Clinical, haematological, and genetic studies of type 2 normal Hb A₂ β thalassaemia

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SUMMARY The clinical and haematological phenotype as well as chain synthesis data were studied in 35 doubly heterozygous patients with either normal Hb A₂ and Hb F, type 2 β thalassaemia and β (high A₂) thalassaemia (26 patients), or type 2 and other rare β or $\delta\beta$ variants (nine patients). Patients doubly heterozygous for type 2 and β^0 or $\delta\beta^0$ thalassaemia variants had no detectable Hb A, indicating that the type 2 normal A₂ β thalassaemia is primarily the result of a β^0 gene. The clinical phenotype varied from severe thalassaemia major to mild thalassaemia intermedia, and was mainly related to the thalassaemia variant with which the type 2 normal A₂ β thalassaemia was combined, and the proportion of Hb A produced in β^+ thalassaemia patients.

Haematological and chain synthesis data were similar in heterozygotes with type 2 and β^0 or β^+ (high A₂) thalassaemia. Hb A₂ levels were within the normal range (2.3 to 3.6%) though absolute values (Hb A₂ per RBC) ranged from low normal (0.5 pg/RBC) to increased levels (1.0 pg/RBC.) The variation of Hb A₂, as well as the presence of Hb A₂ in a type 2/ $\delta\beta$ high F patient and the complete absence of HbA₂ in a homozygous type 2 patient, indicate that there are at least two genotypes of type 2, one β^0 and the other $\delta\beta^0$. This has been recently proven by gene mapping studies.

For clinicians, routine haematological and family studies are sufficient for the proper treatment and prevention of doubly heterozygous type 2 patients.

Molecular studies in the β and $\delta\beta$ thalassaemias have disclosed a number of DNA mutations. However, despite the scientific significance of these mutations, the clinical and haematological phenotypes still remain the principal guides for diagnosis, treatment, and prevention of the β and $\delta\beta$ thalassaemias.

An interesting thalassaemia variant requiring further investigation is heterozygous β thalassaemia with normal Hb A₂ and Hb F which exists in at least two distinct types: type 1 ('silent type') with no or minor red cell abnormalities and normal osmotic fragility and type 2 with distinct red cell abnormalities (low MCV and MCH) and decreased osmotic fragility. Of the two types, type 2 normal A₂ β thalassaemia has a more severe clinical phenotype than type 1 normal A₂ when combined with classical β thalassaemia.¹

To clarify the clinical and haematological phenotype of the type 2 normal A₂ β thalassaemia variant, we studied 35 doubly heterozygous patients, with one parent heterozygous for the type 2 condition and the other heterozygous for the β or $\delta\beta$ thalassaemia variant.

Material and methods

SELECTION OF PATIENTS

Subjects were selected from over 600 thalassaemic patients by careful assessment of clinical and haematological data. Hb chain synthesis studies were then carried out on these patients and their parents. The main prerequisite for selection was that one parent was heterozygous for type 2 normal A₂ β thalassaemia and the other for β or $\delta\beta$ thalassaemia.

A total of 35 patients fulfilled the above criteria. According to the parental β or $\delta\beta$ variant, they were classified in two major groups. Group A comprised 26 patients with one parent heterozygous for type 2

normal $A_2 \beta$ thalassaemia and the other with classical (high A_2) β thalassaemia (either β^0 or β^+). Group B comprised nine patients, with one parent heterozygous for type 2 normal $A_2 \beta$ thalassaemia and the other with one of the rare β or $\delta\beta$ thalassaemia variants.

METHODS

The haematological phenotype was characterised using generally accepted haematological and Hb chain synthesis data. Hb A_2 was measured by elution from cellulose acetate strips after electrophoresis and Hb F was measured as previously described.²⁻⁵ Isoelectric focusing and chromatography were used in a number of samples to identify the presence of Hb A when cellulose acetate and starch gel electrophoresis were not conclusive.

