Hemoglobin α Chain Deficiency in Black Children with Variable Quantities of Hemoglobin Bart's at Birth

C. ALTAY,⁽³⁰⁾ B. RINGELHANN⁽³¹⁾ G. I. YAWSON, A. A. BRUCE-TAGOE, F. I. D.
KONOTEY-AHULU, L. JAMES, M. GRAVELY, AND T. H. J. HUISMAN⁽³²⁾

Laboratory of Protein Chemistry and Comprehensive Sickle Cell Center, Medical College of Georgia, Augusta, Georgia, USA; National Institute for Rheumatism, Budapest, Hungary, and Ghana Institute of Clinical Genetics, Accra, Ghana

Summary

Hematologic and globin chain synthesis studies have been made in 21 children, aged 2 to 6 years, many of their parents, and several normal adults and α -thalassemia heterozygotes. At birth, 11 children had about 5% hemoglobin (Hb) Bart's, 5 had about 2% Hb Bart's, and 5 had no trace of Hb Bart's. A significant decrease in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values and an increase in the β/α ratio was observed in the first group; microcytosis and hypochromia were absent in the children of the second group although the β/α ratio was significantly increased. The α chain deficiency is familial. Increased α/α ratios were present in many parents although only two parents of children with 5% Hb Bart's at birth had hematologic findings suggestive of the presence of the same type of defect as observed in the children with the larger amount of Hb Bart's at birth.

Speculation

It is postulated that the absence or presence of duplicated Hb $_{\alpha}$ structural genes is the underlying mechanism for the variable α chain deficiency in black infants. Children with about 5% Hb Bart's at birth have the genotype $-\alpha/-\alpha$ or, rarely, the $-/\alpha\alpha$ genotype; when only two Hb $_{\alpha}$ structural loci instead of four are active, a modest deficiency in α chain production will be the result. The presence of the $-\alpha/\alpha\alpha$ genotype could be predicted from the small amounts of Hb Bart's at birth and from data of the hemoglobin synthesis analyses in older children and adults; the $-\alpha/\alpha$ genotype however, is also suggested from data obtained by MCH and MCV determinations. It is concluded that although the $-\alpha/\alpha$ genotype always produces Hb Bart's at birth in moderate amounts, the $-\alpha/\alpha\alpha$ genotype may or may not. The rarity of the $-/\alpha\alpha$ genotype in this population is responsible for the absence of the Hb Bart's hydrops fetalis syndrome.

INTRODUCTION

The presence of Hb Bart's or γ_4 in blood of newborn babies is regarded as an indication for some form of a chain deficiency which is often termed α -thalassaemia (for a review and references see (28)). The expression of this type of deficiency includes extremes; for instance, a total α chain deficiency exists in the infant with hydrops fetalis whereas others appear clinically and hematologically normal. The various forms of α -thalassaemia (discussed in (28)) can best be differentiated by the relative amounts of Hb Bart's at birth. In the Far East, α -thalassaemia-1 is recognized because it is associated with complete suppression of the α chain synthesis and α -thalassaemia-2 is associated with partial suppression of a chain synthesis; homozygosity for each of these conditions and a double heterozygosity contribute to the heterogeneity of the α chain deficiency syndromes (23,25).

Hb Bart's has also been found in blood from Black newborns (5,7,8,9,10 and others) and in newborns from various other racial and/or ethnic origins such as Saudia Arabia (22), Cuba (19), Malaya (18) (see also Table I of ref. 5). About 5% of Nigerian newborns had Hb Bart's and a comparable incidence has been observed in others. The quantities of Hb Bart's vary considerably which, at least in part, can be explained by differences in methods used for quantitation. However, the amount does not exceed 10%, and Hb H disease and hydrops fetalis have never been found among African Blacks. Some investigators (7,8,10,27) suggest that the presence of Hb Bart's indicates an inherited α chain deficiency (although a previous limited study by one of the present authors (10) did not substantiate this hypothesis) but others (5) believe that its occurrence is a developmental abnormality.

A survey of 14,053 newborns which was conducted by one of the authors over a period of 15 years (13) showed that 366 (or 2.65%) had moderate amounts of Hb Bart's, (later to be defined as the "children with 5% Hb Bart's") whereas 1330 babies (9.45%) had small amounts of Hb Bart's (the children with less than 2% Hb Bart's). None of the 14,000 newborns had high amounts of Hb Bart's, comparable to quantities found in newborns with Hb H disease (25). In this communication, we describe data on 21 Black children, aged 2 to 6 years, who had variable amounts of Hb Bart's (from zero to 5%) at birth using both hematological and *in vitro* globin chain synthesis studies. In order to further evaluate the significance of the Hb Bart's anomaly in the newborn period, many of the parents of these children were also studied. The data are interpreted to indicate the presence of either single or duplicated Hb_B chain loci on one or both chromosomes.

