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The Occurrence of Different Levels of $^{G}\gamma$ Chain and of the $^{A}\gamma^{T}$ Variant of Fetal Hemoglobin in Newborn Babies From Several Countries

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The γ chain compositions of the fetal hemoglobins of 2453 newborn babies from East Asian countries (1350 babies), from Italy, Yugoslavia, Bulgaria, and Georgia (417 Caucasian babies), and 686 black babies from Georgia were determined by high pressure liquid chromatography. Unusual results for a limited number of babies were confirmed by chemical analyses, and were evaluated further by family studies.

Statistical analyses indicated high gene frequencies for the $^{A}\gamma^{T}$ chain in Italian (f = 0.237), Yugoslavian and Bulgarian (f = 0.238), and white Georgia babies (f = 0.224), a lower frequency in Japan (f = 0.178), and India (f = 0.173), and particularly in mainland China (f = 0.079). The $^{A}\gamma^{T}$ gene frequency in normal (AA) Black babies was 0.102. When a β^{S} or β^{C} mutation was also present this frequency was greatly decreased, particularly in babies with the AC condition (f = 0.036). These results suggest the near absence of the $^{A}\gamma^{T}$ mutation on the chromosome also carrying the β^{C} determinant.

Most babies had the expected $^{G}\gamma$ values which vary between 60 and 80%, but several (mainly black) babies had higher values (between 80 and 90%), while one normal black baby had a $^{G}\gamma$ value of (nearly) 100%. This condition may be a form of $^{A}\gamma^{+1}$-thalassemia and has been discussed in detail elsewhere (Blood *58*:491–500, 1981). Thirty-five clinically normal (mainly Chinese, Indian, and Japanese) babies had $^{G}\gamma$ values of about 40%. Twenty-six babies had $^{A}\gamma^{1}$ values of about 60%, while the remaining nine babies had $^{A}\gamma^{T}$

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and $^{A}\gamma^{I}$ chains in a ratio of either 1 to 2 or 1 to 1. Two additional newborns did not produce any $^{G}\gamma$ chains, but had only $^{A}\gamma^{I}$ chains or $^{A}\gamma^{T}$ chains. Family studies failed to indicate a specific hematological abnormality. These unusual ratios between the $^{G}\gamma$ and $^{A}\gamma$ (either $^{A}\gamma^{I}$ or $^{A}\gamma^{T}$) chains have led to speculations regarding possible genetic abnormalities present in these infants.

Key words: three types of γ chain, Hb F, HPLC, $^{A}\gamma$-thalassemia, gene conversion, frequency of $^{A}\gamma^{T}$ chain, population genetics

INTRODUCTION

The development of rapid high pressure liquid chromatographic (HPLC) methodology [1–3] for the separation of three types of γ chains of human fetal hemoglobin (Hb F), ie, the $^{G}\gamma$ (75Ile, 136Gly), the $^{A}\gamma^{I}$ (75Ile, 136Ala), and the $^{A}\gamma^{T}$ (75Thr, 136Ala) chains, has made it possible to study the γ chain heterogeneity of the Hb F of newborns on a large scale. In a previous publication we reported data for 203 black babies born in hospitals in the Augusta area [4]. The study concerned an evaluation of the frequency of the $^{A}\gamma^{T}$ chain as well as a description of a few newborns with high or low $^{G}\gamma$ levels, which were interpreted to indicate the possible presence of either an $^{A}\gamma$- or a $^{G}\gamma$-thalassemia. In a subsequent publication results for newborns with β chain abnormal Hb types, such as Hb S and Hb C, were reported [5]. These preliminary data made us decide to extend these investigations to cord blood samples from newborn babies of different populations mainly to evaluate the frequencies of the $^{A}\gamma^{T}$ anomaly and the γ-thalassemia-like conditions. Two normal infants with an Hb F not containing any $^{G}\gamma$ chains were discovered; data from structural analyses of the γ chains and from family studies are included in this report. Some of the data obtained for Chinese babies have been reported before [6].

MATERIALS AND METHODS

Newborn Babies

Cord blood samples (2–4 ml) were collected in ACD or EDTA, and were obtained from various parts of the world through the activities of different investigators, as follows.

Georgia, USA. The cord blood testing laboratory of the Comprehensive Sickle Cell Center at the Medical College of Georgia, which serves as a reference laboratory for the state-wide newborn testing program, provided blood samples from 686 black babies (343 with AA, 155 with AS, 84 with AC, 66 with SS, 31 with SC, and 7 with CC), 78 Caucasian babies, and 26 babies from Vietnamese immigrants.

Italy. Cord blood samples from 150 babies were collected at the Center for the study of thalassemia at the Ospedali Galliera of the University of Genova.

Yugoslavia. These samples were collected from 189 newborns of Yugoslavian and Bulgarian origin, and were shipped from Skopje, Macedonia.

India. The number of samples from babies of the Bombay area was 208.

China Mainland. A large number (841) of samples were collected over a period of eight months in the Children's Hospital of Shanghai.

Japan. The 275 cord blood samples were mainly from babies born in the metropolitan area of Tokyo.

All foreign samples were air-mailed in batches as whole blood (sterile) or as packed red cells, were washed upon arrival with 0.9 g/dl NaCl, and were stored at 4°C. Red cell lysates were stored at -70°C.

Additional blood samples (2–3 ml) were collected from the Chinese babies #2, #53, #56, #753, and from India #147. These, as well as those of the parents of babies China #753 and India #147 (20 ml each), were shipped, air-mail special delivery, to Augusta, Georgia where they arrived within 2 to 7 days.

Methods

Cord blood red cell lysates were analyzed by cellulose acetate electrophoresis at alkaline pH. The presence of an Hb S or Hb C heterozygosity or homozygosity and of the SC condition was confirmed by citrate agar electrophoresis at acidic pH and by high pressure liquid chromatography (HPLC), as described [7,8]. Quite a few samples contained either an α-chain variant or a γ-chain variant; these samples were excluded from this study and are not included in the tabulation listed above. The γ chain compositon of the Hb F (0.5–1 mg) was analyzed by reverse-phase HPLC using Bondapack C_{18} columns (Waters Assoc.), and an elution system described before [1–3]. The percentages of the three types of γ chains were calculated from the weights of cutouts of appropriate parts of photocopies of the chromatograms (ie, $\% {}^{A}\gamma^{T} + \% {}^{G}\gamma + \% {}^{A}\gamma^{I} = 100\%$ total γ). The separation of the β chains from the α and the γ chains was often complete, and allowed a quantitation of the relative amounts of β and γ chains (ie, $\% \beta + \% {}^{A}\gamma^{T} + \% {}^{G}\gamma + \% {}^{A}\gamma^{I} = 100\%$). Such calculations have relative values only since the extinction coefficients of the four different chains at 220 nm have not been determined.