Assessment of the clinical phenotype was based on the criteria proposed by Kattamis *et al*⁶ in use in our Unit. By these criteria, the clinical phenotypes were classified as two major types: thalassaemia major (M) and thalassaemia intermedia (I). Each major type was subdivided into subtype I, the severe form, and subtype II, the mild form. The criteria used for evaluation were the haemoglobin level, age at diagnosis, and age at which transfusions started, as well as the degree of impairment of growth, bones, heart, the size of liver and spleen, and the overall clinical condition.

Results

Based on the presence or absence of Hb A as detected by electrophoresis, chromatography, or isoelectric focusing, patients were divided into two groups. The first group (group 1) of 16 patients had no detectable haemoglobin A, while the second group (group 2) of 19 patients had various amounts of Hb A. Table 1 summarises genotypes assumed from parental haematological phenotypes.

From these data it is evident that in six patients the association of type 2 normal A_2 with one of the known β^0 thalassaemia genes, namely the β Dutch (high A_2 and high F) and the $\delta\beta^0$ high F, leads to a complete reduction of β chain synthesis, as indicated by the absence of Hb A. The same holds true for eight more patients where type 2 is associated with classical (high A_2) β thalassaemia. The absence of Hb A in these patients clearly shows that type 2 thalassaemia is the result of a β^0 gene. That type 2 normal $A_2 \beta$ thalassaemia gene is the result of a β^0 gene is further confirmed from the findings of a unique patient homozygous for the type 2 condition where no Hb A was detected. Group 1 also includes a double heterozygote for types 1 and 2 normal $A_2 \beta$

TABLE 1 Presumed genotypes based on parental haematological phenotypes in the 35 double heterozygotes with type 2 normal $A_2 \beta$ thalassaemia.

Presumed genotypes	No	Group 1 (no Hb A)	Group 2 (with Hb A)
Type 2 (normal A_2)/ β^0 *(high A_2)	8	8	0
Type 2 (normal A_2)/ β^+ (high A_2)	17	0	17
Type 2 (normal A_2)/ β^0 Dutch (high A_2 + F)	4	4	0
Type 2 (normal A_2)/ $\delta\beta^0$ (high F)	2	2	0
Type 2 (normal A_2)/type 1 (normal A_2)	3	1†	2
Type 2 (normal A_2)/type 2 (normal A_2)	1	1	0
Total	35	16	19

*Presumed β^0 .

†Coexistence of α thalassaemia?

thalassaemia. The absence of Hb A in this patient is exceptional, as the type 1 gene is a β^+ gene.

Group 2, on the other hand, comprises 17 patients with the type 2 normal A_2 and β^+ (high A_2) genotype and two double heterozygotes for types 1 and 2 normal $A_2 \beta$ thalassaemia. In these patients Hb A varies and as it is not possible to prove whether or not the type 2 gene participates in the synthesis of Hb A, they cannot be used as a strong argument that the type 2 gene is a β^0 one. Thus the exclusive evidence that type 2 is a β^0 gene comes from the 16 patients in group 1, where type 2 combines with known β^0 thalassaemic genes.

The clinical phenotype of double heterozygotes for the type 2 and β^0 thalassaemia was, as a rule, severe, mainly M_1 (type I thalassaemia major),

FIGURE The relation of clinical phenotype to haematological phenotype and the percentage of Hb A in 35 doubly heterozygous type 2 normal $A_2 \beta$ thalassaemia patients.

while that of double heterozygotes for β type 2 and β^+ thalassaemia was more heterogeneous. Of eight patients with type 2 (normal A₂)/ β^0 (high A₂), seven had thalassaemia major and only one had the severe form of thalassaemia intermedia. In contrast, nine out of 17 patients with normal A₂ type 2/ β^+ (high A₂) had thalassaemia major and eight had thalassaemia intermedia (six severe and one mild).

The milder phenotype of patients with type 2/ β^+ thalassaemia could be attributed to the ability of these patients to produce varying amounts of Hb A, in contrast to the type 2/ β^0 thalassaemia genotype, which is not capable of synthesising Hb A. As seen in the figure, Hb A in the type 2/ β^+ thalassaemia genotype ranged from 25 to 60% and the milder phenotype was seen in the patients with the higher percentage of Hb A.