MATERIALS AND METHODS

Children and Adults Participating in the Study

Fifteen Black children from the Augusta area were selected from a roster of cases who had been tested in 1973 and 1974 by one of the authors (T.H.J.H.) for the possible presence of Hb Bart's and other hemoglobin variants. Five children, aged 19-36 months, had about 5% Hb Bart's at birth, five, aged 24-37 months, had 1-2% Hb Bart's at birth, while five, aged 21-24 months, had no Hb Bart's. Quantitation of Hb Bart's at that time was based in part on the visual inspection of a starch gel block stained with α -dianisidine (3) and on data obtained by column chromatography using the cation exchanger CM-cellulose (14). The variability in the amounts of Hb Bart's has been described before (10); the group referred to in that study as having "small quantities of Hb Bart's" will be termed here "children with 2% Hb Bart's" and that having "moderate amounts of Hb Bart's" will be termed "children with 5% Hb Bart's." The actual level of Hb Bart's varied considerably also because the Hb Bart's zone isolated by column chromatography was often contaminated with small amounts of other hemoglobins such as the minor Hb F₁ and Hb A₁ components. Methods to improve the quantitation of Hb Bart's are presently under study; however, it appears that the amount of Hb Bart's in newborns of the "2% Hb Bart's" group varies between 1% and 2%, and that in newborns of the "5% Hb Bart's" group between 3.5% and 7% (14). Parents of the 10 children with Hb Bart's were also available; none had an abnormal α or β chain variant or a β -thalassaemia trait. Parents of the children who had no Hb Bart's at birth were not studied.

A fourth group of children were studied in Ghana in 1974. These 6 children were found to have 4 to 6% Hb Bart's at time of birth, and were reinvestigated at the age of about 4 years together with their mothers. Two children were also heterozygous for Hb S, and one for Hb C, while three mothers had a Hb S heterozygosity.

Eight healthy Caucasian adults and 14 healthy Black adults (mostly laboratory workers and their relatives) served as control groups. In addition, 5 persons with a definite α thalassaemia trait were also available. One was a Black female described before (20), and the others were of Greek or Chinese descent and related to persons with Hb H disease.

Hematological and Hemoglobin Studies

Blood samples were collected in vacutainers and either transported in ice to the laboratory or studied in part at the home of the donor. Hematological measurements were made with a Coulter Counter model S electronic cell counter. Other hematological determinations were made by standard methods (29). Red cell hemolysates were prepared as described before (4) and analyzed by starch gel electrophoresis for the detection of abnormal hemoglobin (3), by micro-column chromatography for the quantitation of Hb A₂ (12) and by an alkali denaturation procedure for the quantitation of Hb F (12). Hemoglobins S and C were quantitated by column chromatography using DEAE-Sephadex (2,11).

Hemoglobin Synthesis Studies

Globin synthesis determinations were made by the method of Friedman *et al.* (6) as modified in our own laboratory (13). Washed cells, enriched in reticulocytes by fractional centrifugation, were incubated for 120 minutes with ¹⁴C leucine in an appropriate medium either in the home of the donor or soon after arrival in the laboratory (13). Separation of the α and β chains was made by column chromatography, and the β/α ratio was calculated using the total radioactivity present in the chromatographic fractions corresponding to each of the hemoglobin chains.

Statistical Studies

The significance of the differences between two mean values was assessed by the Student "t" test.

RESULTS

Hematologic and Hemoglobin Studies

Starch gel electrophoretic examination showed that none of the children from Georgia who had Hb Bart's at birth had any hemoglobin variant at the time of examination; notably, no trace of Hb H or Hb Bart's was detectable.

The same was true for the children from Ghana except that 3 were heterozygous for a β chain variant.

A summary of the most pertinent hematological data is given in Table I; Tables II, III, and IV which are part of the addendum to this paper give details on the individual cases. Serum iron levels were normal although in 2 children the level (10 μ mol/l) was in the low range of our normal values (9-30 μ mol/l). Similar data were not available for the children from Ghana. β -thalassaemia was absent and the level of Hb F (as F_{AB}) was less than 2% in all cases. The levels of Hb S (or Hb C together with Hb A₂) in the 3 children from Ghana were 23, 25 and 30%. In one of the mothers the level was low (23%) and in two the levels were 31 and 32%. Examination of peripheral blood smears showed a mild hypochromia, anisocytosis and poikilocytosis in all children who had 5% Hb Bart's at birth, in two of their parents, and in all 5 subjects with a thalassaemia trait. Fig. 1 gives an example.

Most significant were the differences in the MCV and MCH values and the β/α synthetic ratios. The individual data for the children from Georgia, their parents, and the persons serving as adult controls are plotted in Figures 2 and 3. The rather marked microcytosis with hypochromia in the children with 5% Hb Bart's was comparable to that found for the 5 adults with a thalassaemia trait. The microcytosis and hypochromia were absent in the children of the 2% Hb Bart's group; their values were not different from those found for the 5 children of the 0% Hb Bart's group. The parents of the "5% Hb Bart's" children showed a wide range in MCV and MCH values; two persons (the father of child II and the mother of child III) had a marked hypochromia and microcytosis. The values for the parents of the children with the 2% Hb Bart's fell in the range of normal and were not different from the data found in the persons who served as controls.

Hemoglobin Synthesis Studies

The results of the *in vitro* biosynthetic analyses (Fig. 3) again indicate significant differences between the various groups. A distinct α chain deficiency was evident for all 5 children with 5% Hb Bart's (average β/α ratio of 1.51). This deficiency was also present in the children with 2% Hb Bart's but much less marked (average β/α ratio of 1.27). The average β/α ratio of the children without Hb Bart's showed a balanced chain synthesis; however, at least in one child a mild α chain deficiency is apparent. The majority of the parents of the children with the 5% and the 2% Hb Bart's showed a mild α chain deficiency (the β/α ratio in 14 of the 18 persons with acceptable results fell between 1.2 and 1.5), 2 parents (both of children of the 5% Hb Bart's group and both having a distinct microcytosis and hypochromia) had an overt α chain deficiency, and 2 had a balanced chain synthesis. The β/α ratios of the 5 persons with the α -thalassaemia trait were higher with the exception of a ratio of 1.33 in one case; this person might well have been misdiagnosed and likely has a milder form of α -thalassaemia. The differences in β/α ratio between the Black and Caucasian adults, although statistically not significant, are of interest; it appears that 5 of the 14 Black controls had a mild α chain deficiency with a β/α ratio ranging between 1.17 and 1.35.