Hemolysates, prepared from washed red cells of the additional samples from babies China #753, and India #147 and of their parents, were analyzed by DEAE-cellulose chromatography [9] to quantitate the hemoglobins A and F. The alkali denaturation method of Betke et al was sometimes also used [10]. Hb F was isolated by DEAE-cellulose chromatography and HPL chromatography as described before [11], and analyzed for γ chain composition by a second type of HPL chromatography [1–3]. Sufficient amounts of Hb F were isolated from the original cord blood samples of baby China #753 and of the second sample of Indian baby #147 to allow structural analyses. The γ chain was isolated by CM-cellulose chromatography [12] and digested with TCPK trypsin for 4 hours at room temperature. The tryptic peptides were separated by an HPLC method described by Wilson et al [13] and characterized by amino acid analysis. The γ chain of baby China #690 with three types of γ chains ($^{A}\gamma^{T} = 29.8\%$; $^{G}\gamma = 38.1\%$; $^{A}\gamma^{I} = 32.1\%$) served as control.

Several newborn babies had fetal hemoglobins with $^{G}\gamma$ values which were higher or lower than observed in the great majority of cases; some of these data were confirmed through analyses of the γCB-3 peptides using the procedure developed by Schroeder et al [14,15].

RESULTS

Analyses of Nearly 2500 Newborn Blood Samples

The separation of the γ chains by HPLC. Figures 1 and 2 illustrate the γ chain sections of eight different chromatograms. The most commonly occurring pattern is that of China #816 (Fig. 1; third chromatogram) with a $^{G}\gamma$ to $^{A}\gamma$ ratio of 68 to 32. For baby China #788 (Fig. 1; second chromatogram) this ratio is reversed (34 to 66), while the $^{G}\gamma$ chain is completely absent in the Hb F of Baby China #753. Presumably, babies China #788 and #753 are heterozygous and homozygous for a

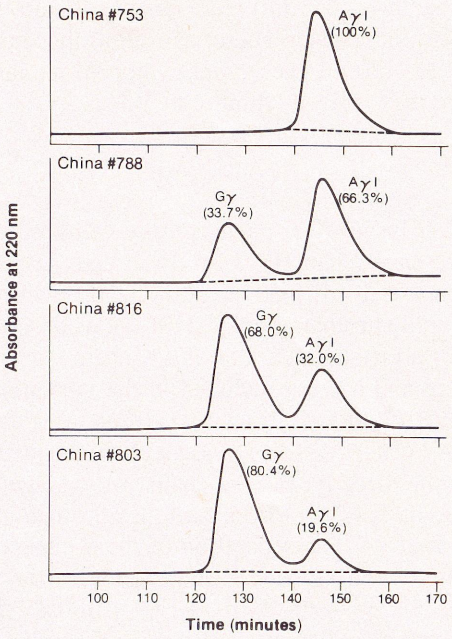

Fig. 1. Sections of the HPL chromatograms showing the separation of the $^{G}\gamma$ and $^{A}\gamma^{I}$ chains. The four samples were $^{A}\gamma^{T}$ negative. For further details see text.

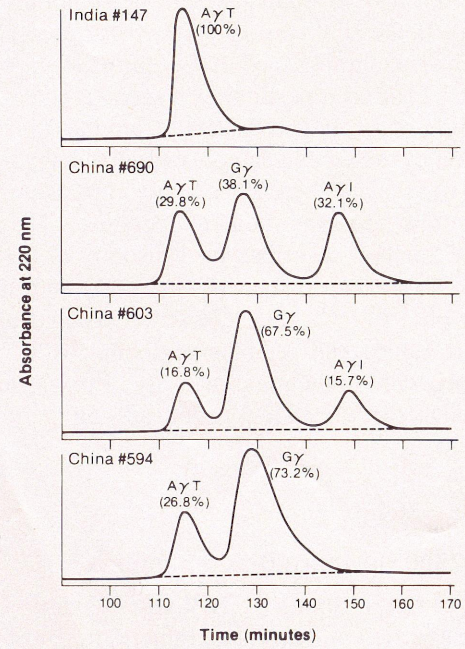

Fig. 2. Sections of the HPL chromatograms showing the separation of the $^{A}\gamma^{T}$, $^{G}\gamma$, and $^{A}\gamma^{I}$ chains. Two babies (India #147, China #594) were $^{A}\gamma^{T}$ homozygotes, while babies China #690 and #603 were $^{A}\gamma^{T}$ heterozygotes. For further details see text.

"low $^{G}\gamma$ level" condition, respectively. The pattern in baby China #803 indicates a decreased level of $^{A}\gamma(^{A}\gamma^{I})$ chains, perhaps representing an $^{A}\gamma$-type of thalassemia. A presumably homozygous form of this condition was described in an earlier publication [4].

Similar patterns are shown in Figure 2; however, all babies are also heterozygous or homozygous for the $^{A}\gamma^{T}$ variant. Most common is the chromatogram of baby China #603 (Fig. 2; third chromatogram) who had a normal $^{G}\gamma$ chain level of 67.5% and an $^{A}\gamma^{T}$ heterozygosity. Baby China #594 had an $^{A}\gamma^{T}$ homozygosity and a normal $^{G}\gamma$ chain level of 73%. The pattern for baby China #690 was characterized by a decreased level of $^{G}\gamma$ chain and equal amounts of $^{A}\gamma^{T}$ and $^{A}\gamma^{I}$ chains (Fig. 2; second chromatogram). This baby apparently had a heterozygosity for the low $^{G}\gamma$ level condition as well as for the $^{A}\gamma^{T}$ variant. Only $^{A}\gamma^{T}$ chains were observed in the Hb F of baby India #147 (Fig. 3; top chromatogram). This baby apparently had homozygosities for both the low $^{G}\gamma$ level condition and the $^{A}\gamma^{T}$ variant. Conclusions based on chromatographic patterns are preliminary, and supporting data will be presented below.

The levels of $^{G}\gamma$ chain in the Hb F of $^{A}\gamma^{T}$ negative newborns. Figure 3 (bottom section) lists the data for 1891 babies. The results fell into five categories, namely (nearly) only $^{G}\gamma$ chains (one baby); high levels of $^{G}\gamma$ chains (80–90%); normal $^{G}\gamma$ levels (55–80%); low $^{G}\gamma$ levels (30–50%); and 0% $^{G}\gamma$ chains (one baby). The division between the second and third groups was not always clear and overlap of data appeared unavoidable. The data are also listed according to the racial and/or ethnic origins of the babies. High $^{G}\gamma$ values were mainly observed in black newborns (including those with AS, and AC, but not in SS, SC, and CC babies), as well as in a few babies born in China. The only homozygote ($^{G}\gamma \geqslant 96\%$) was a black newborn [4]. Low $^{G}\gamma$ values (30–50%) were primarily seen in Chinese babies but were also observed in other populations. The only homozygote ($^{G}\gamma = 0\%$) was Chinese baby #753 (Fig. 1).

The $^{A}\gamma^{T}$ heterozygous newborns. Figure 3 (middle section) lists the levels of the $^{G}\gamma$ chain in the Hb F of 514 heterozygous babies. All values are comparable to those observed for $^{A}\gamma^{T}$ negative newborns. $^{G}\gamma$ values above 80% were seen in five babies (four black; one Japanese), while the nine infants with $^{G}\gamma$ values between 32 and 52% originated from Yugoslavia (one), India (one), China (three), and Japan (four).

