

α -Thalassemia in the American Negro

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ABSTRACT In Italian and Chinese patients with the α -thalassemia syndromes the production of α -chain of normal hemoglobin is decreased relative to that of β -chain in reticulocytes. In this study the relative rates of α - and β -chain synthesis were determined in members of three Negro families with α -thalassemia. Two of the families had members with hemoglobin H disease and α -thalassemia trait, while the mother of several children with α -thalassemia trait in the third family was doubly heterozygous for α -thalassemia and an α -chain mutant. The α/β ratios of globin synthesis in the patients with hemoglobin H disease and α -thalassemia trait indicated less severe biochemical defects in the peripheral blood than those previously determined in Italian and Chinese patients. In the third family, there was a heterogeneity of expression of the gene for α -thalassemia, including patients with normal red cell indices and synthesis ratios. These findings differ from those previously described in patients with α -thalassemia from other racial groups. Hydrops fetalis due to homozygous α -thalassemia may not occur in the Negro because of the relatively mild thalassemic defect.

INTRODUCTION

The expression of the α -thalassemia syndromes includes the extremes of the infant dying with hydrops fetalis in utero and the clinically and hematologically normal "silent carrier." Extensive population studies in Thailand have clarified the genetic basis of these syndromes (1-3). The infant with hydrops fetalis totally lacks α -chain production (4, 5), and is homozygous for α -thalassemia. Both parents of an affected fetus have abnormal red cell morphology, including microcytosis, mild hypochromia, and poikilocytosis. They each have α -thalassemia trait. Hemoglobin H (Hb H)¹ disease is a hypochromic hemolytic anemia in which there is a

variable amount of an abnormal hemoglobin, Hb H, which is a tetramer of normal β -chains. One parent of a patient with Hb H disease usually has α -thalassemia trait, while the other, called the "silent carrier," is hematologically normal, and carries a mild thalassemic defect. Biochemical studies of the relative synthesis of normal α - and β -globin chains in these four syndromes have demonstrated deficient α -chain synthesis, the degree of abnormality corresponding to the severity of the clinical disorder (6-8).

α -Thalassemia is found in many parts of the Far East, the Mediterranean area, and Africa (9). The presence of α -thalassemia in the American Negro is suggested by the finding of greater than trace amounts of Hb Barts, a tetramer of normal γ -chains, in 2-7% of newborn Negro infants (10, 11), indicating deficient α -chain synthesis. Despite the relatively high incidence of this genetic abnormality, Hb H disease and hydrops fetalis due to homozygous α -thalassemia have not been reported in this group.

In this study two Negro families with Hb H disease and one with α -thalassemia and an α -chain structural mutant (HbI [$\alpha^{16} \text{Iys} \rightarrow \text{g}^{16}$]) are described. Studies of globin synthesis in these families indicate a difference between the genetic abnormalities causing α -thalassemia in the Negro and in other racial groups.

METHODS

Patients. The "M" family consists of many members living in Philadelphia, New Jersey, and New York. Before moving to these areas, the family lived in Cambridge, Md. The propositus was first found to have a hemolytic anemia at age 77 when she was hospitalized for treatment of pyelonephritis. Examination of a hemolysate revealed the presence of a rapidly moving hemoglobin, Hb H. There was no family history of newborn infants dying with hydrops fetalis, nor of gallstones. Anemia had not been known to be a family problem until the present investigation. None of the persons examined had physical evidence of expansion of the intramedullary spaces of the skull. The spleens were not palpable in the family members with Hb H disease. The pedigree and hematologic values are

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¹ Abbreviation used in this paper: Hb H, hemoglobin H.