Statistical Studies

Statistical analyses of all data are presented in Table V of the Addendum.

DISCUSSION

The data obtained in this study indicate that an α chain deficiency is the underlying cause for the presence of Hb Bart's that is present in some Black children at birth. This deficiency persists throughout life, is genetically determined, and is not a developmental abnormality as suggested by Esan (5). Friedman *et al.*, reached a similar conclusion and state the "the hematologic data, globin synthesis ratios, and a chain pool studies indicate that Black infants with greater than 2% Hb Bart's have α -thalassaemia" (7).

The synthesis of adult hemoglobin is under the control of genes for the α chain and for the β production. Lehmann (16,17) has proposed that the α chain gene in man can be duplicated, and results from studies on persons with abnormal α chain variants (for references and review see (20)) have indicated that multiple α chain loci do occur in some populations but not in all. The genetic heterogeneity of a chain deficiency (or α -thalassaemia) therefore can also be explained on the basis that production of a chain depends upon control by duplicated α chain gene loci, $\alpha\alpha/\alpha\alpha$ (26). The absence or the lack of function of from one to all four genes would account for all the different phenotypes (17,26). Thus, the two types of a thalassaemia trait (α thalassaemia-1 and a thalassaemia-2) can be considered to represent the genotypes $-\alpha/\alpha$ and $-\alpha/\alpha$, Hb H disease, that of the genotype $-\alpha/-\alpha$ and the hydrops fetalis syndrome of the genotype $---/--$. This concept has been supported by data from hybridization studies (15,21,24) indicating that a chain genes are deleted in some of these conditions.

This concept allows two genotypes for an individual with abnormalities characteristic for a thalassaemia-1 trait, namely $-\alpha/\alpha$ and $-\alpha/-\alpha$. In both, only two active α chain structural loci are present. Parents with the $-\alpha/\alpha$ genotype will have a 1 in 4 chance of having an offspring with genotype $---/--$, i.e. a baby with hydrops fetalis; however, such a baby will not be born to parents with genotype $-\alpha/-\alpha$. Assuming an incidence of about 2.5% of a thalassaemia-1 trait, i.e. either the genotype $-\alpha/\alpha$ or $-\alpha/-\alpha$, in the black population of Georgia (13) and considering the fact that the Hb Bart's hydrops fetalis syndrome with genotype $---/--$ is absent in this Black population one has to assume that the $-\alpha/-\alpha$ genotype is the major genotype causing a deficiency in a chain production. Thus, the presence of genotype $-\alpha/-\alpha$ results in the presence of about 5% Hb Bart's at birth, in a persistent α chain deficiency with β/α ratios averaging about 1.5 and with a modest hypochromia and microcytosis. The $-\alpha/\alpha$ genotype is responsible for the presence of much smaller amounts of Hb Bart's at birth (perhaps some babies with this genotype do not produce any Hb Bart's at all), for a most modest α chain deficiency with an average β/α ratio of about 1.25 without significant microcytosis and hypochromia. It often appears difficult to distinguish between the expression of genotypes $-\alpha/\alpha$ and $\alpha\alpha/\alpha\alpha$; babies with the latter do not have an α chain deficiency.

Based on these assumptions, one is able to assign a specific genotype to the individuals who participated in this study (Table II of the Addendum). It was not always possible to decide with reasonable confidence between specific genotypes; moreover, the presence of genotype $-\alpha/\alpha$ cannot be excluded because of its possible introduction in this population through admixture with persons of Asian origin (see also 20).

The pedigrees of Fig. 4 illustrate again the familial aspects of the α chain deficiency (the data are from Table III; family X refers to the fifth family of the group of children with 5% Hb Bart's and Family Y to the first family of the group with 2% Hb Bart's). The parents in Family X have a mild α chain deficiency but their child has a β/α chain ratio of 1.50 with a distinct microcytosis and hypochromia. The likely genotypes are listed in the figure. The presence of $-\alpha/\alpha$ genotype in both parents of Family Y (despite

a high β/α ratio of 1.49 in the mother) is supported by two different genotypes observed in the offspring. These data and the hematological findings clearly indicate that while the $-a/\alpha\alpha$ genotype does not produce significant hematological abnormalities, the $-a/-a$ type is not only detectable by an increased *in vitro* synthesis ratio but also by decreased MCH and MCV values.

The results of these studies also explain the rather wide variability in β/α chain synthesis ratios observed in normal (Black) controls. The presence of a relatively high incidence of the $-a/\alpha\alpha$ genotype (estimated at about 10% in ref. 13 but probably higher because small amounts of Hb Bart's at birth are not always critically evaluated) is likely responsible for the β/α ratios above 1.2 (33,34).

