Hb S, Hb G-PHILADELPHIA AND α -THALASSEMIA-2 IN A BLACK FAMILY

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SUMMARY

A Black family is described in which Hb S, Hb G-Philadelphia and α -thalassaemia-2 determinants occurred in different combinations. The propositus was a healthy fullterm neonate who had 46% Hb G-Philadelphia and about 5% Hb Bart's in cord blood together with a relative microcytosis (MCV = 85 fl) and hypochromia (MCH = 28 pg). This is consistent with a diagnosis of Hb G-Philadelphia trait in association with a homozygous α -thalassaemia-2 (α^0/α^0) and β^A/β^A . The mother and another son also had Hb G-Philadelphia in association with Hb S trait but with 37% Hb G-Philadelphia and with 39% Hb S. Hematological and biosynthetic studies confirm the assignment of the α^0/α^0 and β^A/β^S genotype in both and that of the α^0/α^0 and β^A/β^A genotype in the father. Despite this evidence for a moderate α chain deficiency in the propositus, the biosynthetic α /non- α value in the neonatal period was a high 1.2. Similar values were observed in 8 control cord blood samples if the incubation was not delayed longer than 3 hours after collection (α /non- α = 1.28 $\pm$ 0.14). When the propositus was studied again, but at six months of age, the proportion of Hb G-Philadelphia in peripheral blood was unchanged, a marked microcytosis and hypochromia were observed, and a distinct deficiency of α chain synthesis (α /non- α = 0.56) was present.

SPECULATION

The occurrence of a decreased number of active α chain genes due to the inheritance of α -thalassaemia-2 determinants causes the proportion of the α chain variant Hb G-Philadelphia to increase and the proportion of the β chain variant Hb S to decrease. Thus, the levels of these two abnormal hemoglobins help to define the number of active α chain genes and the occurrence of α -thalassaemia. Despite the existence of a moderate α chain deficiency, the failure to detect any decreased *in vitro* synthesis of α chains in this neonate as well as the finding of excessive *in vitro* α chain synthesis in normal neonates suggest that during the transition from fetal to adult type of erythropoiesis, the synthesis of γ chains may decline more rapidly than that of α chains before the synthesis of β chains is established.

INTRODUCTION

The occurrence of genotypes with four (normal; $\alpha\alpha/\alpha\alpha$), three (α -thalassaemia-2 heterozygote; $\alpha^0/\alpha\alpha$) or two (α -thalassaemia-2 homozygote; α^0/α^0) active α chain genes of human hemoglobin (Hb) among Black Americans has been associated with correspondingly decreased MCV and MCH values, decreased α /non- α *in vitro* synthesis ratios (2,8), zero, 'low' (less than 2%) or 'high' (up to 5%) levels of Hb Bart's (γ_2) in cord blood (2), a trimodality in the concentration of certain β chain variants such as Hb S, Hb C and Hb Leslie in heterozygotes (12), and a variable modal distribution in the proportion of α chain variants in heterozygotes (4,6,15). It has also been shown that this type of hereditary deficiency of α chain synthesis alters the post-translational interaction between nascent chains such as to influence their assembly into asymmetric dimers of the type $\alpha^0\beta^A$, $\alpha^0\beta^S$, or $\alpha^0\beta^A$ (1,6,17). The *in vitro* assembly of β^S , β^C and β^L chains into $\alpha^0\beta^A$ dimers is diminished compared to that of the β^A chains (1,17). Thus, the concomitant occurrence of an α -thalassaemia promotes the production of Hb A over that of the β chain variants Hb S, Hb C and Hb Leslie in heterozygotes (7,12). The α chains of variants such as Hb St. Luke's and Hb G-Georgia and, to a lesser extent, Hb Montgomery fail to form dimers with normal β^A chains as efficiently as the normal α^A chains (6). However, when an α -thalassaemia and an α chain variant occur together, the relative production of the Hb variant is increased over that of Hb A because the synthesis of normal α^A chains is decreased (6,15).

These considerations imply that an evaluation of the concentration of α chain variants and of certain β chain variants together with hematological parameters can help define the number of active α chain genes in heterozygotes. This was again demonstrated when the production of Hb Bart's, Hb S and Hb G-Philadelphia was evaluated in some members of a Black family, including a neonate, in which α -thalassaemia-2, Hb S and Hb G-Philadelphia traits occurred in different combinations. *In vitro* chain synthesis data on a blood sample of the neonate were compared with those of 17 normal newborn.