Table I presents the relative percentages of the two types of $^{A}\gamma$ chains in the 498 $^{A}\gamma^{T}$ heterozygous newborns with normal $^{G}\gamma$ values. The average values of 13.7 and 16.4% for the $^{A}\gamma^{T}$ and $^{A}\gamma^{I}$, respectively, confirm earlier data [4,5] that the $^{A}\gamma^{T}$/total $^{A}\gamma$ percentage (45.5%) is usually slightly less than the $^{A}\gamma^{I}$/total $^{A}\gamma$ percentage (54.5%). No significant differences were observed between the babies of different racial or ethnic backgound.

The black and Japanese babies with elevated $^{G}\gamma$ values (79% and higher) had normal $^{A}\gamma^{T}$ levels (average 14.0%), but distinctly decreased levels of $^{A}\gamma^{I}$ chain (average 3.3%). Individual data for four black babies and three Japanese babies (including two with $^{G}\gamma$ values slightly below 80%) are given in Table II. It is indeed difficult to confirm the presence of the $^{A}\gamma^{I}$ chain in these cases particularly when this percentage is below 2% [16,17]. However, comparisons of these chromatograms with those of babies with an $^{A}\gamma^{T}$ homozygosity (not showing any $^{A}\gamma^{I}$ chain) suggested the presence of a small amount of the $^{A}\gamma^{I}$ chain.

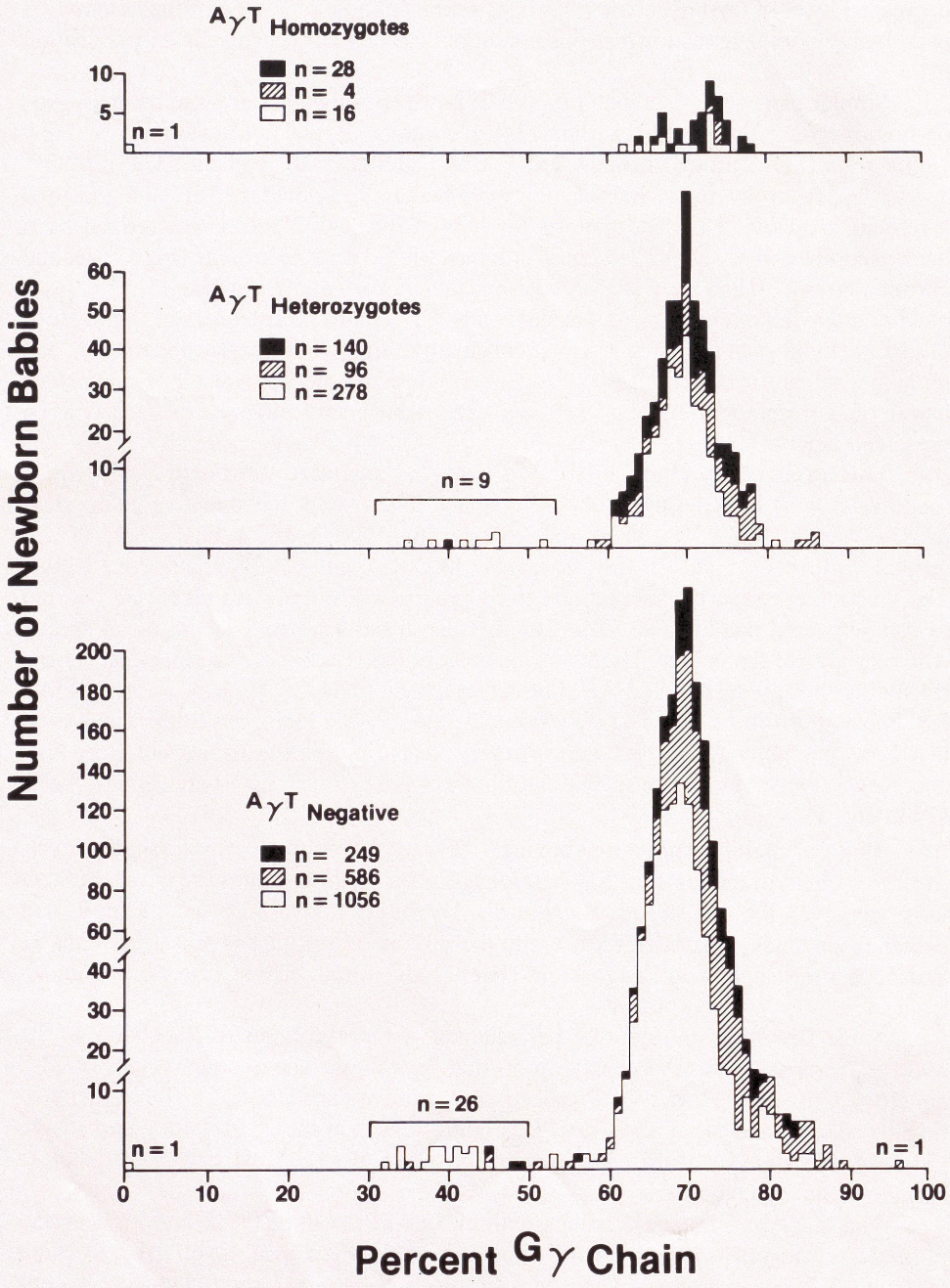

Fig. 3. The distribution of the $^{G}\gamma$ chain values in the Hb F of 2453 newborn babies from various parts of the world. □: babies from East Asia (Japan, China, Vietnam, and India); ▨: Black babies including those with the hemoglobin abnormalities AS, AC, SS, CC, and SC; ■: Caucasian babies from Georgia, Italy, Yugoslavia, and Bulgaria.

TABLE I. The Percentages of the $^{A}\gamma^{T}$ and $^{A}\gamma^{I}$ Chains in the Hb F of $^{A}\gamma^{T}$ Heterozygous Newborns With Normal $^{G}\gamma$ Chain Values

Country of origin	Total number of babies	$^{A}\gamma^{T}$ % (SD)	$^{A}\gamma^{I}$ % (SD)
Georgia; Black AA	58	12.6 ± 2.1	15.4 ± 2.7
Georgia; Black[a]	34	13.2 ± 2.0	15.9 ± 2.5
Georgia; Caucasian	27	14.3 ± 2.5	16.2 ± 2.0
Italy	55	13.9 ± 2.3	16.9 ± 2.9
Yugoslavia	57	13.4 ± 2.3	16.4 ± 3.1
India	53	14.9 ± 2.7	17.2 ± 2.8
Georgia; Vietnamese	7	13.5 ± 0.8	16.5 ± 1.7
China	124	14.3 ± 2.2	16.7 ± 2.5
Japan	83	13.8 ± 2.5	15.9 ± 2.7
Total	498	13.7 ± 2.1	16.4 ± 2.5

[a]Includes babies with AS (20), AC (6), SS (6), and SC (2).

TABLE II. The Percentages of the Three Types of γ Chains in the Hb F of $^{A}\gamma^{T}$ Heterozygous Newborns With an Elevated Level of $^{G}\gamma$ Chain

Country of origin	Case number	$^{A}\gamma^{T}$ (%)	$^{A}\gamma^{I}$ (%)	$^{G}\gamma$ (%)
Georgia; Black AA	S.J.[a]	12.4	3.2	84.4
	A.W.W.[a]	13.6	0.4	86.0
	B.G.	11.8	3.8	84.4
	I.T.	13.6	0.8	85.6
Japan	8203329	14.2	4.8	81.0
	8202432	17.6	3.9	78.5
	no number	14.8	5.9	79.3
Average and S.D.		14.0 ± 1.8	3.3 ± 1.8	82.7 ± 2.9

[a]From reference [4].