REFERENCES

- Betke, K., Marti, H. R. and Schlicht, I.: Estimation of small percentages of foetal haemoglobin. *Nature*, 184: 1877, 1959.
- Dozy, A. M., Kleihauer, E. F. and Huisman, T.H.J.: Studies on the heterogeneity of hemoglobins. XIII. Chromatography of various human and animal hemoglobin types on DEAE-Sephadex. *J. Chromatogr.*, 32: 723, 1968.
- Efremov, G. D., Huisman, T.H.J., Smith, L. L., Wilson, J. B., Kitchens, J. L., Wrightstone, R. N. and Adams, H. R.: Hemoglobin Richmond, a human hemoglobin which forms asymmetric hybrids with other hemoglobins. *J. Biol. Chem.*, 244: 6105, 1969.
- Efremov, G. D. and Huisman, T.H.J.: The laboratory diagnosis of hemoglobinopathies. *Clin. in Hematol.*, 3: 527, 1974.
- Esan, G.J.F.: Haemoglobin Bart's in newborn Nigerian. *Brit. J. Haematol.*, 22: 73, 1972.
- Friedman, S., Hamilton, R. W. and Schwartz, E.: β -thalassaemia in the American Negro. *J. Clin. Invest.*, 52: 1453, 1973.
- Friedman, S., Atwater, J., Gill, F. M. and Schwartz, E.: α -thalassaemia in Negro Infants. *Pediat. Res.*, 8: 955, 1974.
- Henrickse, R. G., Boyo, A. E., Fitzgerald, P. A. and Ransome Kutí, S.: Studies on the haemoglobins of newborn Nigerians. *Brit. Med. J.*, 1: 611, 1960.
- Honig, G. R., Gunay, U., Mason, R. G., Vida, L. N. and Ferenc, C.: Sick cell syndromes. I. Hemoglobin SC- α -thalassaemia. *Pediat. Res.*, 10: 613, 1976.
- Horton, B. F., Thompson, R. B., Dozy, A. M., Nechtman, C. M., Nichols, E. and Huisman, T.H.J.: Inhomogeneity of hemoglobin. VI. The minor hemoglobin components of cord blood. *Blood*, 20: 302, 1962.
- Huisman, T.H.J. and Dozy, A. M.: Studies on the heterogeneity of hemoglobin. IX. The use of tris (hydroxymethyl) aminoethane HCl buffers in the anion exchange chromatography of hemoglobins. *J. Chromatogr.*, 19: 160, 1965.
- Huisman, T.H.J., Schroeder, W. A., Brodie, A. R., Mayson, S. M. and Jakway, J.: Microchromatography of hemoglobins. III. A simplified procedure for the determination of hemoglobin A₂. *J. Lab. Clin. Med.*, 80: 700, 1975.
- Huisman, T.H.J. and Jonxis, J.H.P.: The Hemoglobinopathies, Techniques of Identification. Marcel Dekker Inc., New York, *in press*.
- Huisman, T.H.J.: Unpublished observations.
- Kan, Y. W., Dozy, A. M., Varmus, H. E., Taylor, J. M., Holland, J. P., Lie-Injo, L. E., Ganesan, J. and Todd, D.: Deletion of α -globin genes in haemoglobin H disease demonstrates multiple α -globin structural loci. *Nature*, 255: 255, 1975.
- Lehmann, H. and Carrell, R. W.: Differences between α - and β -chain mutants of human haemoglobin and between α - and β -thalassaemia. Possible duplication of the α -chain gene. *Brit. Med. J.*, 4: 748, 1968.
- Lehmann, H.: Different types of alpha-thalassaemia and significance of haemoglobin Bart's in neonates. *Lancet*, 2: 78, 1970.
- Lie-Injo, L. E. and Ti, T.S.: The fast moving haemoglobin component in healthy newborn babies in Malaya. *Med. J. Malaya*, 16: 107, 1961.
- Martinez, G., and Colombo, B.: α -Thalassaemia in Cuba. *Acta Haematol.*, 55: 36, 1976.
- Milner, P. F. and Huisman, T.H.J.: Studies on the proportion and synthesis of haemoglobin G-Philadelphia in red cells of heterozygotes, a homozygote, and a heterozygote for both haemoglobin G_a and α -thalassaemia. *Brit. J. Haematol.*, 34: 207, 1976.
- Ottolenghi, S., Lanyon, W. G., Paul, J., Williamson, R., Weatherall, D.J., Clegg, J. B., Pritchard, J., Pootrakul, S. and Wong, H. B.: Gene deletion as the cause of α -thalassaemia. *Nature*, 251: 389, 1974.
- Pembrey, M. E., Weatherall, D. J., Clegg, J. B., Bunch, C., and Perrine, R. P.: Haemoglobin Bart's in Saudi Arabia. *Brit. J. Haematol.*, 29: 221, 1975.
- Pootrakul, S., Sappara, S., Wasi, P., Na-Nakorn, S., and Suwanik, R.: Haemoglobin synthesis in 28 obligatory cases for α -thalassaemia traits. *Humangenetik*, 29: 121, 1975.
- Taylor, J. M., Dozy, A. M., Kan, Y. W., Varmus, H. E., Lie-Injo, L. E., Ganesan, J., and Todd, D.: Genetic lesion in homozygous α -thalassaemia (hydrops fetalis). *Nature*, 251: 392, 1974.
- Wasi, P.: The alpha thalassaemia genes. *J. Med. Ass. Thailand*, 53: 677, 1970.
- Wasi, P., Na-Nakorn, S. and Pootrakul, S.: The α -thalassaemia. *Clin. in Haematol.*, 3: 383, 1974.
- Weatherall, D. J.: Abnormal haemoglobins in the neonatal period and their relationship to thalassaemia. *Brit. J. Haematol.*, 9: 265, 1963.
- Weatherall, D. J., and Clegg, J. B.: The thalassaemia syndromes. Blackwell Scientific Publications, Oxford, 1972.
- Wintrobe, M. M., Lee, G. R., Boggs, D. R., Bithell, T. C., Athens, J. W. and Foerster, J.: *Clinical Hematology*, Ed. 7, Lea and Febiger, Philadelphia, Pa., USA, 1974.
- On leave from the Department of Pediatrics, Hacettepe University, Ankara, Turkey.
- Dr. Ringelmann was, in part, responsible for the studies conducted in Ghana while temporarily stationed in that country.
- Correspondence and request for reprints should be addressed to: Dr. T.H.J. Huisman, Laboratory of Protein Chemistry, Medical College of Georgia, Augusta, GA 30902, USA.
- Informed consent was obtained for all subjects in this study.
- Supported in part by U. S. Public Health Service Research Grants HL-05168, and HL-15158.

