The abnormalities of ears, nose, genitalia, hands, and feet were similar in both twins.

Case 3 (J.C., VI.9). An 8-year-old male brother of the twin girls. He did not have cryptophthalmos but there was flattening of the supraorbital ridges with pseudo-hypertelorism, cleft palate, abnormal teeth, unilateral cryptorchidism, and syndactyly. Assuming autosomal recessive inheritance these findings suggest either that he was a homozygote with less severe manifestation or a heterozygote.

Case 4 (G.C., VI.7). A stillborn male delivered prematurely. From his parents' description it seems likely that he had bilateral cryptophthalmos, syndactyly, and genital abnormalities.

Family History showed that the parents were second cousins once removed and the mother's parents were first cousins. Clinical examination (by E.S.A.) of all individuals shown in the pedigree did not disclose any abnormality. The mother said that the abortions (VI.8 and VI.14) did not show any features to suggest the syndrome.

Discussion

The twins in family 2, their parents, and two normal sibs were studied for ABO, DCEc, MN, P, Kell, and Duffy blood types. The ABO and MN were segregating allowing an overall estimation of 0.976 probability for monozygosity.

The clinical descriptions of the cases reported by Key (1920), Ashley (1947), Gupta and Saxena (1962), Sugar (1968), François (1969), and Ide and Wollschlaeger (1969), together with those described here, show cryptophthalmos to be a syndrome with a wide range of expressivity. In addition, the finding of different ocular manifestations in the monozygotic twins suggests that genetical factors alone do not account for this syndrome. The anomalies described in case 3 in family 2 may represent one of the mildest cases of the syndrome so far reported. Unfortunately, there is no way available to investigate whether the case with mild anomalies is one of low expressivity in a homozygous individual or partial manifestation in a heterozygous one.

The finding of consanguinity between the parents in both families increases the evidence favouring autosomal recessive inheritance. Both families came from the same town in the State of Bahia, had different family names, and were not known to be related. However, their birthplace was within the same 'município'. Because the kinship coefficient is higher for individuals born at shorter distances in this region, north-eastern Brazil (Azevêdo, 1969), it is easier to assume a common ancestor for both families than to admit independent mutations.

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The Genetic Variability of Thalassaemia. A Family Study

Summary. Two step-brothers, homozygotes for β -thalassaemia, have been studied. One of them showed the characteristics of Cooley's anaemia, whereas the other was almost symptomless. The existence of two different β -thalassaemic genes is discussed in relation to the haematological and clinical findings.

The thalassaemia syndromes are a group of hereditary haemolytic anaemias due to a more or less severe disorder in haemoglobin synthesis (Weatherall and Clegg, 1972). Molecules of the main human

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adult haemoglobin are built up of two α -peptide chains and two β chains ($\text{HbA} = \alpha_2\beta_2$), each being synthesized under the control of an autosomic gene (Baglioni, 1963). A third gene is also active in the adult life which leads to the synthesis of a second normal haemoglobin, HbA_2 ($\alpha_2\delta_2$), which represents 2.5% of the total haemoglobin (Weatherall and Clegg, 1972). Accordingly, different types of thalassaemic genes, which either partially or completely depress the synthesis of a specific polypeptide chain, have been found (Fessas, 1968; Weatherall and Clegg, 1972).

The primary defect in the most common thalassaemic syndromes affects the synthesis of either α or β chain, the two resulting conditions being called α - and β -thalassaemia, respectively. Each of these conditions exist in the heterozygous or in the homozygous form, the first being usually symptomless, the latter being a very severe haemolytic anaemia. Thus it appears that the thalassaemic syndromes are a large group of different conditions both from a clinical and a genetic point of view. Moreover, each group presents a broad spectrum of haematological and clinical manifestations. Studies of different cases of β -thalassaemia thus demonstrate that this condition can express itself in different ways, suggesting that different mutations are responsible for the alteration of the β -chain synthesis. In fact, different haematological entities all called β -thalassaemia have been found, and accordingly different classifications of β -thalassaemia have been proposed, based mainly on the levels of HbA_2 and HbF (Fessas, 1968; Went and Shokker, 1967).

In spite of many investigations carried out to understand the genetic defect leading to the β -thalassaemia syndromes, no explanation has been reached for the molecular mechanism responsible for the decrease of the synthesis of the β chain (Nathan *et al.*, 1971; Nienhuis, Laycock, and Anderson, 1971). Furthermore it should be noted that different mutations, leading in different ways to the same effect, ie, low β -chain synthesis, exist and thus each investigation must be considered, at most, as a possible explanation of a single case; the results cannot be generalized.

This paper reports the study of a family which shows the existence of at least two different genes for β -thalassaemia.

Methods

Routine haematological data were obtained by standard methods (Dacie and Lewis, 1966). Red blood cells were counted in TOA automatic blood cell counter.*

* Microcell Counter, Model CC-1002.

HbF was determined according to the method described by Betke, Marti, and Schlicht (1959), and quantification of HbA_2 carried out on diethylaminoethyl (DEAE)-cellulose (Bernini, 1968/1969). Serum iron was determined following the technique of Barkan and Walker (1940).