The nine babies who had low $^{G}\gamma$ values as well as an $^{A}\gamma^{T}$ heterozygosity fell into two groups (Table III). The $^{A}\gamma^{T}$ level in the first group (Type I) was normal (average 15.4%), while the $^{A}\gamma^{I}$ level (average 39.8%) was as high as the $^{G}\gamma$ value and at least twice the $^{A}\gamma^{I}$ level observed in simple $^{A}\gamma^{T}$ heterozygotes. The babies of the second group (Type II), had greatly increased $^{A}\gamma^{T}$ values (average 29.2%), while the $^{A}\gamma^{I}$ percentages (average 29.5%) were significantly less than those seen in the Hb F of the babies of Type I. The $^{G}\gamma$ values of both categories were the same and also did not differ from the average value of the 26 newborns with low $^{G}\gamma$ values, but without the $^{A}\gamma^{T}$ abnormality.

The $^{A}\gamma^{T}$ homozygous newborns. Forty-eight of these babies have been discovered, mainly among Italian, Bulgarian, Yugoslavian, and Indian infants (Fig. 3, top section). All but one had $^{G}\gamma$ values in the normal range of 60–80% and not different from the values seen in newborns without the $^{A}\gamma^{T}$ anomaly. One baby (India #147) produced only Hb F with $^{A}\gamma^{T}$ chains (Fig. 2; top chromatogram) and apparently had a homozygosity for the condition characterized by a low $^{G}\gamma$ value as well as an $^{A}\gamma^{T}$ homozygosity.

TABLE III. The Percentages of the Three Types of γ Chain of the Hb F of Newborn Babies With a Heterozygosity for the Low $^{G}\gamma$ Level Condition

Country of origin	Newborn baby (#)	$^{G}\gamma$ (%)	$^{A}\gamma^{T}$ (%)	$^{A}\gamma^{I}$ (%)	Comments
Different countries[a]		40.5	0	59.5	
(n = 26)	(SD)	(4.4)	—	(4.4)	
Yugoslavia	BC-92	40.0	18.3	41.7	Type I[c]
India	CB-14[b]	52.5	15.7	31.8	
China	337	45.0	15.0	40.0	
China	549	44.0	15.4	40.6	
Japan	8203580	42.2	12.8	45.0	
	Average	44.8	15.4	39.8	
	(SD)	(4.2)	(1.8)	(4.4)	
China	690	38.1	29.8	32.1	Type II[c]
Japan	8202461	46.1	31.7	22.2	
Japan	8202482	46.3	26.0	27.7	
Japan	8203625	34.6	29.1	36.3	
	Average	41.3	29.2	29.5	
	(SD)	(5.1)	(2.1)	(5.2)	
Controls (n = 498)		69.9	13.7	16.4	
Table II	(SD)	(3.4)	(2.1)	(2.5)	

[a]Includes babies from China Mainland [17], Japan [2], India [2], Yugoslavia [2], Italy [1], and Blacks [2].
[b]The only baby with a $^{G}\gamma$ value in excess of 50%.
[c]See text for further discussion.

Gene frequencies of the different conditions in the various populations. Table IV summarizes the results of the survey concerning the frequencies of the $^{A}\gamma^{T}$ anomaly in Caucasian, black, and (East) Asian populations. High $^{A}\gamma^{T}$ frequencies were observed in Italian babies, Caucasian babies from Georgia, and Yugoslavian and Bulgarian babies. The frequencies in newborns from Japan, India, and Vietnam were significantly lower. Low $^{A}\gamma^{T}$ frequencies were seen in Chinese and in black babies, particularly in those with a Hb S and/or Hb C heterozygosity or homozygosity. The value for the black Hb C heterozygote was significantly less than those for the black Hb S heterozygote and the normal black baby.

The frequency for genetic condition resulting in low $^{G}\gamma$ values was the highest in Chinese (f = 0.013), Indian (f = 0.012), and Japanese (f = 0.011) babies. Only a few heterozygotes were observed among black babies from Georgia (two cases among 686 babies), Italian babies (one case among 150 babies), and Yugoslavian babies (three babies among 189 babies, f = 0.008).

Special Studies of a Few Selected Newborn Babies

Blood samples from babies with significantly different levels of $^{G}\gamma$ chains (with or without an additional $^{A}\gamma^{T}$ heterozygosity or homozygosity) were evaluated in further detail. Data for the one black baby with a $^{G}\gamma$ level of at least 96% and her parents have been reported in an earlier publication [4]. Therefore, this section will be limited to babies with low levels of $^{G}\gamma$ chains.

Chemical analyses of the Hb F from babies with low $^{G}\gamma$ values. The low $^{G}\gamma$ levels observed in the Hb F of several newborns were confirmed through analyses of the γCB-3 peptides using the method developed by Schroeder et al [14,15]. The data for five babies with low $^{G}\gamma$ values and four with normal to high $^{G}\gamma$ values are listed in Table V, and indicate an acceptable agreement between the results obtained with the two methods.

The level of β chain (or Hb A) in cord bloods of infants with low levels of $^{G}\gamma$ chains. These percentages were calculated from the HPL chromatograms for 238 babies with normal $^{G}\gamma$ levels, 17 newborns with $^{G}\gamma$ levels between 30 and 50%,

TABLE IV. The $^{A}\gamma^{T}$ Gene Frequency in Newborn Babies of Different Populations

Country of origin	Total number of babies	$^{A}\gamma^{I}$ Homozygote[a]	$^{A}\gamma^{T}$ Heterozygote[a]	$^{A}\gamma^{T}$ Homozygote[a]	Gene frequency $^{A}\gamma^{T}$
Georgia; Black AA	343	278	62	3	0.102
Georgia; Black AS	155	134	20	1	0.071
Georgia; Black AC	84	78	6	0	0.036
Georgia; Black SS	66	60	6	0	0.045
Georgia; Black SC	31	29	2	0	0.032
Georgia; Black CC	7	7	0	0	0
Georgia; Caucasian	78	47	27	4	0.224
Italy	150	87	55	8	0.237
Yugoslavia	189	115	58	16	0.238
India	208	145	54	9	0.173
Georgia; Vietnamese	26	19	7	0	0.135
China	841	711	127	3	0.079
Japan	275	181	90	4	0.178

[a]Babies with different levels of $^{G}\gamma$ chain are grouped together.