TABLE I. The mean values, standard deviations, and ranges of some hematological findings.

Subject	Age in mos. and (no. of cases)	Hb g/dl	RBC $10^{12}/l$	PCV l/l
Children with 5% Hb Bart's	28 $\pm$ 6.2 (5)	11.64 $\pm$ 1.52 (9.6 - 13.6)	5.39 $\pm$ 0.51 (4.58 - 5.66)	0.333 $\pm$ 0.03 (0.290 - 0.376)
Children with 2% Hb Bart's	31 $\pm$ 5.4 (5)	12.34 $\pm$ 0.89 (10.9 - 13.3)	4.55 $\pm$ 0.27 (4.24 - 4.85)	0.362 $\pm$ 0.02 (0.337 - 0.377)
Children from Ghana with 5% Hb Bart's	48 (6)	10.78 $\pm$ 0.29 (10.4 - 11.1)	5.14 $\pm$ 0.42 (4.65 - 5.75)	0.356 $\pm$ 0.01 (0.345 - 0.370)
Children without Hb Bart's	23 $\pm$ 1.4 (5)	12.58 $\pm$ 1.12 (11.3 - 14.2)	4.59 $\pm$ 0.54 (4.00 - 5.20)	0.357 $\pm$ 0.03 (0.333 - 0.400)
Parents of children with 5% Hb Bart's	- (10)	13.40 $\pm$ 1.89 (10.9 - 16.9)	4.90 $\pm$ 0.56 (3.89 - 5.28)	0.394 $\pm$ 0.05 (0.320 - 0.464)
Parents of children with 2% Hb Bart's	- (9)	14.41 $\pm$ 1.90 (11.9 - 17.4)	4.65 $\pm$ 0.48 (4.01 - 5.59)	0.419 $\pm$ 0.05 (0.360 - 0.481)
Mothers of children from Ghana	- (6)	11.73 $\pm$ 1.13 (10.2 - 13.3)	4.12 $\pm$ 0.50 (3.57 - 4.98)	0.378 $\pm$ 0.05 (0.330 - 0.445)
Caucasian Adults	- (8)	14.04 $\pm$ 2.00 (10.6 - 16.2)	4.68 $\pm$ 0.65 (3.38 - 5.61)	0.415 $\pm$ 0.06 (0.311 - 0.500)
Black Adults	- (14)	12.99 $\pm$ 1.56 (10.3 - 15.1)	4.38 $\pm$ 0.54 (3.64 - 5.76)	0.380 $\pm$ 0.04 (0.201 - 0.461)
Adults with α -thal. trait	- (5)	12.00 $\pm$ 1.59 (10.8 - 14.8)	5.20 $\pm$ 0.86 (4.56 - 6.71)	0.379 $\pm$ 0.04 (0.335 - 0.442)

Subject	MCV fl	MCH pg	MCHC g/dl	Hb Synthesis non-a/a
Children with 5% Hb Bart's	63.6 ± 3.2 (60 - 67)	22.02 ± 1.36 (20.6 - 23.8)	35.1 ± 1.50 (33.5 - 36.9)	1.514 ± 0.096 (1.40 - 1.63)
Children with 2% Hb Bart's	79 ± 2.12 (77 - 82)	26.68 ± 1.59 (25.1 - 28.9)	34.24 ± 1.85 (32.4 - 35.8)	1.27 ± 0.070 (1.20 - 1.36)
Children from Ghana with 5% Hb Bart's	68.4 ± 5.68 (60 - 75)	21.25 ± 1.64 (18.5 - 23)	30.67 ± 0.26 (30.5 - 31.0)	1.47 ± 0.234 (1.20 - 1.90)
Children without Hb Bart's	78 ± 4.95 (74 - 86)	27.12 ± 2.05 (25.1 - 30.5)	35.2 ± 1.41 (33.3 - 37.0)	1.07 ± 0.149 (0.86 - 1.26)
Parents of children with 5% Hb Bart's	79.5 ± 6.88 (66 - 89)	27.02 ± 3.12 (21.6 - 32.2)	34.29 ± 1.39 (32.6 - 36.6)	1.379 ± 0.267 (1.10 - 1.84)
Parents of children with 2% Hb Bart's	89 ± 4.69 (85 - 98)	30.5 ± 2.19 (28.3 - 35.6)	34.50 ± 1.71 (32.3 - 36.9)	1.28 ± 0.152 (0.94 - 1.49)
Mothers of children from Ghana	91 ± 5.29 (85 - 98)	29 ± 1.82 (27 - 31)	31 ± 1.82 (30 - 35)	1.127 ± 0.259 (0.84 - 1.43)
Caucasian Adults	89 ± 5.70 (79 - 98)	29.85 ± 2.05 (26.4 - 31.6)	33.60 ± 2.04 (29.0 - 35.3)	1.038 ± 0.10 (0.88 - 1.18)
Black Adults	86.86 ± 5.03 (78 - 95)	29.71 ± 1.72 (27.2 - 32.1)	34.51 ± 1.05 (32.0 - 36.8)	1.11 ± 0.121 (0.99 - 1.35)
Adults with α-thal. trait	72.8 ± 0.83 (65 - 76)	23 ± 0.83 (21.7 - 24)	31.82 ± 1.25 (30.4 - 33.7)	1.65 ± 0.202 (1.33 - 1.87)