In the other rare genotypes (group B), the clinical phenotype seems to be related to the rare thalassaemic variant combined with the type 2 condition. In these patients the severity of the clinical phenotype resembles that of a double heterozygote for high A₂ β thalassaemia and the rare β or $\delta\beta$ thalassaemia variant. Of interest is the heterogeneity observed in the four patients with the type 2/ β Dutch genotype and the mild thalassaemia intermedia in the type 2/ $\delta\beta$ (high F) patient.

The relevant haematological data of patients of group 1 are shown in table 2 and that of group 2 in table 3. The data clearly show the variation in clinical manifestations of type 2 double heterozygotes, as estimated from the age at diagnosis, the Hb and Hct levels, and the necessity for transfusion treatment. It also shows that the clinical phenotype is mainly

TABLE 2 Analytical data on 16 patients doubly heterozygous for type 2 normal A₂ β thalassaemia without Hb A (group 1).

Case No	Age at diagnosis (y)	Clinical phenotype	Genotype	Hb (g/dl)	Hct (%)	MCH (pg)	MCV (fl)	Hb A ₂ (%)	α/γ
1	3	M _{II}	β type 2/ β^0 thalassaemia	9.8	30	18.5	56.6	1.7	2.54
2	3	M _{II}	β type 2/ β^0 thalassaemia	7.2	22	—	—	2.1	3.70
3	2	I _I	β type 2/ β^0 thalassaemia	9.2	31	20.0	67.4	0.9	5.81
4	1.5	M _I	β type 2/ β^0 thalassaemia	5.6	20	—	—	1.8	—
5	0.5	M _I	β type 2/ β^0 thalassaemia	6.1	20	13.5	44.4	1.4	2.33
6	0.3	M _I	β type 2/ β^0 thalassaemia	3.9	14	—	—	2.1	—
7	0.3	M _I	β type 2/ β^0 thalassaemia	5.8	21	—	—	1.9	—
8	0.8	M _I	β type 2/ β^0 thalassaemia	7.0	23	—	—	3.5	—
9	4	I _I	β type 2/ β^0 Dutch	9.3	32	15.5	53.3	0.7	5.40
10	1	M _I	β type 2/ β^0 Dutch	Transfused	—	—	—	—	—
11	9	M _{II}	β type 2/ β^0 Dutch	Transfused	—	—	—	—	—
12	0.9	M _{II}	β type 2/ β^0 Dutch	9.8	29	23.3	69.0	0.9	3.99
13	11	I _{II}	β type 2/ $\delta\beta$	10.3	32	22.9	71.1	2.2	—
14	2	M _{II}	β type 2/ $\delta\beta$	6.9	19	18.6	51.3	—	—
15	1	M _I	β type 2/ β type 1	7.5	25	18.7	62.5	1.5	3.63
16	3	I _I	β type 2/ β type 2	9.3	29	20.2	63.0	0	5.80

TABLE 3 Analytical data on 19 double heterozygotes, with varying amounts of Hb A (group 2).

Case No	Age at diagnosis (y)	Clinical phenotype	Genotype	Hb (g/dl)	Hct (%)	MCH (pg)	MCV (fl)	Hb A* (%)	Hb A ₂ (%)	$\alpha/\text{non-}\alpha$
1	5	I _I	β type 2/ β type 1	8.0	26	15.7	51.0	77	6.4	2.62
2	3	I _{II}	β type 2/ β type 1	11.0	37	19.0	64.9	30	2.6	2.26
3	6	I _{II}	β type 2/ β^+ thalassaemia	8.0	26	—	—	62	6.5	3.01
4	1.5	I _I	β type 2/ β^+ thalassaemia	6.6	22	22.0	73.0	27	2.5	3.03
5	0.7	M _I	β type 2/ β^+ thalassaemia	Transfused	—	—	—	—	—	3.96
6	2.5	M _{II}	β type 2/ β^+ thalassaemia	7.3	22	17.0	51.0	—	—	—
7	3	M _{II}	β type 2/ β^+ thalassaemia	9.8	31	20.8	65.9	50	3.1	5.04
8†	4	I _I	β type 2/ β^+ thalassaemia	8.8	28	16.3	51.8	50	2.4	—
9†	3	I _I	β type 2/ β^+ thalassaemia	8.8	29	18.3	60.4	47	2.4	—
10†	4	I _I	β type 2/ β^+ thalassaemia	9.3	29	17.7	53.7	48	2.8	—
11	6	I _I	β type 2/ β^+ thalassaemia	8.5	27	—	—	32	2.3	—
12	0.5	I _I	β type 2/ β^+ thalassaemia	3.4	12	—	—	61	5.5	2.46
13	1	M _I	β type 2/ β^+ thalassaemia	7.4	24	17.3	58.5	31	2.4	—
14	0.5	M _I	β type 2/ β^+ thalassaemia	8.6	26	21.5	65	48	2.8	—
15	0.5	M _I	β type 2/ β^+ thalassaemia	8.5	29	21.0	72.5	50	2.4	—
16	0.5	M _I	β type 2/ β^+ thalassaemia	5.4	17	14.6	46.0	31	3.8	4.12
17	0.8	M _I	β type 2/ β^+ thalassaemia	6.7	23	19.0	65.7	56	4.1	—
18	2	I _I	β type 2/ β^+ thalassaemia	9.5	30	19.4	61.2	50	4.2	—
19	6	I _{II}	β type 2/ β^+ thalassaemia	10.2	32	22.2	69.6	61	4.6	—

