Further studies of the frequency and significance of the Tgamma-chain of human fetal hemoglobin.

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J Clin Invest. 1979;63(2):268-275. https://doi.org/10.1172/JCI109299.

Research Article

A further study of the Tgamma-chain in a variety of conditions has revealed its presence in the cord bloods of ethnic groups previously unstudied. Heterozygous newborn average 17-19% Tgamma-chain while the mean value in four presumed homozygotes was 31%. The Tgamma-chain is readily detectable in beta-thalassemia of various ethnic groups (although infrequent in Blacks) as well as in deltabeta-thalassemia. Studies of a few families have provided an opportunity to determine whether or not certain individuals are heterozygous or homozygous for the Tgamma-gene. The Tgamma-chain has not been detected in the human fetal hemoglobin that is synthesized in increased amounts in persons with the hereditary persistence of fetal hemoglobin. Although the Tgamma-chain is detectable in sickle cell anemia, its frequency appears to be lower than in normal individuals. By focusing upon the relationship of the percentage of Tgamma-chain to the sources of human fetal globulin from determinants in cis and in trans, the conclusion has been reached that the Tgamma-chain is the product of a mutant Agamma-locus which should be named the TAgamma-chain.

Further Studies of the Frequency and Significance of the $^{T}\gamma$-Chain of Human Fetal Hemoglobin

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ABSTRACT A further study of the $^{T}\gamma$-chain in a variety of conditions has revealed its presence in the cord bloods of ethnic groups previously unstudied. Heterozygous newborn average 17–19% $^{T}\gamma$-chain while the mean value in four presumed homozygotes was 31%.

The $^{T}\gamma$-chain is readily detectable in β-thalassemia of various ethnic groups (although infrequent in Blacks) as well as in $\delta\beta$-thalassemia. Studies of a few families have provided an opportunity to determine whether or not certain individuals are heterozygous or homozygous for the $^{T}\gamma$-gene. The $^{T}\gamma$-chain has not been detected in the human fetal hemoglobin that is synthesized in increased amounts in persons with the hereditary persistence of fetal hemoglobin.

Although the $^{T}\gamma$-chain is detectable in sickle cell anemia, its frequency appears to be lower than in normal individuals.

By focussing upon the relationship of the percentage of $^{T}\gamma$-chain to the sources of human fetal globulin from determinants in cis and in trans, the conclusion has been reached that the $^{T}\gamma$-chain is the product of a mutant $^{A}\gamma$-locus which should be named the $^{TA}\gamma$-chain.

INTRODUCTION

The γ-chains of human fetal hemoglobin (Hb-F)[1] are produced by nonallelic genes (1, 2). Evidence for this is derived from the observation that Hb-F contains two types of γ-chains; namely, one with glycine in position 136 (the $^{G}\gamma$-chain) and a second with alanine in this position (the $^{A}\gamma$-chain). The recent discovery of the $^{T}\gamma$-chain by Ricco et al. (3) has added yet another facet to the many ways in which the study of Hb-F has provided data about the genetics of this fetal protein. In the $^{T}\gamma$-chain, the isoleucyl residue at position 75 of the γ-chain is replaced by a threonyl residue. Although Ricco et al. (3) particularly examined the frequency of the $^{T}\gamma$-chain in β-thalassemia, they also detected its presence in cord bloods. Our studies of the $^{T}\gamma$-chain (4) substantiated their data in β-thalassemia of individuals of Mediterranean origin, but detected no $^{T}\gamma$-chains in the β-thalassemia of Blacks. Moreover, our data from cord blood suggested that the frequency of the $^{T}\gamma$-chain might be related to ethnic source or geographical locality, and quantitatively was present in greater percentage than reported by Ricco et al. Because of the limited data, conclusions could only be tentative, and statistical evaluation had limited meaning. Consequently, we have extended these studies in several areas and examined other previously unstudied abnormalities.

METHODS

Many samples of Hb-F were available from previous investigations of the $^{G}\gamma$- to $^{A}\gamma$-ratio in Hb-F (1, 2). In most of these, not only γ- and α-chains, but considerable percentages of β-chains were also present. For most samples, the $^{G}\gamma$- to $^{A}\gamma$-ratio was known as well as the percentages of Hb-F and Hb-A_2; procedures for these determinations have been described in detail (1, 2).

The methods of tryptic hydrolysis, column chromatographic isolation of the γT-9 peptides (residues 67–76, inclusive), their amino-acid analysis, and the calculation of results have been described in detail (4) and were used without major modification. To assess the reproducibility of results within and between laboratories in Pasadena, Calif. and Augusta, Ga., a series of samples was repeatedly analyzed (Table I). Major discrepancy of data occurred only in the results from Cases M.D., M.H., and O.J. The original data in Augusta sug-

Received for publication 7 August 1978 and in revised form 25 September 1978.