ADDENDUM

This addendum consists of four tables, as follows: Table II presents the data collected on each of the Black children and their parents. Table III gives similar data on the children studied in Ghana and their mothers while comparable data on normal Caucasian and Black adults and on the five α thalassemia heterozygotes are listed in Table IV. Detailed statistical analyses are given in Table V.

TABLE II Hematological and Hemoglobin Synthesis Data on children and their parents.

Subjects *	RBC 10 ¹² /l	Hb g/dl	PCV l/l	MCV fl	MCH pg	MCHC g/dl	Fe μmol/l	TIBC μmol/l	Suggested Genotype	HEMOGLOBIN SYNTHESIS		
										Total β α	Count β/α	
<u>Children with 5% Hb Bart's and their parents.</u>												
I- M-36	5.39	12.3	0.340	62	22.5	36.9	13	80	-α/-α	739	466	1.59
Mother	4.55	12.5	0.360	78	27	35.2	13	59	-α/-α	1230	878	1.40
Father	5.28	14.2	0.430	81	26.5	33.2	19	69	-α/-α	2286	1826	1.25
II- F-19	4.70	10.8	0.320	67	22.5	33.9	13	57	-α/-α or --/αα	3147	2298	1.40
Mother	3.89	10.8	0.320	81	27.4	34.2	17	103	-α/αα or αα/αα	26885	24406	1.10
Father	5.96	14.4	0.440	74	23.7	32.6	16	62	-α/-α or --/αα	1289	702	1.84
III M-31	4.58	9.6	0.290	62	20.7	33.5	13	46	-α/-α or --/αα	3848	2361	1.63
Mother	4.96	10.9	0.340	66	21.6	32.7	18	61	-α/-α or --/αα	2458	1359	1.81
Father	5.17	13.5	0.400	76	25.7	34.1	22	66	-α/αα	1228	920	1.34
IV M-25	5.66	11.9	0.340	60	20.6	34.8	10	100	-α/-α	3971	2739	1.45
Mother	4.41	11.9	0.345	77	26.5	34.7	22	93	-α/αα	2584	2101	1.23
Father	4.96	14.4	0.435	87	28.7	33.4	-	-	-α/αα	-	-	-
V F-29	5.61	13.6	0.376	67	23.8	36.4	18	63	-α/-α	2050	1363	1.50
Mother	4.64	14.5	0.403	86	30.9	36.2	25	88	-α/αα	3079	2575	1.20
Father	5.16	16.9	0.464	89	32.2	36.6	32	60	-α/αα	614	494	1.24
<u>Children with 2% Hb Bart's and their parents.</u>												
I F-36	4.54	12.4	0.374	82	26.9	33.4	20	59	-α/αα	1370	1040	1.31
Mother	4.27	12.5	0.387	90	28.8	32.3	16	86	-α/αα	4420	3240	1.36
Father	4.39	14.1	0.425	96	31.7	33.4	28	62	-α/αα	2510	1682	1.49
Brother (13 yrs)	4.69	11.5	0.357	75	24.2	32.4	15	63	-α/-α	4305	2359	1.82
II F-30	4.85	12.4	0.377	77	25.1	33.0	10	48	-α/αα	1889	1390	1.36
Mother	4.92	14.1	0.429	86	28.3	33.1	33	93	-α/αα	539	408	1.32
Father	4.94	14.9	0.441	88	29.6	33.9	33	62	-α/αα	2493	2067	1.20
III F-24	4.79	13.3	0.373	77	27.3	35.8	21	71	-α/αα	1269	1058	1.20
Mother	4.63	14.2	0.400	87	30.1	36.3	20	74	-α/αα	2058	1634	1.26
Father	4.83	17.4	0.480	98	35.6	35.6	24	60	-α/αα	1624	1203	1.35
IV M-37	4.34	12.7	0.350	80	28.9	36.6	26	54	-α/αα	2110	1759	1.20
Mother	4.27	13.3	0.364	85	30.8	36.9	33	64	-α/αα	991	787	1.26
Father	5.59	17.3	0.481	85	30.5	36.2	23	64	-α/αα	1057	783	1.35
V F-29	4.24	10.9	0.337	79	25.2	32.4	20	59	-α/αα	2180	1691	1.28
Mother	4.01	11.9	0.360	89	29.1	33.1	21	65	αα/αα	2777	2956	0.94
Father	-	-	-	-	-	-	-	-	-α/αα	-	-	-
<u>Children without Hb Bart's.</u>												
F-24	5.13	13.1	0.380	74	25.1	34.4	15	84	αα/αα	948	903	1.05
F-22	4.00	12.4	0.348	86	30.5	35.8	18	70	αα/αα	1566	1820	0.86
F-24	5.20	14.2	0.400	77	27	35.5	16	83	αα/αα	4358	4230	1.03
F-21	4.32	11.9	0.324	74	27	37	22	77	αα/αα or -α/αα	1700	1478	1.15
M-24	4.28	11.3	0.333	79	26	33.3	19	54	-α/αα	2758	2184	1.26

* M= Male, F=Female; number refers to age in months.