MATERIALS AND METHODS

All samples were collected with EDTA as anticoagulant. Informed consent was obtained prior to collection. Hematological indices were obtained with a Coulter Counter Model S cell counter. Other hematological data were obtained by standard procedures (18).

Red cell hemolysates were analyzed by starch gel electrophoresis and by DEAE-cellulose chromatography. Hb Bart's in cord blood was quantitated by CM-cellulose chromatography using Bis-Tris developers. For details of these methods, see ref. 13. The hemoglobin variant was identified by a radioimmuno-logical procedure, and data were kindly provided by Dr. F. Garver (11).

Reticulocyte rich fractions of peripheral blood collected from adults were prepared for *in vitro* biosynthetic studies by isopycnic centrifugation on a Ficoll-Renograffin gradient (5). Cord blood samples were not enriched. The cells were washed extensively with cold NKM solution (NaCl: 8.18 g/l, KCl: 0.37 g/l, MgCl₂·6H₂O: 1.42 g/l), and next incubated for 2 hours with ¹⁴C-leucine, as described before (13). Aliquots were taken at earlier times as indicated later. Globin was made from whole cell lysates by precipitation with acid acetone and analyzed by chromatography on columns of CM-cellulose (Whatman CM 52) developed with β -mercaptoethanol-phosphate-urea buffers (13). Aliquots of the effluent fractions were assayed by β -spectrometry. The synthesis ratios were calculated from the total counts per minute detected in each Hb chain zone. These biosynthetic analyses were done on samples from the propositus, members of his family, and on cord blood samples from 17 normal babies.

RESULTS

The propositus was a healthy male fullterm baby weighing 2800 g. He was delivered vaginally after an uncomplicated pregnancy. His cord blood was analyzed as part of a routine testing program conducted by the Sickle Cell Center.

A slow-moving Hb variant which comprised about 50% of total Hb was noted by starch gel electrophoresis and a "high" amount of Hb Bart's (approx. 5%) was also present. The variant was immunologically identified as Hb G-Philadelphia or $\alpha_2\beta_2$ Asn-Lys β_2 (3). Hematological values and hemoglobin composition data for samples from the propositus and his relatives are given in Table 1. The propositus had lower MCV and MCH values than the other neonates without Hb Bart's in cord blood. Hb Bart's was quantitated by two procedures. A rather low value of 2.2% was obtained by DEAE-cellulose chromatography from which Hb Bart's elutes last and about 48 hours after the start of the chromatographic experiment. The more rapid procedure using CM-cellulose chromatography and Bis-Tris developers gave a higher value of 6.7% in agreement with the semi-quantitative estimate made by visual inspection of the starch gel electrophoresis.

The same α chain variant was detected in the propositus' mother who also had Hb S. Her hematological values were nearly normal. The proportion of Hb G-Philadelphia was 36% and that of Hb S was 38%. The propositus' brother had the same condition as the mother, and the proportions of Hb G-Philadelphia and of Hb S were also about the same. The father lacked hematological abnormalities and only the normal hemoglobins A and A₂ were detected.

The results of *in vitro* biosynthetic analyses are summarized in Table 2. When first studied at the age of one day, the propositus had α /non- α values of about 1.20 both at 20 and 120 min of incubation. The proportion of radioactivity in the α^0 chains was equal to the concentration of Hb G-Philadelphia in the hemolysate. A larger excess of α chain synthesis was observed in samples from eight normal neonates which were incubated within a short time (less than 3 hours) from delivery as was done for the sample from the propositus. The imbalance in these samples was slightly milder at 120 min (α /non- α = 1.28 $\pm$ 0.14 (S.D.)) than at 5 min (α /non- α = 1.37 $\pm$ 0.18 (S.D.)) of incubation. The samples whose incubation had been delayed for an average of 17 $\pm$ 4 hrs (range 12-22 hrs) gave lower α /non- α values (at 120 min α /non- α = 1.03 $\pm$ 0.14 (S.D.) n = 9). Moreover, the incorporation of radioactivity in hemoglobin was decreased to the extent that only four out of nine experiments gave an acceptable uptake of ¹⁴C-leucine after 5 min of incubation.