TABLE V. The Percent $^{G}\gamma$ Chain in the Hb F of Five Chinese Babies With Low $^{G}\gamma$ Values and of Four Normal Chinese Babies, Determined by Two Procedures

Newborn baby (#)	HPLC method (%) $^{G}\gamma$	γCB-3 method[a] Gly; Ala
2	42.8	0.42–2.74
2[b]	40.2	n.d.
53	40.6	0.36–2.68
53[b]	42.5	0.38–2.67
56	42.8	0.36–2.66
56[b]	39.1	n.d.
105	38.4	0.40–2.68
194	42.5	0.41–2.62
49	69.0	0.78–2.30
71	67.0	0.75–2.28
13	80.5	0.84–2.23
66	78.9	0.79–2.30

[a]Expressed as the numbers of glycyl and alanyl residues in the γCB-3 peptide (residues 133–146, inclusive).
[b]At the age of 3 months.

and for the two newborns without $^{G}\gamma$ chains (Table VI). The values for babies with or without the $^{A}\gamma^{T}$ anomaly were the same. However, babies with $^{G}\gamma$ values between 30 and 50% had slightly increased levels of Hb A (28.5% versus 21.5%), while the two newborns without $^{G}\gamma$ chains had relative β chain values which were twice those found in the normal babies.

The Babies China #753, and India #147

Table VII lists the limited hematological data and the results of the hemoglobin analyses. Data obtained for the parents are also included. The low level of Hb F at time of birth for the two babies without $^{G}\gamma$ chains is striking, as is the rapid decrease in this level during the first few months of life. The chain composition of the Hb F isolated at different times remained the same, ie, 100% $^{A}\gamma^{I}$ in China baby #753, and 100% $^{A}\gamma^{T}$ in India baby #147. The levels of Hb A_2 and Hb F were normal in all four parents. The hematological data for the Indian mother suggested a mild iron and/or nutritional deficiency which was corrected after appropriate therapy. Gamma chain composition analyses were made on the Hb F from the four adults, but were successful for the parents of the Chinese baby. Figure 4 illustrates chromatograms showing the isolation of Hb F from Hb F containing hemoglobin fractions by Synchropak AX-300 HPL chromatography, and the analyses of the γ chain composition by reverse-phase HPL chromatography. The final results for the two Chinese parents were remarkably identical.

Clinical observations on both babies were limited but no overt disease or anemia was present. Both liver and spleen were not palpable.

Structural analyses. Figure 5 provides segments of HPL chromatograms depicting the separations of the tryptic peptides γT-3, γT-9, γT-8,9, γT-14, γT-15, and γT-11. The top chromatogram (baby China #690 with $^{A}\gamma^{T}$, $^{A}\gamma^{I}$, and low $^{G}\gamma$ levels) shows among others the recovery of four zones containing the peptides $^{T}\gamma$T-9 and $^{T}\gamma$T-8,9 with a threonine in position 9 (ie, position 75 of the intact γ chain) and the $^{I}\gamma$T-9 and $^{I}\gamma$T-8,9 peptides with an isoleucine in that position. The peptides $^{G}\gamma$T-15 with a glycine in position 4 (ie, position 136 of the intact γ chain) and $^{A}\gamma$T-15 with an alanine in that position are also well separated from each other and from other peptides. Only the $^{I}\gamma$T-9, $^{I}\gamma$T-8,9 and $^{A}\gamma$T-15 peptides were detected in the chromatogram of China baby #753 while the $^{T}\gamma$T-9, $^{T}\gamma$T-8,9 and $^{A}\gamma$T-15 peptides were present in the chromatogram of India Baby #147 (Fig. 5; middle and bottom chromatograms). The amino acid compositions of these peptides are listed in Table VIII.

TABLE VI. The Percentages of β Chain in the Hemoglobin of Newborn Babies With Different $^{G}\gamma$ Values of the Hb F*

Condition	$^{A}\gamma^{T}$ Negative	$^{A}\gamma^{T}$ Heterozygote	$^{A}\gamma^{T}$ Homozygote
$^{G}\gamma$ between 60 and 80%	21.4 ± 7.7 (n = 140)	22.0 ± 7.7 (n = 85)	21.8 ± 7.0 (n = 13)
$^{G}\gamma$ between 30 and 50%	28.7 ± 8.2 (n = 14)	28.3 (n = 3)	—
$^{G}\gamma$ = 0%	43.4 (n = 1)	—	44.4 (n = 1)

*Averages and Standard Deviations.

TABLE VII. Hematological and Hemoglobin Composition Data for Two Babies With Unusual γ Chain Ratios and Their Parents

Subject	Age	Hb g/dl	PCV 1/1	RBC 10^{12}/1	MCV fl	Retics (%)	A_2[a] (%)	F_{DE}[a] (%)	F_{AD}[b] (%)	$^{A}\gamma^{T}$[c] (%)	$^{A}\gamma^{I}$[c] (%)	$G\gamma$[c] (%)
China												
#753	Newborn	—	—	—	—	—	—	56.4	—	0	100	0
	14 wks	—	—	—	—	—	2.5	3.4	—	0	100	0
	22 wks	13.3	0.41	5.49	74	0.6	—	—	—	—	—	—
Father[d]	33 yrs	12.8	0.40	4.06	97	0.5	2.75	1	0.6	10.0	55.0	35.0
Mother[d]	27 yrs	11.3	0.36	3.63	99	0.8	2.85	1	0.8	18.0	44.8	39.2
India												
#147	Newborn	—	—	—	—	—	—	55.6	—	100	0	0
	5 wks	10.4	0.35	4.06	86	0.2	1.0	47.7	41.6	100	0	0
	8 wks	10.0	0.34	4.17	81	3.2	1.6	10.4	14.2	100	0	0
Father[d]	35 yrs	13.7	0.48	4.87	98	0.2	2.4	1	1.3	—	—	—
Mother[d]	24 yrs	9.1	0.30	3.91	76	0.6	2.3	1	1.4	—	—	—

[a]By DEAE-cellulose chromatography [9].
[b]By the method of Betke et al [10].
[c]By HPL chromatography [1–3].
[d]No relationship was known between the parents of China #753; the parents of baby India #147 are first cousins.