Case Reports

Case 1 (II.1). This child was first admitted to the William Soler Hospital, Habana, at 18 months of age. His past history disclosed that he was observed to have been pale at 6 months; he had been treated at his local hospital with iron ever since. He was the first child of a young mulatto couple. The father had one Chinese antecedent. At the time of his admission the patient appeared very pale, he showed prominent frontal bones and a congenital right short leg. He was unable to stand up from the sitting position and only spoke a few words. A moderate systolic murmur at the apex and the base was noticed; the liver and the spleen were 6 and 10 cm below the costal ribs, respectively. The platelet count fluctuated between 60,000 and 80,000/mm³. Other coagulation tests were normal. The total bilirubin was 1 mg%, with 0.5 mg in the direct form. Cephalin flocculation, tymol turbidity test, serum glutamic-oxaloacetic, and glutamic-pyruvic transaminase were all normal. Radiological studies showed the typical findings of a haemolytic process and agenesis of the right fibula. The bone marrow study showed an erythroid hyperplasia with a positive Prussian-blue reaction, giant metamyelocytes, giant stab cells, and scattered Gaucheroid cells.

The patient died in a rural hospital when he was 3 years old. We were informed that he had had an acute respiratory disease, together with a sudden fall of the haemoglobin level. The clinical picture of the child was suggestive of an aplastic crisis associated with bronchopneumonia, but the short course of the disease did not allow the complementary tests to be performed.

FIG. 1. Family pedigree.

TABLE I
HAEMATOLOGICAL DATA

	Hb (g/100 ml)	PCV (%)	RBC (10 ⁹ /mm ³)	MCV (μ ³)	MCH (μg)	MCHC (%)	Reticulocytes (%)	HbF (%)	HbA ₂ (%)	Serum Iron (μg%)
I.1	9.1	31	4.20	73	21	29	1.8	1.2	4.4	52
I.2	12.6	42.2	5.27	80	23	29	1.4	1.7	4.2	126
I.3	10.0	33	4.47	73	22	30	2.0	2.1	3.93	85
II.1	4.0	17.5	3.49	51	11	22	10.0	64.1	2.9	72
II.2	6.5	22	3.60	61	18	29	6.6	82.6	0.97	90

Case 2 (II.2). This child, the step-sister of case 1, was brought to our attention when she was 9 months old by her father, who asked for a check up, since he knew the nature of the disease of his son. The baby was alert and only slightly pale; she was able to hold the standing position and was able to say a few words. The liver and the spleen were about 2 cm below the costal edge. Otherwise the physical examination was normal. Total bilirubin was 1 mg%, with a direct reading of 0.4 mg%.

Cephalin flocculation, thymol turbidity test, transaminases, and radiological studies were all normal. Bone marrow studies showed marked erythroid hyperplasia and giant metamyelocytes and stabs. The Prussian-blue reaction was negative. She was seen in the outpatients clinic every month. She is now 2 years old and apart from a slight variation of the haemoglobin between 6.5 and 7.9 g% all other clinical and haematological parameters remained unchanged. It is important to note that HbF and HbA₂ did not show any variation. The baby did not require any blood transfusion, and her physical and mental development is normal.

Results and Discussion

The pedigree of the family is shown in Fig. 1. The proband (II.1) is the step-brother of case 2. The two children are offspring of the same father (I.2) who is a β -thalassaemia heterozygote, as are the two mothers (Table I). No difference can be seen from a comparison of the three parents. They are symptomless and show high HbA₂ levels, normal HbF, high osmotic resistance, and a blood smear typical of β -thalassaemia carriers. They would be considered three cases of the same entity, ie, classical high HbA₂ β -thalassaemia heterozygotes.

On the other hand the products of the two matings show remarkable differences which are mainly clinical (see above). The proband shows very severe haemolytic anaemia with the haematological, clinical, and bone changes typical of Cooley's anaemia, whereas his step-sister shows no sign of anaemia. She was never admitted to a hospital, but was studied to ascertain whether she was a β -thalassaemia heterozygote like her father. It was

very surprising to find that she was a case of thalassaemia major, firstly, because she was symptomless and secondly, because it is highly improbable to find a family pedigree such as this in a country where the β -thalassaemia gene is not very frequent (de la Torre and Colombo, 1972).

The haematological data of the two children are summarized in Table I. It can be seen that the main difference is the amount of haemoglobin, which is mainly HbF in the girl, indicating that in this case the activity of the γ -gene is sufficient to compensate for the depression of the synthesis of the β -chain whereas in the boy this compensation is inadequate.

In the pedigree two thalassaemic genes are indicated (by different shading) in the same way, although there is no demonstration that they are the same. The only indication supporting this suggestion is that the two parents (I.1 and I.2) are black, whereas the second wife (I.3), whose β -thalassaemic gene is indicated in a different way, is white. Thus it is tempting to suggest that the thalassaemic gene of negroes is different from that of whites, and that the homozygous state β -thalassaemia 'negro' is a true homozygote which leads to a severe disease, whereas the product of a mating, white $\times$ negro does not produce a true homozygote, this situation being characterized by a less severe, almost asymptomatic condition.

Studies are in progress to elucidate the difference between the two mechanisms which lead to different thalassaemic states.

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Important Announcement

A Ten-Year Cumulative Index, 1964–1973, is in the course of production in response to a continuous demand from subscribers to the *Journal of Medical Genetics*. Earlier entries have, as far as possible, been reclassified according to the recommendations of the Paris Conference (1971), and this forthcoming volume contains both an author and subject index.

For librarians, research workers, and regular readers of the journal this is an essential work of reference.

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