The propositus was studied again at the age of six months. The proportion of Hb G-Philadelphia was unchanged (48.7%), Hb F was 1.6%, Hb Bart's was absent, and Hb H was not detectable. The MCV and MCH had declined to values lower than those of both parents. Following incubation of the peripheral blood reticulocytes for 120 min, a distinct deficiency of α chain synthesis (α /non- α = 0.56) was observed. The proportion of α^0 radioactivity, in contrast, remained unchanged (α^0 ·100/total α = 48%). No γ chain radioactivity was present.

The parents had α /non- α values of 0.72-0.75 (Table 2) which is indicative of a milder α chain deficiency than present in the propositus. Synthesis studies on blood from the propositus' brother were unsuccessful.

DISCUSSION

The *in vitro* biosynthetic data and the erythrocytic indices establish the diagnosis of α -thalassaemia-2 trait in the parents of the propositus, i.e. each had 3 active α chain genes. In the mother and in the brother, one α chain gene is labelled by the Hb G-Philadelphia mutation. As expected, this gene produces one third of all α chains. Both persons are Hb S heterozygotes. Hence, the mother as well as the brother can be assigned the $\alpha^0\alpha^A/\alpha^A$; β^A/β^S genotype, while the father is assigned the $\alpha^0\alpha^A/\alpha^A$; β^A/β^A genotype.

The high quantities (approx. 5%) of Hb Bart's in cord blood, the higher proportion of Hb G-Philadelphia and the low *in vitro* α /non- α synthesis ratio at six months of age are consistent with the diagnosis of homozygous α -thalassaemia-2 in association with Hb G-Philadelphia trait in the propositus, i.e. the $\alpha^0\alpha^0/\alpha^0$; β^A/β^A genotype. One α -thalassaemia-2 determinant has been inherited from the father. The other must have been inherited from his mother in linkage with Hb G-Philadelphia trait, i.e. the ($\alpha^0\alpha^0$) haplotype. Linkage between α -thalassaemia-2 and Hb G-Philadelphia is known to occur among the Black population of the Southeastern USA (6,15).

The proportion of Hb G-Philadelphia in heterozygotes can vary according to the number of active α chain genes on the locus *in trans* (4,6,15). In the propositus there is only one active α chain gene *in trans* to the $\alpha^0\alpha^0$ locus and the output of α^0 chains should be about 50% as observed. There are two normal α genes *in trans* to the $\alpha^0\alpha^0$ locus in the mother and the brother who have about 37% Hb G-Philadelphia in peripheral blood. The presence of three rather than four active α chain genes results in Hb S levels below 40% (39.5% in the mother and 38% in the brother). There is an inverse correlation between the percentage of Hb G-Philadelphia and Hb S when these occur together in heterozygotes (6). Thus, when the number of active α chain genes is decreased, the proportion of Hb G-Philadelphia is increased, but that of Hb S is decreased.

Although all other data indicate that the propositus has only two active α chain genes, the α chain deficiency could not be detected by analysis of *in vitro* Hb chain synthesis in the neonatal period. At birth, the ratio of α to non- α chains was about 1.2. It declined to 0.57 by six months of age. In fact, many other (normal) newborn had a rather similar degree of imbalanced synthesis (α /non- α : 1.28) by reticulocytes and erythroblasts in their cord blood. The imbalance was not detectable if the incubation was delayed longer than 3 hours after collection of the sample. Variable synthesis data have been obtained by others in cord blood (9,10,14). Gahr et al. found an increased production of γ chains in infants after exchange transfusion (16). However, the α /non- α values were balanced in stroma-free material prepared from incubated cord blood of normal neonates (10). It is possible that in these cases the excess of nascent α chains was removed together with the stroma.

The reason for the observed imbalance is not entirely clear. Neither a kinetic study nor the γ /non- α values offer a definitive explanation. However, it seems reasonable to assume that during the transition from fetal to adult erythropoiesis the transcriptional or translational regulation of α chain synthesis is modified so that the production of Hb chains decreases to mature levels in the post-natal period. It is possible for the translation of γ mRNA to decline during the maturation of erythroblasts or that the γ mRNA may be un-

stable. Indeed, the production of α and γ chains in the neonatal period may decline at dissimilar rates before δ chain synthesis becomes established (19).