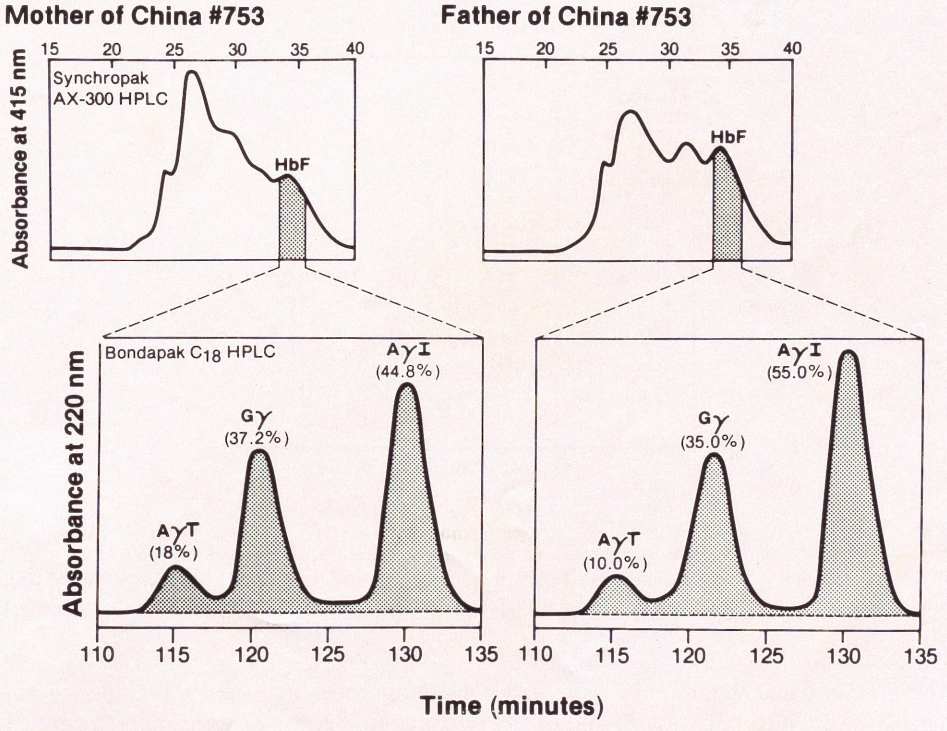

Fig. 4. Isolation and γ chain analyses of the Hb F from two adults with levels below 1%. The Hb F fraction was first isolated by a series of DEAE-cellulose chromatograms. The partially purified Hb F containing zones were next chromatographed on Synchropak AX-300 columns (top two chromatograms), while γ chain composition analyses of the (impure) Hb F zones were made by reverse-phase HPL chromatography (bottom two chromatograms). The methodology is described in detail in reference [11].

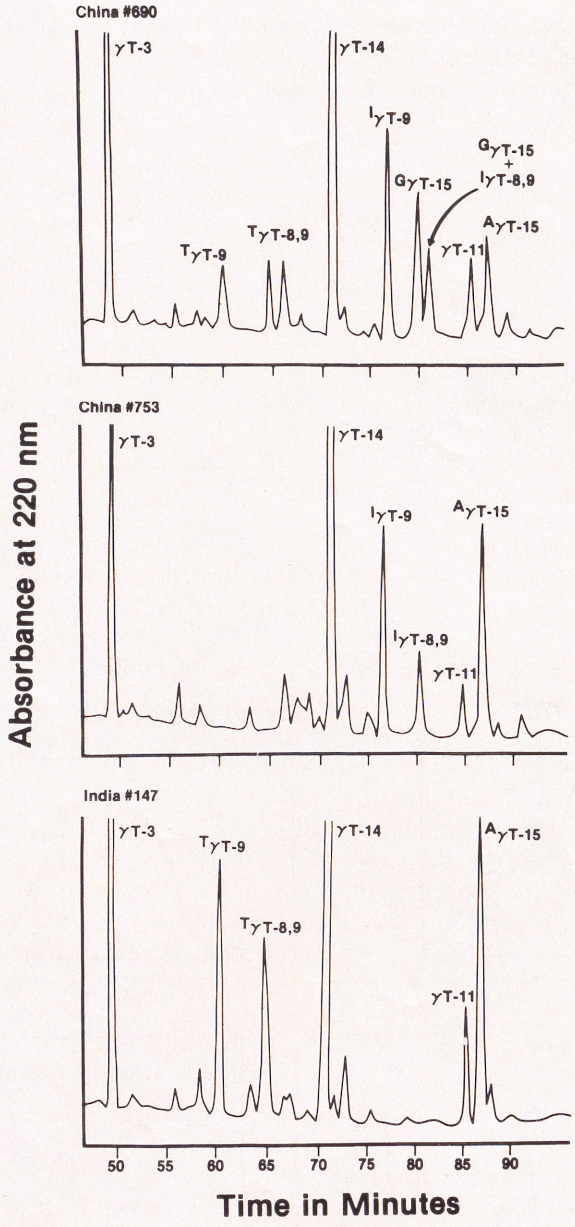

Fig. 5. Sections of HPL chromatograms depicting the separation of selected peptides from tryptic digests of isolated γ chains from the Hb F's of three newborn babies. Details are provided in the text, while the technique is described in reference [13].

These results and the absence of $^{T}\gamma$T-9 containing zones and the $^{G}\gamma$T-15 peptide in the Hb F of China baby #753, and of $^{I}\gamma$T-9 containing zones and the $^{G}\gamma$T-15 peptide in the Hb F of India baby #147 confirm the chain composition data obtained by HPL chromatography as given in Figures 1 and 2.

TABLE VIII. The Amino Acid Composition of the T-9 and T-15 Peptides Isolated From Tryptic Digests of the γ Chains of Hb F of Two Newborn Babies

	China #753			India #147		
	T-9	T-8,9	T-15	T-9	T-8,9	T-15
Aspartic acid	1.03(1)	1.17(1)	—	1.05(1)	1.29(1)	—
Threonine	1.00(1)	0.99(1)	1.04(1)	1.95(2)	1.72(2)	1.03(1)
Serine	1.13(1)	1.16(1)	2.61(3)	1.35(1)	1.14(1)	2.66(3)
Glycine	1.12(1)	1.15(1)	0	1.07(1)	1.02(1)	0
Alanine	1.17(1)	1.70(1)[a]	3.27(3)	1.10(1)	1.00(1)	3.28(3)
Valine	0.87(1)	0.92(1)	2.07(1)	0.86(1)	0.94(1)	2.04(2)
Methionine	—	—	0.47(1)	—	—	0.61(1)
Isoleucine	0.75(1)	0.67(1)	—	0	0	—
Leucine	1.92(2)	1.79(2)	0.94(1)	1.79(2)	1.65(2)	0.94(1)
Lysine	1.01(1)	1.88(2)	—	1.03(1)	1.94(2)	—
Arginine	—	—	1.06(1)	—	—	1.06(1)
n moles	2.7	1.0	3.2	6.3	2.0	4.3
Type of chain		$^{A}\gamma^{I}$ (75Ile; 136Ala)			$^{A}\gamma^{T}$ (75Thr; 136Ala)	

[a]The high alanine level is unexplained.

DISCUSSION

The heterogeneity of the human γ chain, ie, the duplication of the γ chain gene with products being different at postion 136, has been known for nearly 15 years [16]. Additionally, several γ chain mutants have been discovered, and presently as many as 30 such variants have been reported (International Hemoglobin Information Center, Augusta, GA 1982). Most of these variants are rare, but a few such as Hb F-Malta-I [$\alpha_2\gamma_2$ 117His → Arg; 136Gly [18]], Hb F-Hull [$\alpha_2\gamma_2$ 121Glu → Lys; 136Ala [19]], Hb F-Port Royal [$\alpha_2\gamma_2$ 125Glu → Ala; 136Gly [20]], and Hb F-Sardinia [$\alpha_2\gamma_2$ 75Ile → Thr; 136Ala [21]] have been observed at higher frequencies. Most outstanding is Hb F-Sardinia which has been found in the Hb F of many patients with β-thalassemia and other disorders [22–24]. The fact that the γ-F-Sardinia chain, better known as the $^{A}\gamma^{T}$ chain, can readily be separated from the $^{A}\gamma^{I}$ (75Ile; 136Ala) and $^{G}\gamma$ (75Ile; 136Gly) chains using high pressure liquid chromatography, has greatly facilitated the quantitation of these three chains in Hb F containing samples.