* Hb A by subtraction. † Sibs.

TABLE 4 Summary of relevant data of eight patients in group 1 with type 2 normal A_2/β^0 (high A_2) genotype and of 17 patients in group 2 with type 2 normal A_2/β^+ (high A_2) genotype.

	Genotype				p
	Type 2 normal A_2/β^0 (high A_2) (n = 8)		Type 2 normal A_2/β^+ (high A_2) (n = 17)		
	Mean (SD)	Range	Mean (SD)	Range	
Hb (g/dl)	6.8 (1.9)	3.9–9.8	7.9 (1.8)	3.4–10.2	NS
Hct (%)	22.6 (5.5)	14–31	25.4 (5.3)	12–32	NS
MCV (fl/RBC)	56.1 (11.5)	44.4–67.4	61.1 (8.6)	46–73	NS
MCH (pg/RBC)	17.3 (3.4)	13.5–20	19.0 (2.4)	14.6–22.2	NS
Ret (%)	4.8 (1.7)	3–8	4.0 (1.0)	2.5–6	NS
Normoblasts/100 WBC	25.8 (28.6)	2–72	9.2 (7.6)	1–25	p<0.001
Hb A (%)	0	0	46.5 (11.6)	27–60	p<0.001
Hb A ₂ (%)	1.9 (0.7)	0.9–3.5	3.4 (1.3)	2.2–6.5	p<0.01
Hb A ₂ (pg/RBC)	0.23 (0.07)	0.18–0.31	0.60 (0.19)	0.39–1.02	p<0.001
α /non- α	3.6 (1.6)	2.3–5.8	3.6 (0.9)	2.46–5.0	NS
Age at diagnosis (y)	1.4 (1.1)	0.3–3	2.3 (2.0)	0.5–6	NS
Transfusion dependent	7/8		13/17		

TABLE 5 Summary of haematological data of β type 2, β^0 and β^+ heterozygous parents.

	β type 2 (n = 34)		β^0 thalassaemia (n = 8)		β^+ thalassaemia (n = 15)	
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Hb (g/dl)						
Male	13.3 (1.3)	11.0–16	14.7 (1.8)	13.5–16	13.3 (0.7)	12.5–14.4
Female	11.6 (1.4)	9.4–13.1	11.3 (0.9)	10.2–12.7	11.9 (0.7)	10.0–12.7
Hct (%)						
Male	43.1 (3.9)	36–49	48.0 (5.6)	44–52	43.4 (2.3)	40–44
Female	37.4 (4.9)	29–46	37.8 (3.5)	34–42	36.6 (2.3)	33–41
MCV (fl/RBC)	73.9 (10.0)	57.8–100	68.5 (7.9)	59.3–82.3	71.9 (9.1)	63–93
MCH (pg/RBC)	22.8 (3.1)	17.8–30.2	20.7 (2.5)	17.8–24.9	23.1 (3.1)	18.0–29.7
Hb A_2 (%)	3.0 (0.4)	2.3–3.6	5.2 (0.58)	4.2–6.2	5.2 (0.5)	4.7–6.2
Hb A_2 (pg/RBC)	0.70 (0.14)	0.45–1.0	1.1 (0.2)	0.91–1.5	1.2 (0.19)	0.95–1.6
Hb F (%)	0.9 (0.8)	0.1–2.5	0.6 (0.5)	0.1–1.6	0.65 (0.6)	0.1–1.9
α /non- α	2.2 (0.5)	1.0–3.0	2.7 (0.05)	2.6–2.7	1.7 (0.3)	1.5–2.0