** Normal value is 9-30 μmol/l.

TABLE III Hematological and Hemoglobin Synthesis Data on Children from Ghana and their Mothers.

Subject *	RBC 10 ¹² /l	Hb g/dl	PCV l/l	MCV fl	MCH pg	MCHC g/dl	Hb Type	Suggested Genotype	HEMOGLOBIN SYNTHESIS		
									β^S or β^C	Total Count	α non- α/α
I - Child	4.65	10.6	0.355	75	23	30.5	A	- α / α		739	486 1.52
Mother	4.98	13.3	0.445	89	27	30	AS(23)	- α / $\alpha\alpha$	204	442	457 1.42
II - Child	5.03	11.1	-	-	22.5	-	AA	- α / α		746	392 1.90
Mother	3.57	10.2	0.330	92	29	31.5	A	- α / α		579	405 1.43
III - Child	4.74	10.4	0.345	72	22	30.5	A	- α / α or - α / $\alpha\alpha$		1422	995 1.43
Mother	4.21	11.3	0.365	86	27	31.5	A	- α / $\alpha\alpha$ or $\alpha\alpha$ / $\alpha\alpha$		2028	1946 1.04
IV - Child	5.75	10.6	0.350	60	18.5	31	AC(30)	- α / $\alpha\alpha$	236	608	608 1.39
Mother	3.75	11.4	0.365	96	31	32		$\alpha\alpha$ / $\alpha\alpha$		2136	2547 0.84
V - Child	5.31	10.9	0.370	67	20.5	30.5	AS(25)	- α / α or - α / $\alpha\alpha$	295	755	878 1.20
Mother	4.29	12.8	0.425	98	30	30.5	AS(31)	- α / $\alpha\alpha$ or $\alpha\alpha$ / $\alpha\alpha$	768	1627	2070 1.16
VI - Child	5.36	11.1	0.370	68	21	31	AS(23)	- α / $\alpha\alpha$	93	320	295 1.40
Mother	3.89	11.4	0.335	85	30	35	AS(32)	$\alpha\alpha$ / $\alpha\alpha$	345	765	1275 0.87

* The children were 3 to 5 years old at the time of the study.

** Values between parenthesis indicate the percent of the abnormal hemoglobin.

*** Data from Hb synthesis analyses make it unlikely that the mothers have the - α / $\alpha\alpha$ genotype; therefore, the children carry a duplicated α chain locus on at least one chromosome and likely have the - α / $\alpha\alpha$ genotype. The low MCV and MCH values are perhaps due to a mild iron deficiency anemia.

TABLE IV Hematological and Hemoglobin Synthesis Data on Adult Controls.

Subject and Sex	RBC 10 ¹² /l	Hb g/dl	PCV l/l	MCV fl	MCH pg	MCHC g/dl	Hb Synthesis β/α	Subject and Sex	RBC 10 ¹² /l	Hb g/dl	PCV l/l	MCV fl	MCH pg	MCHC g/dl	Hb Synthesis β/α
Normal Caucasian Adults								Normal Black Adults							
1-M	5.04	16.2	0.500	98	31.6	32.5	1.04	1-M	4.73	15.1	0.434	92	31.9	34.9	1.17
2-F	5.35	12.8	0.379	87	29.6	34.1	1.18	2-M	4.21	12.5	0.361	86	29.7	34.7	1.05
3-M	5.61	15.2	0.443	79	27	35.0	0.92	3-M	5.76	15.8	0.461	81	27.5	34.5	1.07
4-M	4.66	14.2	0.466	85	30.7	34.7	0.88	4-M	4.27	13.3	0.390	92	31.0	34.0	1.08
5-F	3.38	10.6	0.311	92	31.5	34.2	1.11	5-F	3.64	10.3	0.301	82	28.4	34.5	1.30
6-M	4.98	15.4	0.450	90	30.9	34.0	1.05	6-F	3.96	11.3	0.329	83	28.7	34.7	1.22
7-F	4.88	15.2	0.488	88	31.1	35.3	1.11	7-M	3.96	12.3	0.353	89	31.3	35.2	1.04
8-F	4.53	12.1	0.453	93	26.4	29	1.01	8-F	4.06	13.0	0.388	95	32.1	33.7	1.24
α-thalassemia trait								9-F	4.06	11.0	0.347	85	27.2	32.0	1.35
								10-M	4.63	14.2	0.404	87	30.8	35.2	1.03
1-M	6.71	14.8	0.442	65	21.7	33.0	1.62	11-M	4.86	14.6	0.405	84	29.5	36.8	1.01
2-F	4.56	10.8	0.335	74	23.0	32.0	1.75	12-F	4.77	13.0	0.380	78	27.0	35.0	0.99
3-F	4.97	11.4	0.378	75	23.0	30.4	1.69	13-M	4.14	12.3	0.370	89	30.0	34.0	0.99
4-M	5.00	11.6	0.376	74	23.3	31.0	1.87	14-F	4.24	13.2	0.400	93	31.0	34.0	1.02
5-F	4.78	11.4	0.365	76	24.0	32.0	1.33								