This is again evident from the results of our most recent survey. The $^{A}\gamma^{T}$ chain was present in all racial and/or ethnic groups although at widely different frequencies (Table IV). The occurrence of the $^{A}\gamma^{T}$ chain among normal black children of the South Eastern USA has been discussed before [4,5]. The frequency is considerably less than that seen in Caucasian babies born in the same area. Of interest is also the low frequency among SS newborns and persons with sickle cell anemia living in the same area [3]. A most recent survey of 178 SS patients gave 10 heterozygotes and one homozygote ($f\cdot^{A}\gamma^{T} = 0.034$). These low f values can be explained by assuming that chromosomes carrying the β^{S} mutation are relatively free of the $^{A}\gamma^{T}$ mutation (the $^{G}\gamma$-$^{A}\gamma^{I}$-δ-β^{S} genic arrangement), and that crossovers involving a chromosome with the $^{G}\gamma$-$^{A}\gamma^{T}$-δ-β^{A} genic arrangement had occurred infrequently. In fact, the data for newborns with a Hb S heterogeneity ($f\cdot^{A}\gamma^{T} = 0.071$) suggest that the genotype with the $^{A}\gamma^{T}$ mutant in trans to the β^{S} mutation occurs about three times more frequently than that in which this mutant is in cis to the β^{S} mutation [5].

Analyses of the Hb F from more than 80 SS patients from Ghana failed to identify any with the $^{A}\gamma^{T}$ heterozygosity [25]. This suggests that the population of the West African Coast is relatively free of the $^{A}\gamma^{T}$ anomaly. Support for this suggestion is also provided by the low $^{A}\gamma^{T}$ frequncy of the Hb C heterozygous newborns ($f \cdot {}^{A}\gamma^{T} = 0.036$), which is nearly half of that of black AS babies and about one-third of that of black AA babies from the same area (Table IV). This low $^{A}\gamma^{T}$ frequency indicates that the $^{A}\gamma^{T}$ mutant is primarily in trans to the β^{C} mutation; the latter likely originated in the West African population (mainly Ghana), where its incidence is higher than anywhere else in the world.

These comparisons suggest a wide variation in the distribution of the $^{A}\gamma^{T}$ anomaly with high frequencies in Mediterranean (and other European) populations and in populations of the Far-East, and with low frequencies in populations of the African West Coast and of Mainland China.

A very high percentage of the 2453 babies tested had $^{G}\gamma$ values between 55 and 80%. The $^{G}\gamma$ levels were not influenced by the presence of an $^{A}\gamma^{T}$ heterozygosity or homozygosity, and were neither different for the various racial and/or ethnic groups tested (Fig. 3). High $^{G}\gamma$ values, ie, 80% and above, were found in 58 babies (2.4%), but a considerable overlap with normal values was observed (Fig. 3). The one black baby with an apparent homozygosity for this condition had the high$^{G}\gamma$ value of at least 96%. Presently, this infant is nearly 3 years old and in excellent health. DNA structural analyses to establish the exact nature of the anomaly are in progress.

The previous publication [4] also reported on two, related, black infants with low $^{G}\gamma$ values (35.0 and 44.5%, respectively). Two possibilities were suggested: a $^{G}\gamma^{\circ}$-thalassemia (the $^{G}\gamma \cdot {}^{A}\gamma \cdot /\!\!-\!\!- {}^{A}\gamma$ genic arrangement) or a condition in which the Ala → Gly exchange at position γ136 is not present (the $^{G}\gamma \cdot {}^{A}\gamma / {}^{A}\gamma \cdot {}^{A}\gamma$ genic arrangement). The present study has identified as many as 35 babies with $^{G}\gamma$ values between 30 and 50%, while two babies did not produce any Hb F with $^{G}\gamma$ chains at all (Fig. 3). Structural analyses have confirmed the low $^{G}\gamma$ values in some of these infants, as well as the observation that the Hb F of one of these babies has only $^{A}\gamma^{I}$ chains and the other a Hb F with only $^{A}\gamma^{T}$ chains (Fig. 5; Table VIII). Several other aspects are of interest.

1) The parents of these two babies were clinically and hematologically normal (Table VII) which excludes the possibility that an $^{A}\gamma$ type of HPFH [26] is present.

2) The level of Hb F in the two babies at time of birth averaged a low 56%, while this Hb F was nearly completely replaced by Hb A in 8 to 10 weeks, which may be faster than is normally observed. It is indeed surprising that these two babies are clinically well. Unfortunately, the hematological data collected after birth were limited and not sufficient for a definitive clinical characterization of the condition.

3) Babies with a heterozygosity for this condition also exhibited decreased levels of Hb F, although the average increase in the Hb A level was relatively small (from 21.5% in normal babies to 28.5%; Table VI).

4) The additional presence of an $^{A}\gamma^{T}$ heterozygosity was observed in nine babies. In five (Type I) the $^{A}\gamma^{T}$ level averaged only 15.4% and was similar to that observed in simple $^{A}\gamma^{T}$ heterozygotes (Table III); the $^{A}\gamma^{I}$ level averaged 38.5%. The $^{A}\gamma^{T}$ level in the other four babies (Type II) was nearly twice that value (29%), while that of the $^{A}\gamma^{I}$ chain was decreased to 29.5% (Table III).

These various observations could be explained by assuming either the deletion or complete inactivation of a $^{G}\gamma$ chain gene or the replacement of a $^{G}\gamma$ chain gene by

an $^{A}\gamma$ chain gene. Preliminary, DNA structural analyses have shown a gene conversion in one of the two black babies with low $^{G}\gamma$ values resulting in a $^{G}\gamma\cdot{}^{A}\gamma^{I}/{}^{A}\gamma^{I}\cdot{}^{A}\gamma^{I}$ genic arrangement [27]. A similar arrangement might be present in (most of) the babies of the other populations. The five babies with low $^{G}\gamma$ values and an $^{A}\gamma^{T}$ heterozygosity (Table III; Type I), for instance, may have the $^{G}\gamma\cdot{}^{A}\gamma^{I}/{}^{A}\gamma^{I}\cdot{}^{A}\gamma^{T}$ or $^{G}\gamma\cdot{}^{A}\gamma^{T}/{}^{A}\gamma^{I}\cdot{}^{A}\gamma^{I}$ condition. Another possiblity would be the $^{G}\gamma\cdot{}^{A}\gamma^{T}/{}^{A}\gamma^{I}\cdot$ —— genic arrangement in which it is assumed that one chromosome carries one active $^{A}\gamma^{I}$ gene producing this $^{A}\gamma^{I}$ chain at the same rate as the $^{G}\gamma$ chain by a $^{G}\gamma$ gene of a normal chromosome, while the normally occurring $^{A}\gamma^{I}$ gene is either deleted or not active. Additional support for this hypothesis comes from the observation on the Hb F's from baby China #753 with the possible $^{A}\gamma^{I}\cdot$ ——/$^{A}\gamma^{I}\cdot$ —— genic arrangement, from baby India #147 with the $^{A}\gamma^{T}\cdot$ ——/$^{A}\gamma^{T}\cdot$ —— genic arrangement (the same condition as present in baby China #753, but with an additional Ile → Thr replacement at position 75), and from a baby with a Hb F-Yamaguchi heterozygosity who has the $^{G}\gamma\cdot{}^{A}\gamma^{I}/{}^{A}\gamma^{T\text{-}X}\cdot$ —— (X refers to the γ80 Asp → Asn substitution) genic arrangement (this baby is discussed in detail in the following paper). The four babies with low $^{G}\gamma$ values and about the same percentages of the $^{A}\gamma^{T}$ and $^{A}\gamma^{I}$ chains (Table III: Type II) might have a homozygosity for the $^{A}\gamma^{T}$ chain (the $^{G}\gamma\cdot{}^{A}\gamma^{T}/{}^{A}\gamma^{I}\cdot{}^{A}\gamma^{T}$ genic arrangement) or the $^{G}\gamma\cdot{}^{A}\gamma^{I}\cdot/{}^{A}\gamma^{T}\cdot{}^{A}\gamma^{I}$ condition in which the $^{A}\gamma^{T}$ chain gene occupies the $^{G}\gamma$ chain position and produces the $^{A}\gamma^{T}$ chain at a rate slightly less than that of a $^{G}\gamma$ chain gene. It is obvious that DNA structural analyses will be needed to further define the detailed genetic anomalies in these infants.

