Eur J Pediatr (1982) 139 : 135–138

European Journal of
Pediatrics

© Springer-Verlag 1982

The Clinical Phenotype of β and $\delta\beta$ Thalassemias in Greece

C. Kattamis, A. Metaxotou-Mavromati, V. Ladis, H. Tsiarta, S. Laskari, and E. Kanavakis

Thalassemia Unit, 1st Department of Pediatrics, University of Athens, St. Sophie Children's Hospital Athens 608, Greece

Abstract. Based on precise evaluation of hematological findings and clinical manifestations, the relationship between genotype and clinical phenotype was studied in 475 Greek patients with β and $\delta\beta$ thalassemias. Almost all known genotypes are included in this series, but the most frequent was homozygous β^{th} high A_2 (71.6%), β^{th}/β^{th} silent (7.4%), $\beta^{th}/\delta\beta^{oth}$ high F (6.3%) and β^{th}/β^{th} Dutch (6.3%).

In general, the phenotype was related to the genotype, though clinical heterogeneity was detected among patients with the same genotype. The severe type of thalassemia major was most commonly found in homozygous β^{th} patients mainly of β^o/β^o and β^o/β^+ genotypes while homozygous β^+patients had milder clinical manifestation. Furthermore a small group of patients, characterized as homozygous β^{++} (HbF$<$30%) had mild thalassemia intermedia. In addition mild thalassemia intermedia was principally related with homozygous $\delta\beta^{oth}$, and compound heterozygous β^{th}/β^{th} silent I, and less frequently with other genotypes such as compound heterozygous with β^{th}/β^{th} Dutch, β^{th}/β^{th} silent II, $\beta^{th}/\delta\beta^{oth}$ high F or Lepore.

It was shown that precise genetic characterization and clinical evaluation is of primary importance in predicting the prognosis and formulating the proper treatment for the individual patient with thalassemia.

Key words: β, $\delta\beta$ thalassemias – Clinical phenotype – Genotypes

Introduction

Long before research workers realized the extreme genetic heterogeneity of thalassemias, clinicians recognized the wide variety of phenotypic expression of these syndromes and applied the terms thalassemia "major" and "intermedia" to define the existing differences in the severity of clinical manifestations [1, 9]. At present, clinicians are greatly interested in exploring the patient's genotype as there is strong evidence suggesting that the phenotype of the thalassemia syndrome, is basically related to the genotype. This seems to be particularly true for thalassemia intermedia [11].

In an attempt to clarify the relation between the genotype and the clinical phenotype of patients with thalassemias, the clinical, hematological and genetic data of 475 Greek children with β and $\delta\beta$ thalassemias were analysed and the results are reported here.

Material and Methods

The study includes 475 fully investigated genetically classified patients with thalassemia, followed at the Thalassemia Unit of the 1st Department of Pediatrics of Athens University. The genetic characterization of β and $\delta\beta$ thalassemias was based on the detailed hematological investigation of the patient and both parents. All patients with evidence of interaction with α-thalassemia or other abnormal hemoglobin were excluded.

Hematological analysis included complete blood count and red cell indices, one tube osmotic fragility, quantitation of HbF and HbA_2, hemoglobin electrophoresis on starch gel, and a search for excess β-chains in red cells. In selected cases column chromatography and hemoglobin chain synthesis were carried out [2, 4]. The criteria used for the genetic classification are shown in Table 1.

In contrast to genetic characterization there are no definite criteria for classification of the clinical phenotype of the thalassemias; thus somewhat arbitrary criteria such as the hemoglobin level, the age of diagnosis and the age at which transfusions started were adopted. Furthermore for clinical evaluation, the general condition, the degree of bone and heart involvement, the size of liver and spleen, and the degree of growth impairment were also considered.

Based on the above criteria patients were classified into the two major clinical types, thalassemia major and intermedia, but each type was further subdivided into two subgroups: I(severe) and II(mild). More precisely:

Thalassemia Major

Type I: Included patients with the most severe clinical manifestations, in which diagnosis was made very early in life and treatment started at the age of 3–24 months because of low Hb.

Type II: Included patients with rather milder clinical manifestations; hemoglobin was preserved at satisfactory levels (>8 g/dl) during the first two years of life, but later deteriorated and transfusions were started at the age of 3–5 years.

Thalassemia Intermedia

Patients with milder clinical manifestations, preserving Hb levels above 8 g/dl, at least for the first 6 years of life.