[1] *Abbreviations used in this paper*: AS, sickle cell trait; Hb, hemoglobin; Hb-F, human fetal Hb; HPFH, hereditary persistence of fetal Hb; SC, Hb-S,Hb-C disease; SS, sickle cell anemia.

 J. Clin. Invest. © *The American Society for Clinical Investigation, Inc.*, 0021-9738/79/02/0268/08 $1.00
Volume 63 February 1979 268–275

TABLE I

Results of Replicate Determinations within and between Laboratories

Designation	Percentage of $^{T}\gamma$-chain	
	Pasadena	Augusta
F.N.	22, 20	
L.H.	11, 16	
P.W.	16, 18	
C.K.	42, 42	
L.P.		16, 22
India 6,233		18, 22
P.B.	23, 22*, 21‡	25, 21*, 22‡
Chinese 1	18, 17*, 15‡	13, 14, 17*, 13‡
B.V.V.	19, 18, 16	16
N.W.	31, 31	32
M.D.	16, 12	22
M.H.	16, 13	28
O.J.	17	33

In these experiments, the peptides $^{T}\gamma$T-9 and $^{L}\gamma$T-9 were isolated in Pasadena, Calif., but portions were analyzed both in Pasadena, Calif. and Augusta, Ga.

* Data from Pasadena, Calif.

‡ Data from Augusta, Ga.

gested that technical problems existed in addition to the use of the one-column system (4). The remaining data reveal that duplicate determinations may be expected to agree within ±3%. This degree of concordance between duplicate determinations is somewhat unexpected, because neither of the γT-9 peptides is pure as isolated, and the interpretation of the amino-acid analyses is subjective to a degree.

RESULTS

The $^{T}\gamma$-chain in cord bloods. Table II summarizes the results from the examination for $^{T}\gamma$-chains in cord bloods from a variety of ethnic and geographic sources. These data include the information previously reported (4). Initially, Hb-F from 98 newborn was studied; to these data may now be added the results from 96 samples. Two other ethnic groups, Kenya Blacks and Asiatic Indians, have been examined in these additional samples; positive samples were detected in both.

Of the original 98 samples, 70 were negative. Consequently, the frequency of the $^{T}\gamma$-gene in this group was 0.14. There are 149 negative results in the present 194 so that the $^{T}\gamma$-frequency (0.12) is about the same. It is noteworthy that Blacks as well as Caucasians from several geographic areas had positive samples in frequencies that probably were not greatly different from each other.

The distribution of positive data in terms of the percentage of $^{T}\gamma$-chain is presented in Fig. 1. Two groups were observed: One group of four averaged 31% $^{T}\gamma$-chain, and the remaining 39 averaged 18% (17% for 23 Black babies and 19% for 16 Caucasian newborn).

TABLE II

The $^{T}\gamma$-Chain in Newborn Babies of Different Populations

Population	Condition	Total No.	No. of positive
Georgia Blacks	AA; AS; AC; AR; Fx*	81	21
Georgia Blacks	SS; SC‡	13	2
Los Angeles Blacks	AA; AS§	10	2
Ghana Blacks	AA; AS; AC‖	21	1
Kenya Blacks	AA	17	3
Total		142	29
Los Angeles Whites	AA	12	5
Holland Whites	AA	11	4
Malta Whites	AA; F_M heterozygotes¶	17	3
Others	**	12	4
Total		52	16

* 10 babies with AS, AC, or A-Richmond; 5 babies with F-Port Royal (Fx); AA, normal.

‡ Two babies with SC; both negative.

§ One baby with AS; negative.

‖ Three babies with AS or AC; all negative.

¶ Seven babies with Hb F-Malta I; one positive.

** From India (five babies), and one each of Chinese, Japanese, Eskimo, Egyptian, Turkish, American Indian, and Australian aborigine origin.

The 31% $^{T}\gamma$-chain corresponds rather well with the average value of 29% $^{A}\gamma$-chain in the newborn babies (5). It had previously been suggested that one sample with 35% $^{T}\gamma$-chain (this is N.W. of Table II [4] which now averages 33% in three determinations) represented a $^{T}\gamma$-homozygote. The detection of three others in this sample range strengthens this conclusion. One

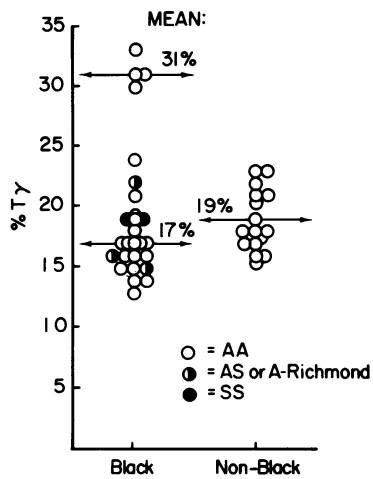

FIGURE 1 Distribution of the percentage of $^{T}\gamma$-chains in positive cord bloods. Data from two definitely positive samples from Blacks are omitted because of technical problems in the quantitation. AA, normal.

of these putative homozygotes is from Kenya whereas the remaining three are from a Georgia community. A family study was possible for one assumed homozygous newborn who had 31% $^{T}\gamma$-chain. Both parents had Hb-F levels of <2% and were positive for the $^{T}\gamma$-chain.