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REFERENCES

1. Huisman THJ, Wilson JB: Recent advances in the quantitation of human fetal hemoglobins with different gamma chains. Am J Hematol 9:225, 1980.
2. Huisman THJ, Webber B, Okonjo K, Reese AL, Wilson JB: The separation of human hemoglobin chains by high pressure liquid chromatography. In Hanash SM, Brewer GJ (eds): "Advances in Hemoglobin Analysis." New York: Alan R. Liss, 1981, pp 23–38.
3. Huisman THJ, Altay C, Webber B, Reese AL, Gravely ME, Okonjo K, Wilson JB: Quantitation of three types of γ chain of Hb F by high pressure liquid chromatography; application of this method to the Hb F of patients with sickle cell anemia or the S-HPFH condition. Blood 57:75, 1981.
4. Huisman THJ, Altay C: The chemical heterogeneity of the fetal hemoglobin of black newborn babies and adults; a reevaluation. Blood 58:491, 1981.
5. Huisman THJ, Webber B: The frequency of the $^{A}\gamma^{T}$ gene in the presence and absence of the β^{S} or β^{C} gene in the black population of the Southeastern USA. Hemoglobin 5:441, 1981.
6. Zeng Yi-tao, Huang Shu-zheng, Huisman THJ: Types and quantities of fetal hemoglobin in Chinese newborn babies. Scientia, in press.
7. Gardiner MB, Carver J, Abraham BL, Wilson JB, Huisman THJ: Further studies on the quantitation of the hemoglobins A, S, C, and F in newborn babies with different hemoglobinopathies using high pressure liquid chromatography. Hemoglobin 6:1, 1982.
8. Huisman THJ, Gardiner MB, Wilson JB: Experiences with the quantitation of human hemoglobin types by high pressure liquid chromatography. In Hanash SM, Brewer GJ (eds): "Advances in Hemoglobin Analysis." New York: Alan R. Liss, 1981, pp 69–82.

9. Abraham EC, Reese A, Stalling M, Huisman THJ: Separation of human hemoglobins by DEAE-cellulose chromatography using glycine-KCN-NaCl developers. Hemoglobin 1:27, 1976.
10. Betke K, Marti HR, Schlicht I: Estimation of small percentages of foetal haemoglobin. Nature 184:1877, 1959.
11. Gardiner MB, Reese AL, Headlee ME, Huisman THJ: The heterogeneity of the γ chain of fetal hemoglobin in Hb S heterozygotes. Blood, 60:513, 1982.
12. Clegg JB, Naughton MA, Weatherall DJ: Abnormal human haemoglobins. Separation and characterization of the α and β chains by chromatography, and the determination of two new variants, Hb Chesapeake and Hb J-(Bangkok). J Mol Biol 19:91, 1966.
13. Wilson JB, Lam H, Pravatmuang P, Huisman THJ: Separation of tryptic peptides of normal and abnormal α, β, γ, and δ hemoglobin chains by high performance liquid chromatography. J Chromat 179:271, 1979.
14. Schroeder WA, Huisman THJ, Shelton JR, Shelton JB, Kleihauer, Dozy AM, Robberson B: Evidence for multiple structural genes for the γ chain of human fetal hemoglobin. Proc Natl Acad Sci 60:537, 1968.
15. Huisman THJ, Jonxis JHP: "The Hemoglobinopathies. Techniques of Identification." New York: Marcel Dekker, 1977, pp. 237–241.
16. Huisman THJ, Gravely ME, Webber B, Okonjo K, Henson J, Reese AL: The gamma chain heterogeneity of fetal hemoglobin in black β-thalassemia and HPFH heterozygotes. Blood 58:62, 1981.
17. Efremov GD, Ibarra B, Gurgey A, Sukumaran PK, Altay C, Huisman THJ: The gamma chain heterogeneity of fetal hemoglobin in non-black β- and $\delta\beta$-thalassemia and HPFH heterozygotes and homozygotes. Am J Hematol 12:367, 1982.
18. Cauchi MN, Clegg JB, Weatherall DJ: Haemoglobin F (Malta) a new foetal haemoglobin variant with a high incidence in Maltese infants. Nature 223:311, 1969.
19. Sacker LS, Beale D, Black AJ, Huntsman RG, Lehmann H, Lorkin PA: Haemoglobin F Hull (γ121 glutamic acid→lysine), homologous with haemoglobin O and O-Indonesia. Br Med J 3:531, 1967.
20. Brimhall B, Vedvick TS, Jones RT, Ahern E, Palomino E, Ahern V: Haemoglobin F Port Royal ($\alpha_2{}^G\gamma$ 125Glu → Ala). Br J Haematol 27:313, 1973.
21. Grifoni V, Kamuzora H, Lehmann H, Charlesworth D: A new Hb variant: Hb F Sardinia γ^{75} (E19) Isoleucine→threonine found in a family with Hb G Philadelphia, β-chain deficiency and a Lepore-like haemoglobin indistinguishable from Hb A_2. Acta Haematol 53:347, 1975.
22. Ricco G, Mazza U, Turi RM, Pich PG, Camaschella C, Saglio G, Bernini LF: Significance of a new type of human fetal hemoglobin carrying a replacement isoleucine → threonine at position 75 (E19) of the γ chain. Hum Genet 32:305, 1976.
23. Huisman THJ, Schroeder WA, Reese A, Wilson JB, Lam H, Shelton JR, Shelton JB, Baker S: The $^T\gamma$ chain of human fetal hemoglobin at birth and in several abnormal hematological conditions. Pediatr Res II: 1102, 1977.
24. Schroeder WA, Huisman THJ, Efremov GD, Shelton JR, Shelton JB, Phillips R, Reese A, Gravely M, Harrison JM, Lam H: Further studies of the frequency and significance of the $^T\gamma$ chain of human fetal hemoglobin. J Clin Invest 63:268, 1979.
25. Bruce-Tagoe AA, Zago MA, Huisman THJ: In preparation.
26. Huisman THJ, Schroeder WA, Stamatoyannopoulos G, Bouver N, Shelton JR, Shelton JB, Apell G: Nature of fetal hemoglobin in the Greek type of hereditary persistence of fetal hemoglobin with and without concurrent β-thalassemia. J Clin Invest 49:1035, 1970.
27. Powars P, Smithies O: Personal communication.