Type I: Included patients, which during the 6th–12th year of life, though they may have preserved a hemoglobin of 7–8 g/dl,

0340-6199/82/0139/0135/$ 01.00

Table 1. Relevant findings in β and $\delta\beta$ thalassemias

Type of thalassemia	Laboratory findings			
	Heterozygous		Homozygous	
	Hb A_2%	Hb F%	Hb A%	Hb F%
β^o (high A_2)	>3.5	<4	0	~100
β^o Ferrara	>3.5	<4	0	~100
β^o (high F) Dutch	<3.5	>4	0	~100
β^+ (high A_2)	>3.5	<4	40	> 60
β^{++} (mild)[a]	>3.5	<4	70	< 30
β^+ Negro	>3.5	<4	40	25-60
β (silent)[b]	<3.5	<2	Not described	
(Type I)	Minimal > Red cell changes			
(Type II)	Obvious > Red cell changes			
$(\delta\beta)^o$ high F	<3.5	4-20	0	100
$(\delta\beta)$ Lepore	<3.5	>5	0	~ 80
	+		+	
	Hb Lepore	(8-10%)	Lepore	(~20%)

[a] Unpublished cases; parents β^+ (high A_2) heterozygote
[b] Chain synthesis for diagnosis

Table 2. β and $\delta\beta$ thalassemia genotypes in 475 greek patients

Genotypes	Patients	
	Number	%
I. Frequent		
β^{th}/β^{th}	340	71.6
β^{th}/β^{th} silent	35	7.4
$\beta^{th}/(\delta\beta)^{oth}$ high F	30	6.3
β^{th}/β^{oth} Dutch (F>4%)	30	6.3
β^{th}/β^{th} (F:3-4%)	21	4.4
$\beta^{th}/\delta\beta$ Lepore	14	2.9
II. Rare		
$(\delta\beta)^{oth}/(\delta\beta)^{oth}$	2	
$(\delta\beta)^{oth}/\beta^{oth}$ Dutch	1	
β^{th} silent/β^{oth} Dutch	1	1.0
β^{oth} Dutch/β^{oth} Dutsch	1	
Total	475	100.0

All $\beta^o + \beta^+$ variants with increased HbA^2 and F<3%

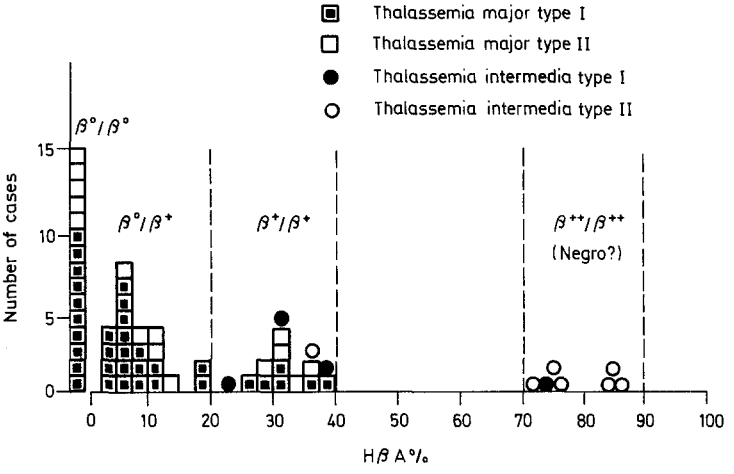


Fig. 1. Clinical phenotypes in 4 genotypes of homozygous β^{th} high A_2 thalassemia (β^o/β^o, β^o/β^+, β^+/β^+ and β^{++}/β^{++})

showed considerable bone changes, enlargement of the spleen, as well as a certain degree of growth retardation, and might have needed transfusions or splenectomy because of hypersplenism.

Type II: This is the milder type of thalassemia intermedia, preserving a high level of Hb (8-10 g/dl), and only minor signs of bone and spleen impairment at the age of 6-12 years.

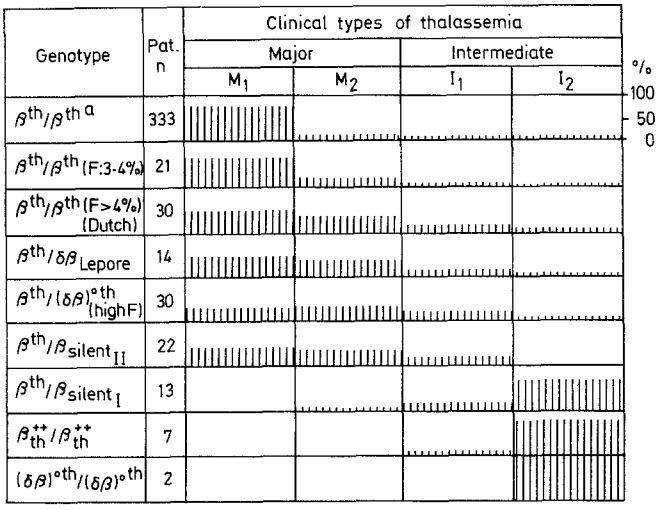
Results

Table 2 shows the spectrum of β and $\delta\beta$ thalassemia genotypes in our series, which is more or less representative of the prevalence of each genotype in Greece.