regulated by the type of thalassaemia with which the type 2 variant is combined. The data in table 4, which compares relevant haematological data of patients doubly heterozygous for β^0 thalassaemia (group 1) or β^+ thalassaemia (group 2), illustrate this point. The age at diagnosis in patients in group 1 is 1.4 ± 1.1 years as compared to 2.3 ± 2.0 years for group 2. Seven out of eight patients from group 1 are transfusion dependent while this applies only to 13 out of 17 patients in group 2.

Of the other haematological parameters, it is interesting to note Hb A and Hb A_2 levels and the globin chain synthesis ratio. Hb A_2 in type 2/ β^0 thalassaemia is low ($1.9 \pm 0.7\%$), ranging from 0.9 to 3.5%, as compared to type 2/ β^+ thalassaemia ($3.4 \pm 1.3\%$) ranging from 2.2 to 6.5%. The α /non- α ratio is 3.6 ± 1.6 for group 1 and 3.6 ± 0.9 for group 2.

Of interest are the haematological data of heterozygotes with type 2 β thalassaemia. A comparison of relevant haematological data in heterozygous type 2

normal A_2 parents with those of β^0 (high A_2) and β^+ (high A_2) heterozygous parents is shown in table 5.

In type 2 normal A_2 heterozygotes, MCV and MCH varied, but all except two had lower than normal indices. However, all heterozygotes had abnormal red cell morphology and decreased osmotic fragility.

Discussion

Type 2 doubly heterozygous thalassaemia patients constitute an interesting group of patients with clinical manifestations ranging from severe thalassaemia major to mild thalassaemia intermedia.¹ As indicated from the data in this study, the severity of the clinical manifestations is directly related to the type of thalassaemia with which the type 2 variant is combined. The most severe clinical phenotype was found in combination with β^0 (high A_2) thalassaemia. The type 2/ β^0 (high A_2) genotype had similar clinical manifestations to homozygous β^0

(high A₂) thalassaemia and type 2/ β^+ thalassaemia was similar to that of homozygous β^+ thalassaemia or doubly heterozygous β^0/β^+ thalassaemia.^{6,7} Milder clinical phenotypes were also found in certain genotypes with no detectable Hb A. The milder clinical phenotype and the relatively higher levels of haemoglobin could be attributed to activation of γ chain synthesis which is known to occur in certain genotypes, as in the case of $\delta\beta^0$ (high F) thalassaemia. The similarity of the clinical manifestations to homozygous β thalassaemia leads to the conclusion that type 2 β thalassaemia is as important as classical high A₂ β thalassaemia in evaluating the prognosis, treatment, and prevention of thalassaemia.

Speculating further on the haematological data of our patients, it is evident that the type 2 normal A₂ gene is a β^0 thalassaemia gene, which is not capable of producing β chains. This is substantiated by the complete absence of Hb A in patients of group 1, where the type 2 gene was combined with known β^0 or $\delta\beta^0$ thalassaemias. The varying amount of Hb A in patients of group 2 seems to be related to the ability of the β^+ thalassaemia variant to synthesise β chains. However, it is not possible to exclude the fact that type 2 may contribute to the synthesis of Hb A in these patients, though the existing evidence favours the assumption that this is not feasible as type 2 is the result of a β^0 gene. Attention should be focused on the one of three double heterozygotes for types 1 and 2 normal A₂ β thalassaemia patients who has no Hb A. As the type 1 gene is considered to be a β^+ gene, the absence of Hb A indicates either the presence of heterogeneity of the type 1 variant, or a β^0 gene with a haematological phenotype similar to type 1, as is the case with coexistence of type 2 with α thalassaemia. Family studies now in progress strongly favour the second assumption.