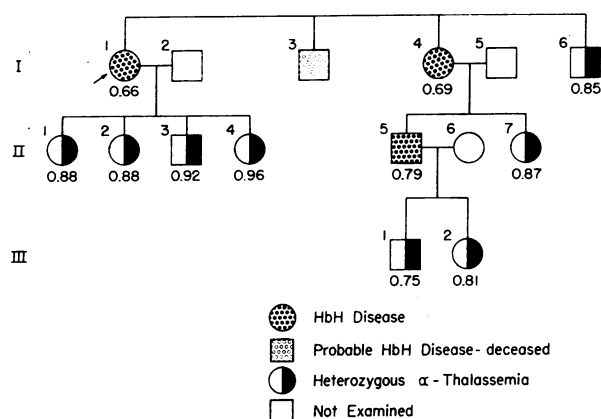


FIGURE 1 Pedigree of family M. The numbers below the symbols are the α/β ratios of specific activities.

shown in Fig. 1 and Table I. Three persons (I-1, I-4, II-5) were found to have Hb H disease, confirmed by the presence of a fast moving hemoglobin consisting only of β -chains, marked hypochromia, microcytosis, anisocytosis and poikilocytosis, reticulocytosis, and characteristic inclusions in the red cells after incubation for 1 hr with brilliant cresyl blue. In addition to the three patients with Hb H disease who were studied, a brother (I-3) who had died several years previously is believed to also have had Hb H disease. Review of the records of his hospitalization at another hospital indicated that he had mild anemia, reticulocytosis, hypochromia and microcytosis, and a persistent increase in "Hb A₂." A determination of hemoglobin A₂ level was not done initially on patient I-4, who has subsequently died.

The "R" family lives in Delaware. The proband is a 6 yr old girl who was first noted to be anemic when she was 3 yr old. Evaluation at that time at another hospital did not reveal the cause of her anemia (hemoglobin 7.6 g/100 ml). Despite therapy with iron on several occasions, her anemia persisted, and she was referred for further evaluation. Physical examination was entirely within normal limits. She was in the 50th percentile for height and weight. Laboratory studies revealed that she had Hb H disease, confirmed by the presence of Hb H, hypochromia, microcytosis, poikilocytosis, reticulocytosis, and characteristic red cell inclusions after incubation with brilliant cresyl blue. The mother and one sibling had morphologic evidence of α -thalassemia trait. The pedigree and hematologic values of this family are shown in Fig. 2 and Table I. Analysis of blood types revealed paternal exclusions in the Lutheran, MNS, and Rh systems, indicating that the putative father was not the true father.

The "W" family lives in Philadelphia. The proband, the mother, has been described previously (12). She has α -thalassemia trait and an α -chain mutant, Hb I ($\alpha^{26} \text{Leu} \rightarrow \text{Glu}$). She had four children at the time of the previous study, and has given birth twice since that time. None of the six children have Hb I on electrophoresis. Only the four youngest children were available for the present study. Three of these children had been studied in the neonatal period, and had approximately 10% of Hb Barts present on hemoglobin electrophoresis. Physical examination of each child was normal. The pedigree and hematological values for this family are shown in Fig. 3 and Table I.

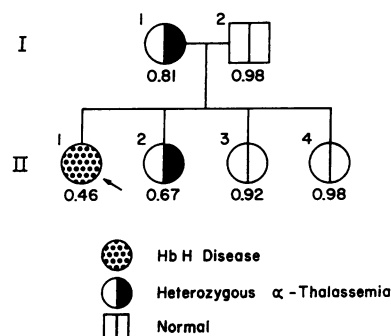


FIGURE 2 Pedigree of family R. The numbers below the symbols are the α/β ratios of specific activities.

The low hemoglobin concentration (7.7 g/100 ml) and mean corpuscular volume ($55.4 \mu^3$) of one daughter (II-3) in 1960 (12) were subsequently shown to be due to iron deficiency. Her present values are shown in Table I.

Laboratory studies. Hematologic values were determined by standard methods (13). The percentage of hemoglobin A₂ was determined by starch block electrophoresis (14). The normal range by this method in our laboratory is 1.8 to 3.3% (mean ± 2 sd). Hemoglobin F was measured by the alkali denaturation method (15). Normal subjects have less than 2% fetal hemoglobin.