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Table 1. Hematologic and Hemoglobin Composition Data

Subject	Age	Hb ^a g/dl	PCV ^a l/l	RBC ^a 10 ¹² /l	MCV ^a fl	MCH ^a pg	MCHC ^a g/dl	Retic %
Propositus	1 d	17.8	0.53	6.45	85	27.8	32.0	9.3
	6 mo	10.9	0.32	5.42	61	20.2	32.4	0.9
Brother	3 y	13.4	0.38	5.37	73	25.3	34.1	0.3
Father	27 y	15.5	0.42	5.44	79	28.7	35.5	1.1
Mother	25 y	13.7	0.39	4.86	83	28.4	33.6	1.8

Table 1 (cont.)

Subject	Age	G ₂ %	A ₂ %	G %	S %	A %	FG %	F %	Bart's %	G% Total	S% Total
Propositus	1 d	--	--	13.2	--	12.9	32.6	39.1	2.2 ^b	45.8	--
	6 mo	1.00	1.4	47.3	--	47.6	0.4	1.6	--	47.7	--
Brother	3 y	1.55	18.8 ^c	20.3	22.5	36.8	--	--	--	37.5 ^d	39.7
Father	27 y	--	2.3	--	--	97.7	--	--	--	--	--
Mother	25 y	1.13	15.5 ^c	22.0	23.7	37.7	--	--	--	36.4 ^d	38.1

^aControl values for 17 neonates without Hb Bart's are: Hb 14.6 $\pm$ 2.4 g/dl; PCV 43.5 $\pm$ 9.8%; RBC 4.97 $\pm$ 0.96 $\times$ 10¹²/l; MCV 108.9 $\pm$ 5.5 fl; MCH 35.8 $\pm$ 1.8 pg; MCHC 32.8 $\pm$ 1.9 g/dl.

^bHb Bart's was 6.7% by CM-cellulose chromatography (see Text).

^cIncludes Hb SG ($\alpha_2\beta_2S$).

^dThese values are adjusted assuming that the percentages of G₂ and A₂ are the same.

Table 2. *In Vitro* Biosynthetic Data

Subject	Age	# ^a	Time of Incubation (min)	Total CPM $\times$ 10 ³ /zone					Ratios			
				γ	β^A	β^S	α^A	α^G	$\alpha/\text{non-}\alpha^b$	$\gamma/\text{non-}\alpha^b$ $\times$ 100	$\beta^S/\text{non-}\alpha$ $\times$ 100	α^G/α $\times$ 100
Propositus	1 d	1	20	24.3	29.0	--	33.0	28.3	1.15	45.6	--	46.2
		1	120	79.5	97.0	--	111.0	96.1	1.17	45.1	--	46.6
		2	120	70.3	86.6	--	97.7	89.1	1.19	44.8	--	47.7
	6 mo	1	120	--	35.0	--	10.2	9.3	0.56	--	--	47.7
		2	120	--	38.7	--	11.5	10.5	0.57	--	--	47.6
Mother ^c	25 y	1	120	--	15.7	9.0	12.9	5.6	0.75	--	36.4	30.2
		2	120	--	16.8	9.4	12.9	5.6	0.75	--	36.0	30.4
Father	27 y	1	120	--	6.4	--	4.7	--	0.73	--	--	--
		2	120	--	9.5	--	6.8	--	0.72	--	--	--

^aNumbers refer to multiple analyses of the same whole cell globin sample.

^bBiosynthetic studies on 8 freshly collected cord blood samples gave an $\alpha/\text{non-}\alpha$ value of 1.37 $\pm$ 0.18 and a $\gamma/\text{non-}\alpha$ percentage of 60.2 $\pm$ 7.3 at 5 min. The $\alpha/\text{non-}\alpha$ value was 1.28 $\pm$ 0.14 and the percentage of $\gamma/\text{non-}\alpha$ was 58.4 $\pm$ 4.3 at 120 min.

^cTwo members of another family with Hb G-Philadelphia, Hb S, and an α -thalassemia-2 homozygosity had 46% Hb G-Philadelphia, 29% Hb S, a MCV value of 76 fl, and a MCH value of 22.3 pg. *In vitro* synthesis studies gave $\alpha/\text{non-}\alpha$ values of 0.57 and 0.55 after 2 hrs of incubation.