TABLE V Statistical Analyses

Subject	Hb	RBC	PCV	MCV	MCH	MCHC	Hemoglobin Synthesis β/α
Children with 5% Hb Bart's versus							
Children with 2% Hb Bart's	t=0.891 n=8 P>0.2	t=3.248 n=8 P<0.02	t=1.812 n=8 P>0.1	t=8.95 n=8 P<0.001	t=4.994 n=8 P<0.01	t=0.811 n=8 P>0.5	t=4.595 n=8 P<0.01
Children without Hb Bart's	t=1.115 n=8 P>0.2	t=2.407 n=8 P<0.05	t=1.263 n=8 P>0.2	t=5.45 n=8 P<0.001	t=4.67 n=8 P<0.01	t=0.938 n=8 P>0.2	t=5.606 n=8 P<0.001
Adult α -thal. trait	t=0.366 n=8 P>0.5	t=0.416 n=8 P>0.5	t=2.090 n=8 P>0.5	t=3.756 n=8 P<0.01	t=1.376 n=8 P>0.2	t=3.746 n=8 P<0.001	t=1.468 n=8 P>0.1
Children with 2% Hb Bart's versus							
Children without Hb Bart's	t=0.376 n=8 P>0.5	t=0.128 n=8 P>0.5	t=0.312 n=8 P>0.5	t=0.416 n=8 P>0.5	t=0.379 n=8 P>0.5	t=0.924 n=8 P>0.2	t=2.716 n=8 P<0.05
Adult α -thal. trait	t=0.665 n=8 P>0.5	t=0.612 n=8 P>0.1	t=0.894 n=8 P>0.2	t=2.831 n=8 P<0.05	t=4.6 n=8 P<0.01	t=2.427 n=8 P<0.05	t=4.02 n=8 P<0.01
Parents of Children with 5% Hb Bart's versus							
Parents of Children with 2% Hb Bart's	t=1.160 n=17 P>0.2	t=1.029 n=17 P>0.2	t=1.190 n=17 P>0.2	t=3.600 n=17 P<0.01	t=2.786 n=17 P<0.02	t=0.276 n=17 P>0.5	t=0.960 n=16 P>0.2
Adult α -thal. trait	t=1.417 n=13 P>0.1	t=0.833 n=13 P>0.2	t=0.6 n=13 P>0.5	t=1.961 n=13 P>0.05	t=2.785 n=13 P<0.02	t=3.342 n=13 P<0.01	t=3.992 n=12 P>0.05

TABLE V continued

Subject	Hb	RBC	PCV	MCV	MCH	MCHC	Hemoglobin Synthesis B/a
Black Adults	t=0.581 n=22 P>0.5	t=2.309 n=22 P<0.02	t=0.333 n=22 P>0.5	t=3.041 n=22 P<0.01	t=2.717 n=22 P<0.02	t=1.388 n=22 P>0.1	t=3.300 n=21 P<0.01
Caucasian Adults	t=0.694 n=16 P>0.2	t=0.766 n=16 P>0.2	t=0.875 n=16 P>0.2	t=3.465 n=16 P<0.01	t=2.210 n=16 P<0.05	t=0.851 n=16 P>0.2	t=3.41 n=15 P<0.01
Parents of Children with 2% Hb Bart's versus							
Adult α -thal. trait	t=2.410 n=12 P<0.05	t=2.345 n=12 P<0.05	t=1.674 n=12 P>0.1	t=6.43 n=12 P<0.001	t=7.821 n=12 P<0.001	t=3.089 n=12 P<0.01	t=3.905 n=12 P<0.2
Black Adults	t=1.955 n=21 P>0.05	t=1.251 n=21 P>0.02	t=0.886 n=21 P>0.2	t=1.196 n=21 P>0.05	t=0.98 n=21 P>0.2	t=0.57 n=21 P>0.5	t=2.982 n=21 P<0.01
Caucasian Adults	t=0.392 n=15 P>0.5	t=.106 n=15 P>0.5	t=0.166 n=15 P>0.5	t=0.135 n=15 P>0.5	t=0.6 n=15 P>0.5	t=1.026 n=15 P>0.2	t=4.05 n=15 P<0.01
Adult α -thal. trait versus							
Black Adults	t=1.123 n=17 P>0.2	t=2.540 n=17 P<0.05	t=0.047 n=17 P>0.5	t=5.4 n=17 P<0.001	t=8.28 n=17 P<0.001	t=4.613 n=17 P<0.001	t=7.213 n=17 P<0.001
Caucasian Adults	t=1.920 n=11 P>0.05	t=1.258 n=11 P>0.2	t=1.214 n=11 P>0.2	t=5.4 n=11 P<0.001	t=7.05 n=11 P<0.001	t=1.729 n=11 P>0.1	t=7.390 n=11 P<0.001
	t=1.369 n=20 P>0.1	t=1.188 n=20 P>0.2	t=1.750 n=20 P>0.05	t=0.922 n=20 P>0.2	t=0.166 n=20 P>0.3	t=1.399 n=20 P>0.2	t=1.46 n=20 P>0.1

Fig. 1 Peripheral blood smear of a child with a mild microcytosis and hypochromia who had 5% Hb Bart's at birth.

Fig. 2 The MCH and MCV values of the Black children, their parents and the control persons. The mean value for each group and the standard deviation of the mean are also given.

Fig. 3 The globin synthesis data (as B/a ratio) of the Black children, their parents and the control persons. The mean value for each group and the standard deviation of the mean are also given.

Fig. 4 Pedigrees of two families. Family X and Family Y are examples of the proposed inheritance of single and duplicated Hb_α loci. The numbers above the squares (= males) or circles (= female) refer to the ages of the individuals. For further details see text.