Globin chain synthesis in the peripheral blood was studied by methods previously described (7, 16, 17). Whole blood was incubated with leucine-¹⁴C at 37°C for 2 hr. The globin chains were separated by column chromatography on carboxymethyl cellulose at pH 6.7 in 8 M urea with increasing concentration of sodium phosphate. The optical density and radioactivity of each of the collected fractions were measured, and specific activity (cpm/OD) for each chain was obtained by averaging the specific activities of the tubes comprising the height of each peak. The absorption of β -chains exceeds that of α -chains by a factor of 1.52 at pH 6.7 (7), necessitating an appropriate correction in the calculation of specific activities. The relative synthesis of α - and β -chains was expressed as the α/β ratio. In the four patients with Hb H disease, α/β radioactivity ratios were also calculated, after measuring the total cpm in each globin chain peak.

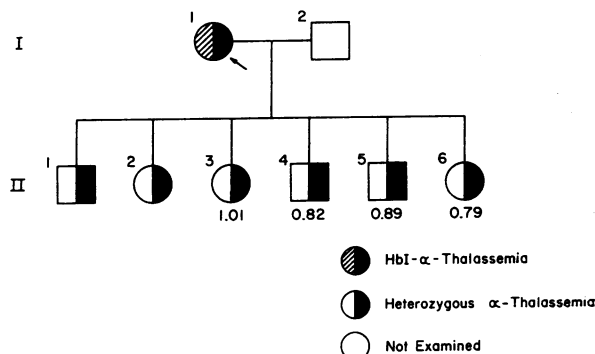


FIGURE 3 Pedigree of family W. The numbers below the symbols are the α/β ratios of specific activities.

TABLE I
Hematologic and Subunit

Subject	Age	Hemoglobin	Hematocrit	Red blood cells	Reticulocytes	Mean corpuscular volume
	yr	g/100 ml	%	$10^{-6}/mm^3$	%	μ^3
Family M						
I-1	77	10.9	34.7	6.19	4.3	56.1
I-4	68	8.5	30.0	5.60	21.0	53.6
I-6	63	12.8	40.0	5.76	1.5	69.4
II-1	59	14.3	42.0	5.19	1.3	80.9
II-2	57	12.2	40.3	5.74	2.1	70.2
II-3	50	13.5	41.7	4.60	4.3	90.7
II-4	48	13.5	43.5	5.73	2.0	75.9
II-5	48	13.8	46.0	7.33	3.3	62.8
II-7	37	11.2	34.0	4.14	1.3	82.1
III-1	28	14.8	44.0	5.66	2.4	77.7
III-2	26	15.1	46.0	6.36	2.9	72.3
Family R						
I-1	30	12.0	38.0	5.44	0.4	69.9
I-2	25	14.2	41.3	4.60	0.5	89.8
II-1	6	8.7	31.0	5.38	2.8	57.6
II-2	5	10.9	36.0	5.08	0.7	70.9
II-3	4	11.4	34.5	4.12	0.5	83.7
II-4	3	12.8	37.0	4.65	0.4	79.6
Family W						
I-1	39	12.4	40.0	4.97	3.2	80.4
II-3	15	12.3	39.3	4.35	0.2	90.3
II-4	12	11.5	38.4	4.97	1.7	77.3
II-5	9	11.7	38.2	4.60	0.3	83.0
II-6	7	11.1	36.9	4.97	0.9	74.2

* Not done.

RESULTS

The results are shown in Table I. The three patients in family M with Hb H disease had α/β specific activity ratios (0.66, 0.69, 0.79) higher than those found in any patients of Italian or Chinese descent previously studied (7, 8). The corresponding α/β radioactivity ratios were 0.55, 0.64, and 0.69. The chromatogram of patient I-4 is shown in Fig. 4. In family R, the girl with Hb H disease had an α/β specific activity ratio (0.46) near the mean of the non-Negro patients previously studied (0.47 ± 0.12 [1 sd]) (7, 8). Her α/β radioactivity ratio was 0.45. Her mother, who has α -thalassemia trait, is Negro, but it has not been possible to find the racial background of her true father. The mean ratio of these four Negro patients with Hb H disease is 0.65.