As for the haematological and chain synthesis data of heterozygotes with type 2 β thalassaemia, there are no significant differences from those of β^0 or β^+ thalassaemia, with the exception that Hb A₂ in type 2 β thalassaemia was within the normal range (2.3 to 3.6%), but in absolute values (Hb A₂ per RBC) there was wide variation ranging from the low level of 0.45 pg/RBC to an increased level of 1.0 pg/RBC.

The wide range of absolute Hb A₂ values points to the presence of genetic heterogeneity of the type 2 gene which in certain cases may be associated with β thalassaemia. This is substantiated from the data of two patients. Hb A₂ was found in low levels in the one with type 2 $\delta\beta^0$ high F thalassaemia. The presence of Hb A₂ must be attributed to the type 2 gene, as it is known that the $\delta\beta^0$ gene is not capable of producing δ chains. The second concerns

the type 2 homozygous patient where no Hb A₂ was detected and haemoglobin consisted exclusively of Hb F. DNA analysis disclosed a novel deletion of 6.5 kb starting between the $\psi\beta$ and δ genes which comprises part of the δ gene and ends inside the δ gene.⁸ A previous study of gene mapping in 17 patients with type 2 showed no evidence of deletion.⁹ However, recently, more complete DNA studies in a number of our patients disclosed the same DNA defect in two more patients (unpublished data).

From these data it is evident that at least two distinct forms of type 2 normal A₂ β thalassaemia exist: one is a β^0 thalassaemia and the other is a $\delta\beta^0$ thalassaemia. Thus, further DNA studies are needed for a precise characterisation of the molecular defects which lead to the haematological phenotype of β thalassaemia with normal Hb A₂ and Hb F type 2. However, for the clinician, routine haematological and biosynthesis investigations combined with family studies are sufficient for proper treatment and prevention of this group of thalassaemic patients.

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References

- Kattamis C, Metaxotou-Mavromati A, Wood W, Nash J, Weatherall D. The heterogeneity of normal Hb A₂- β -thalassaemia in Greece. *Br J Haematol* 1979;42:109-23.
- Weatherall D, Clegg J, Na-Nakorn S, Wasi P. The pattern of disordered haemoglobin synthesis in homozygous and heterozygous β -thalassaemia. *Br J Haematol* 1966;16:257-67.
- Kattamis C. Screening for haemoglobinopathies (thalassaemias and other abnormal haemoglobins). In: Bickel H, Guthrie R, Hammersen G, eds. *Neonatal screening for inborn errors of metabolism*. Berlin: Springer-Verlag, 1980:133-47.
- Pembrey M, McWade P, Weatherall D. Reliable routine estimation of small amounts of foetal haemoglobin by alkali denaturation. *J Clin Pathol* 1972;25:738-40.
- Clegg J, Naughton M, Weatherall D. Abnormal human hemoglobins. Separation and characterization of the α and β chains by chromatography and the determination of two new variants Hb Chesapeake and Hb J (Bangkok). *J Mol Biol* 1966;19:91-9.
- Kattamis C, Metaxotou-Mavromati A, Ladis V, Tsiarta H, Laskari S, Kanavakis E. The clinical phenotype of β and $\delta\beta$ thalassaemias in Greece. *Eur J Pediatr* 1982;133:135-8.
- Kattamis C, Karambula K, Metaxotou-Mavromati A, Ladis V, Constantopoulos A. Prevalence of β^0 and β^+ thalassaemia genes in Greek children with homozygous β -thalassaemia. *Hemoglobin* 1978;2:29-46.
- Wainscoat J, Thein S, Wood W, et al. A novel deletion in the β -globin gene complex. *Ann NY Acad Sci* 1985;445:20-7.
- Kanavakis E, Metaxotou-Mavromati A, Kattamis C, Aksoy M, Weatherall D, Wood W. Globin gene mapping in normal Hb A₂ types of β -thalassaemia. *Br J Haematol* 1982;51:59-64.

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