More than 70% of the patients were homozygous for β(high A_2) thalassemia. This is the most common variant which predominates, not only in Greece, but all over the world. The remaining patients resulted from the mating of the common β thalassemia gene to one of the less common β or $\delta\beta$ thalassemia genes, namely the β-silent, the $(\delta\beta)^o$ high F, the $\delta\beta$ Lepore, and the β^o (Dutch) variant while homozygosity of the rare variants was exceptional.

The clinical manifestations of the most common genotype, the homozygous β-thalassemia (high A_2) were as a rule severe corresponding to the clinical subtypes I and II, of thalassemia major. But even in this genotype a heterogeneity in clinical phenotype was found. Studies with column chromatography, and chain synthesis in 57 patients, with homozygous β-thalassemia showed that this heterogeneity was related, to a certain extent, to the presence of the β^o or β^+ genes and to the amount of HbA (Fig. 1).

Homozygous β^o thalassemia, with no HbA, and compound heterozygous with β^o and β^+genes with low HbA (4-20%) had



a all β° and β^{+} (high A_2 and F<3%) variants

Fig. 2. Prevalence of clinical phenotypes in 475 Greek patients with β and $\delta\beta$ thalassemia

as a rule the most severe type I thalassemia major, while homozygous β^{+}(with rather high HbA>20%) had equally type I and II thalassemia major and occasionally type I, thalassemia intermedia. Furthermore, all 7 patients with high HbA (>70%) had mild thalassemia intermedia, mainly type II.

The relation observed in this series between thalassemic genotype and clinical phenotype is shown in Fig. 2. This figure illustrates also the clinical heterogeneity which was found in patients with the same genotype. The most severe cases of thalassemia were related to homozygous β(high A_2) thalassemia, and β and $(\delta\beta)^{\circ}$ thalassemias, while the thalassemias intermedia, type I, and II with β^{th}/β^{th} silent type I, the homozygous β^{++}thalassemia (high HbA), and homozygous $(\delta\beta)^{\circ}$; compound heterozygotes with β^{th}/β° Dutch, $\beta^{th}/\delta\beta^{\circ}$ (high F), β^{th}/β^{th} silent type II, had a clinical phenotype of moderate severity, and a wider spectrum ranging from thalassemia major to thalassemia intermedia.

Discussion

As illustrated in Fig. 2 the patient's genotype may prove valuable in assisting the clinician to predict the prognosis and formulate the treatment of individual patients with β or $\delta\beta$ thalassemia. It became clear from this study that certain genotypes are most commonly associated with the severe manifestations of thalassemia major (type I and II), others with the mild manifestations of thalassemia intermedia (type I and II), while others have both severe and mild clinical phenotypes.

Of interest was the clinical heterogeneity observed in patients with the same genotype. Homozygous (high A_2) thalassemic patients had, as a rule, the clinical phenotype of thalassemia major (type I and II). But even in this genotype, heterogeneity in clinical phenotype was found. The variation in clinical severity seemed to be related primarily to the presence of β° or β^{+} genes (Fig. 1); a milder phenotype was observed in homozygous β^{+} thalassemia, namely in patients with a proportion of HbA ranging from 20-40% [5].

The milder clinical manifestations, compatible with thalassemia intermedia type II, were found in 7 patients with very high levels of HbA (>70%) and rather low levels of HbF (<30%). All were older patients aged 6-22 years who preserved, without transfusion, Hb levels of 8-9 g/dl. Both parents had hematological data of β-thalassemia trait with high HbA_2 and imbalanced β-chain synthesis with a ratio of α- to β-chains ranging from 1.9-2.8.

The respective chain ratio in patients ranged from 2.6-3.9, slightly lower than that found in homozygous β° or β^{+} thalassemia of this series. The milder manifestations of these patients could be attributed to the presence of a β^{+} thalassemia gene, which is capable of directing the synthesis of adequate amounts of β-chains, considerably higher than that synthesized by the common β^{+} variant. For distinction, this variant was characterized as β^{++}.

The clinical, hematological and genetic findings of the 7 patients strongly suggest that this is a distinct entity, quite different from the usual homozygous β^{+} thalassemia found in Greece and rather similar to β^{+} thalassemia described in negros [3, 12]. Studies with DNA mapping, now in progress, are expected to give additional information at the molecular level, concerning this β-thalassemia variant, in the Greek population.

Another factor which seemed to influence the clinical phenotype of patients, was the level of HbF, in heterozygous parents. As is known, the HbF levels in β-thal high A_2 heterozygotes varies from normal up to 4%. The variation in the level of HbF may be due to the presence of a genetic heterogeneity which may not be necessarily associated with β° or β^{+} genes. As seen in Fig. 2, the presence of increased levels of HbF (>3%) in one of the parents seems to have a beneficial effect towards a milder phenotype. This assumption is favored by the mild clinical manifestations observed in compound heterozygotes of β^{th}/β^{th} Dutch (high A_2 and high F>4%). Even milder clinical manifestations were described in homozygous β^{th} Dutch patients [8].