14 Negro patients in families M, R, and W with heterozygous α -thalassemia who were identified genetically had a mean α/β specific activity ratio of 0.85 ± 0.09 (1 sd). The α/β specific activity ratio in Italians

and Chinese with α -thalassemia trait is 0.77 ± 0.04 (7, 8). In the control group, the α/β specific activity ratio was 1.01 ± 0.05 . The imbalance of globin synthesis in the peripheral blood of the Negro heterozygotes is less than that previously noted in other racial groups.

Serum iron levels and iron binding capacities were normal in those patients in whom the determinations were done (family M, I-4, I-6, II-1, II-5, II-7, III-1, III-2; family R, entire family).

DISCUSSION

α -Thalassemia trait is the only α -thalassemia syndrome which has been clearly identified in the Negro. 2% of 900 Negro newborns in Baltimore had 5-10% Hb Barts in hemolysates of umbilical cord blood samples (11). Morphologic abnormalities including microcytosis and hypochromia were present in those children who were restudied after they were 1 yr old. Usually, one parent

Synthesis Data

Mean corpuscular hemoglobin	Mean corpuscular hemoglobin concentration	Hemoglobin A ₂	Hemoglobin F	Hemoglobin H	Ratio of specific activities α/β
μg	g/100 ml	%	%	%	
17.6	31.4	1.2	1.6	4.0	0.66
15.2	28.3	*	1.2	3.0	0.69
22.2	32.0	2.0	1.1	—	0.85
27.6	34.0	2.6	0.9	—	0.88
21.3	30.3	2.0	0.9	—	0.88
29.3	32.4	2.0	1.2	—	0.92
23.6	31.0	2.2	1.6	—	0.96
18.8	30.0	1.3	1.3	2.2	0.79
27.1	32.9	2.5	0.3	—	0.87
26.1	33.6	2.1	0.9	—	0.75
23.7	32.8	2.2	1.1	—	0.81
22.1	31.6	2.3	1.6	—	0.81
30.9	34.4	2.6	1.0	—	0.98
16.2	28.1	0.8	1.2	7.6	0.46
21.5	30.3	2.5	1.1	—	0.67
27.7	33.0	2.4	0.7	—	0.92
27.5	34.6	3.1	1.6	—	0.98
24.9	31.0	1.6	0.6	—	—
28.3	31.3	2.8	0.4	—	1.01
23.1	29.9	3.0	0.5	—	0.82
25.4	30.6	3.3	0.6	—	0.89
22.3	30.1	3.2	0.7	—	0.79

of an affected child had abnormal red cell morphology and diminished red cell osmotic fragility. These hematologic and genetic findings correspond to those noted in α -thalassemia trait in other racial groups (3). An earlier study of 449 Negro newborns in St. Louis had shown a 7.1% incidence of infants with Hb Barts, but the morphologic and genetic evidence of the presence of α -thalassemia was not demonstrated in all the affected infants (10).

Hemoglobin H disease and hydrops fetalis due to α -thalassemia have not been described previously in the Negro. In the present report, Hb H disease was detected in the proband in family M for the first time when she was hospitalized at age 77 for pyelonephritis. A brother had been hospitalized several times before his death for gastrointestinal complaints, without Hb H disease being suspected despite abnormalities of red cell morphology and an unusual pattern seen on hemoglobin electrophoresis. The two other affected members of the

family, mother and son, were 68 and 48 yr old at the time of the study. The mother, who was the most anemic member of the family, had been treated with various hematinics for many years. The son had never been known to be anemic, and had donated blood regularly three times a year in a government program. Splenomegaly was not noted in the affected members. Hb H comprised less than 5% of the total hemoglobin in the affected members, while affected patients in other racial groups have 5–30% Hb H (9). The globin synthesis ratios were closer to normal ratios than observed in other patients with Hb H disease (6–8). The mild nature of the clinical disorder in this family accounts for the lack of detection of the disease earlier in the patients' lives despite medical care which included hospitalizations. The absence of previous reports of Hb H disease in the Negro may be related to the mildness of the clinical manifestations of the disorder in this racial group.

