, II.3, and III.4 possess the latter determinant, II.1 having the HPFH determinant only. However, no data were available from I.1 and I.2 to verify this assumption.

Among the heterozygotes of the remaining families, raised Hb A_2 and Hb F were found to be present together. This may indicate that the thalassaemia determinant itself is probably responsible for the rise in both Hb A_2 and Hb F or, if an additional heterocellular HPFH determinant is present, this would be closely linked to the β thalassaemia determinant in our patients.

Detailed analyses performed in some families with β thalassaemia intermedia coexisting with α thalassaemia and homozygous β thalassaemia have been published by Weatherall and Clegg.[1] In our study, the synthetic ratio of α/non-α in peripheral blood (table 1, figure) gave no evidence for the existence of α thalassaemia in the parents of any patients. This is not unexpected because α thalassaemia is not common and the coexistence of α thalassaemia with homozygous β thalassaemia should be rare. The presence of increased numbers of α genes was excluded by the presence of severe hypochromia in the red cells of all the patients and the heterozygotes. The imbalance in chain synthesis was very severe in some of the patients while in some it was milder than that of severe β thalassaemia, the α/non-α ratio being between 4·0 and 2·5 (figure, table 2). However, it was noted that the ratio of imbalance in chain synthesis was more or less equal in the patients of any given family. The mean of the α/non-α synthetic ratio of regular Hb A_2 β thalassaemia heterozygotes and β° thalassaemia heterozygotes with raised Hb A_2 and Hb F was not statistically significant (table 2). This observation is a further indication that the mildness of the thalassaemia determinant cannot always be predicted from the α/non-α synthetic ratio in peripheral blood reticulocytes of heterozygotes and homozygotes. The reason for the heterogeneity in the severity of imbalance in chain synthesis among patients with similar conditions remains unclear.

The degree of bone changes seems to correlate with the degree of splenomegaly and it is quite likely that exacerbation of the disease by hypersplenism has in some cases had an important influence on the degree of bone change.

Review of our 22 patients with homozygous β° thalassaemia in different families showed that at least one of the parents of 11 of the 22 patients (11 of the total 30 parents) had a β° thalassaemia determinant with raised Hb A_2 and Hb F. The thalassaemia was mild in seven of the 11 families who were included in this study.

Raised Hb A_2 and Hb F were found in only three of 140 parents of the 158 patients with β^+ thalassaemia. The thalassaemia was mild in the offspring of these three who were among the subjects of this study. This observation indicates that thalassaemia intermedia characterised by raised Hb A_2 and Hb F is more common among patients with β° thalassaemia than with β^+ thalassaemia.

The $^{G}\gamma^{A}$ ratio of Hb F of the patients and heterozygotes with raised Hb A_2 and Hb F was of the fetal type. The $^{A}\gamma^{T}$ variant of the Hb F was not detected in cis of the β° thalassaemia determinant characterised by raised Hb A_2 and Hb F in six families (table 1). In one study the frequency of $^{A}\gamma^{T}$ variant was reported to be as high as 50 to 70% in β thalassaemia of Mediterranean origin.[20] Our study indicates that the frequency of the $^{A}\gamma^{T}$ variant of Hb F is probably very low in cis of β° thalassaemia characterised by increased Hb A_2 and Hb F.

All of the patients presented here produced between 6·5 to 10 g/dl fetal haemoglobin and this suggests that some additional factor, allowing derepression of the γ genes, may exist. Since segregation of increased Hb F from raised Hb A_2 was not clearly shown in any family examined in this study, it may be possible that the β° thalassaemia determinant itself is responsible for the γ chain activation in cis. Increased production of the γ chain may result from increased or ineffective erythropoiesis, as has been suggested by Trent *et al.*[21] However, the absence of a similar increase in the γ chain in common β° thalassaemia heterozygotes conflicts with this theory.

Cloning analyses performed previously on one of the patients (Tu family, II.1) showed a dinucleotide deletion in the codon for the eighth amino acid of the β chain which produces a termination codon at the position of the new 21st codon.[22] However, it is not known whether this mutation itself is responsible for the increase in Hb F. The haemoglobin levels of the two sibs (Tu family) are much higher than those of the other patients, suggesting that the molecular defect may be different from that of the above mentioned mutation.

Much more study is necessary into severe and mild β thalassaemia in order to understand the molecular mechanism which causes variation in clinical and haematological expression of the disease.

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