The second most common thalassemic genotype with heterogeneity in clinical manifestations was that of double heterozygotes for β^{th} and β silent (normal HbA_2 and HbF) type I and II. This genotype covers 7.4% of the patients; of interest are the differences in clinical phenotype which have been reported, and are confirmed in this series, between the type I, with mild clinical features, and type II, with severe clinical manifestations. Characteristic in this respect were the hematologic findings not only of the heterozygotes but mainly of the double heterozygotes with high HbA (>80%) in type I, and low HbA (<20%), in type II [11].

Other prevalent genotypes in our series were those of double heterozygotes for β^{th} and either $\delta\beta^{\circ}$ high HbF (6.3%) of $\delta\beta$Lepore (2.8%).

The clinical manifestations of both genotypes are generally considered mild, as the first reported series included only older children and adults with thalassemia intermedia [10]. However subsequent publications demonstrated the presence of these genotypes in children, where the clinical manifestations were more severe [4]. These findings are further supported by the extreme variation in clinical severity observed in this series. Of 30 patients, 22 had thalassemia major and were on frequent transfusions, 6 were rarely transfused and only 2 preserved high Hb levels without transfusions. Thirteen of the older patients were splenectomized because of hypersplenism.

From the data collected in this study it is concluded that the heterogeneity in clinical manifestations is basically related to the patient's genotype; it was also shown that the wide spectrum of clinical manifestations in Greek patients is mainly due to presence of nearly all known genetic variants of β and $\delta\beta$

thalassemias in Greece. Furthermore it was demonstrated that the genetic characterization of thalassemia using as criteria relevant hematologic data gives valuable information for predicting the prognosis and establishing the appropriate treatment, especially in patients with thalassemia intermedia. As shown in Fig. 2, the mild thalassemia intermedia type II was mainly found in patients with homozygous β^{++th}, homozygous β^{oth}, and compound heterozygous for β^{th}/β^{th} silent type I, and less frequently in patients double heterozygotes for β^{th}/β^{o} Dutch, $\beta^{th}/\delta\beta^{o}$ (high F), $\beta^{th}/\delta\beta$ Lepore and β^{th}/β^{th} silent type II.

Acknowledgement. This work was supported in part by the Greek Ministry of Social Services and the National Research Institut (Grant 389).

References

1. Chini V, Valeri CM (1949) Mediterranean hemopathic syndromes. Blood 4 : 989–996
2. Dacie J, Lewis SM (1975) Practical haematology, 5th edn Churchill, London
3. Friedman S, Hamilton RW, Schwartz E (1973) β-thalassemia in the American Negro, Clin Invest 52 : 1453–1459
4. Kattamis C, Metaxotou-Mavromati A, Karamboula A, Nasika E, Lehmann H (1973) The clinical and hematological findings in children inheriting two types of thalassemia: High A_2 type beta thalassemia, and high F type or delta beta thalassemia. Br J Haematol 25 : 375–348
5. Kattamis C, Karamboula K, Metaxotou-Mavromati A, Ladis V, Constantopoulos A (1978) Prevalence of β^{o} and β^{+} thalassemia genes in Greek patients with homozygous β-thalassemia. Hemoglobin 2 : 29–46
6. Kattamis C, Metaxotou-Mavromati A, Wood WG, Nash JR, Weatherall DJ (1979) The heterogeneity of normal HbA_2 β-thalassemia in Greece. Br J Haematol 42 : 109–123
7. Kattamis C (1980) Screening for hemoglobinopathies. In: Bickel H, Guthrie R, Hammersen G (eds) Neonatal screening for inborns errors of metabolism, Springer Berlin Heidelberg New York pp 133–147
8. Schokker RC, Went LN, Bok J (1966) A new genetic variant of β-thalassemia. Nature 209 : 44–48
9. Silvestroni E, Bianco J, Vallisneri E (1949) Nuovo constributo allo studio della questione genetica del morbo di Cooley. Minerva Pediar 1 : 136–145
10. Stamatoyanopoulos G, Fessas P, Papayanopoulou TF (1969) Thalassemia: A study of thirty-one families with simple heterozygotes and combination of F thalassemia and A_2 thalassemia. Am J Med 47 : 194–208
11. Weatherall JD, Clegg BJ (1981) The thalassemia syndromes, 3rd edn. Blackwell, Oxford
12. Willcox MC, Weatherall DJ, Clegg JB (1975) Homozygous beta thalassemia in Liberia. J Med Genet 12 : 165–173

Received June 18, 1982/Accepted July 16, 1982