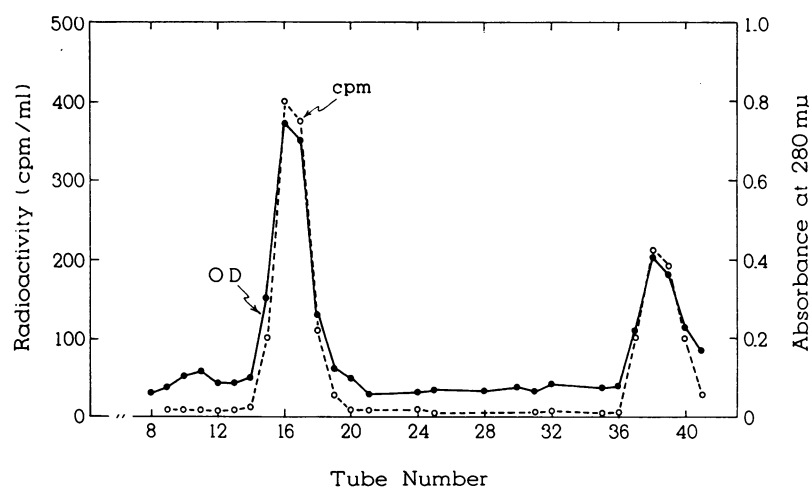


FIGURE 4 Chromatogram of globin from patient I-4 in family M. The α/β radioactivity ratio here is 0.64, and the α/β specific activity ratio is 0.69, indicating a decrease in α -chain synthesis less marked than that seen in Chinese and Italian patients with Hb H disease (5, 6).

Wasi and his coworkers have explained the different clinical and hematologic findings in α -thalassemia on the basis of two allelic genes, α -thal₁ and α -thal₂ (3). Homozygosity for α -thal₁ causes hydrops fetalis and death. α -Chain is not detectable in infants dying of hydrops fetalis due to α -thalassemia (4, 5), indicating that the α -thal₁ allele is totally deficient in directing α -chain production. Further evidence to support this conclusion comes from the observation that Chinese patients doubly heterozygous for Hb Q α and α -thal₁ (18–20) do not produce any normal α -chain. The gene for α -thalassemia trait in family W is different from the α -thal₁ gene in Orientals, since there is production of normal α -chain, although in diminished amounts, in the proband with Hb I α and α -thalassemia. The genetic abnormality is also different from that of the silent carrier (α -thal₂ trait), since three of the children had approximately 10% Hb Barts at birth while infants who are silent carriers have 1–2% Hb Barts. The gene for α -thalassemia found in this family thus appears to differ from the two types described in other races (α -thal₁ and α -thal₂). The red cell morphology and globin synthesis studies also differ from those in other racial groups. The children of the proband in family W are obligatory carriers of α -thalassemia. Their mean cell volumes range from 74.2 to 90.3 μ^3 , with corresponding values of α/β ratios of specific activity from 0.79 to 1.01. The ratios of specific activities overlap those of the non-Negro group who are heterozygous for α -thal₁ and the control group. The findings in persons heterozygous for α -thalassemia in the other two families were similar to those in family R. The heterozygotes included were children of persons with Hb H disease or relatives with

morphologic evidence of α -thalassemia trait. In family R, two siblings (II-3, II-4) with α/β ratios in the normal range were not included in the group of heterozygotes, although the mild decrease of mean cell volume in the absence of iron deficiency suggests that they may indeed have had α -thalassemia. The mean α/β ratio of the heterozygotes in family M and family R (0.84) was similar to that found in family W (0.88), where all the children were obligatory heterozygotes.

In view of the diversity of expression of α -thalassemia observed in family W, it was not possible to distinguish clearly between different groups of heterozygotes in the children of patients with Hb H disease in family M. There were no children born to these patients during the study, thus not allowing a distinction between types of heterozygotes by the amount of Hb Barts in the newborn.

The results of these studies indicate that α -thalassemia trait in the Negro differs from that found in other racial groups and suggest that the genetic basis of Hb H disease in some Negro patients may differ from that previously described. In some Negro patients, Hb H disease may be the expression of homozygosity of the gene for α -thalassemia, rather than a combination of two different genes. Two distinct groups of newborns heterozygous for α -thalassemia have been found in Thais (2, 3), while in the Negro only one group, with levels of Hb Barts corresponding to the more severe of the Thai groups, has been found (11). Hb Barts in the range of 25% has not been found in a Negro newborn, but only one such infant in 10,000 would be expected with an incidence of the trait of 2% if the suggestion of homozygosity of Hb H were correct. The present stud-

ies do not provide sufficient data to decide whether a second, milder α -thalassemia gene is present in the Negro, and whether Hb H disease in this race may be due to a double heterozygosity for α -thalassemia variants in some patients and a homozygosity in others. The absence of reports of hydrops fetalis due to α -thalassemia in the Negro may be due to the milder manifestations of the α -thalassemia gene in this group.

In two previously reported patients with Hb H disease of mixed Negro-Chinese parentage, the Negro mother in each instance had normal red cell morphology (21). The one Chinese father who was studied had a smear typical of thalassemia minor, with a normal Hb F and a slightly decreased Hb A₂, indicating that he was a carrier of the α -thal gene. The absence of previous reports of Hb H disease in the Negro has been thought to be due to an absence of the α -thal gene in the population. In these two Negro-Chinese families, however, the milder α -thalassemia gene appears to have come from the Negro mothers. The studies in this report indicate that the gene for α -thalassemia in the Negro may cause a variable expression of red cell morphology, and that normal indices may be present in a Negro with α -thalassemia trait. The findings in the two Negro-Chinese families support this observation. Examination of the parents and the newborn children of each patient with Hb H disease would provide further data, but this was not possible in the present study. A patient of Negro and American Indian ancestry with Hb H disease has also been reported (22), but red cell morphology of the parents was not described.

One patient with Hb H disease in family M had a son with Hb H disease. Similar occurrences have been reported from Thailand (1), raising the possibility of inheritance of this disease by two abnormal genes at nonallelic sites. A more likely explanation, supported by genetic data (2, 3), is that one parent has Hb H, while the other is heterozygous for α -thal or α -thal, resulting in children with Hb H disease. In family M, the father of the man with Hb H disease in the second generation came from the same town in Maryland as the mother, but it is not known if consanguinity was present.

Recent reports have suggested that there is a duplication of the α -chain locus in man (23-26). An explanation of the genetics of the α -thalassemia syndromes has been proposed on the basis of four loci for α -chains (24, 25). The finding of 100% Hb J α in two Melanesians (27) is difficult to explain in terms of the duplication theory, and suggests that racial differences may exist, with only two α -chain loci being present in some groups. Further evidence is needed to confirm the existence of two, four, or more α -chain

loci in the Negro, and to establish the relative activity of each set of loci. The findings in the α -thalassemia syndromes in Negro and non-Negro patients may be explained by two loci and thalassemia genes of varying severity, such as has been shown to exist in the β -thalassemia syndromes. The presence of the Negro gene for α -thalassemia is associated with the production of diminished amounts of α -chain, resulting in a heterozygous state which differs from α -thalassemia trait and the silent carrier in other racial groups. Further family studies and extensive screening of newborns will be needed to determine the genetic basis of Hb H disease in the Negro.

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