The $^{T}\gamma$-chain in sickle cell anemia. The Hb-F of 73 cases of sickle cell anemia (11 Black newborn, 51 Black adults, 10 Veddoid Indians, and 1 Israeli citizen) has been examined; 7 from Blacks and 0 from Veddoid Indians were positive for the $^{T}\gamma$-chain (Tables II and III). All information on the seven positive cases is detailed in Table IV; initial detection was done in cord blood samples of F.N. and C.N. and several samples from the former (and from two additional sickle cell anemia [SS] infants) could be examined. Although the data are somewhat erratic, no definite trend of increasing percentage of $^{T}\gamma$-chain from birth to adulthood was observed.

The data of Table III indicate that positive results were obtained in one of five adults with sickle cell trait and in one of seven adults with Hb-S,Hb-C (SC) disease. Assuming that one SS patient is homozygous for the $^{T}\gamma$-chain (M.P., to be discussed later) and all others (SS, sickle cell trait [AS], and SC) heterozygous for the $^{T}\gamma$-chain, the frequency of the $^{T}\gamma$-gene in sickle-cell disease approximates 0.06, which suggests that the occurrence of the $^{T}\gamma$-gene in combination with the β^{S}-gene is less than in combination with the β^{A}-gene.

The $^{T}\gamma$-chain in hereditary persistence of fetal hemoglobin (HPFH). 8 homozygotes and 34 heterozygotes of various types, including 6 individuals with Hb-Kenya who have the $^{G}\gamma$-HPFH phenotype, all were negative for the $^{T}\gamma$-chain (Table V). These results do not exclude the presence of a $^{T}\gamma$-gene in trans to the HPFH determinant in the heterozygote, but do suggest that it is absent in cis within the limits of this relatively small number of samples. The data from a newborn infant with the $^{G}\gamma$-HPFH heterozygosity (N. O. C. [6]) suggest that the $^{T}\gamma$-chain can indeed be present in trans to the HPFH determinant (Table VI). At birth, 17% $^{T}\gamma$-chain and ≅14% $^{A}\gamma$-chain were detected. Both values decreased with time, which suggests that cessation of $^{A}\gamma$-chain synthesis by a locus in trans to the $^{G}\gamma$-HPFH determinant parallels the decrease of the $^{T}\gamma$-chain production.

TABLE III
The $^{T}\gamma$ Chain in Persons with Abnormal Hemoglobins*

Population	Condition	Total No.	No. of positives‡
Black	SS	51	5
Black	AS	4	1
Black	AC	1	0
Black	CC	1	0
Black	SC	7	1
India	AS	1	0
India	SS	10	0
Israel	SS	1	0

* 11 months and older.
‡ The $^{T}\gamma$-chain values of the five positive SS patients are listed in Table IV; percent $^{T}\gamma$ in one AS person was 41%, and 17% in one SC individual.

TABLE IV
The $^{T}\gamma$-Chain in SS

Case	Age	$^{T}\gamma$	Gly in γCB-3
		%	
D.C.	Adult	29*, 23*	0.55
M.P.	Adult	27*, 36*	0.77
L.P.	Adult	28	0.42
T.A.‡	28 mo	22	0.50
	31 mo	18	—
	34 mo	25	—
Tw.A.‡	11 mo	24	0.63
	14 mo	22	0.61
	19 mo	22	—
F.N.	Cord	19	0.75
	6 wk	16	0.71
	14 mo	29	—
	21 mo	22, 20§	0.56
C.N.	Cord	19	0.75

* Data from two independently isolated samples of Hb-F for each individual.
‡ Siblings.
§ Duplicate determinations on same sample.

TABLE V
The $^{T}\gamma$-Chain in Adults with HPFH

Population	Condition	Total No.	No. of positives
Black	$^{G}\gamma^{A}\gamma$-heterozygote*	19	0
Black	$^{G}\gamma^{A}\gamma$-homozygote	7	0
India	$^{G}\gamma^{A}\gamma$-heterozygote	1	0
India	$^{G}\gamma$-heterozygote‡	2	0
India	$^{G}\gamma$-homozygote	1	0
Black	$^{G}\gamma$-heterozygote§	3	0
Caucasian	$^{A}\gamma$-heterozygote‖	3	0
Black	$^{G}\gamma$-heterozygote¶ with Hb-Kenya	6	0

* Includes all types; two subjects were also heterozygous for Hb-S.
‡ Was also heterozygous for Hb-E.
§ See (6).
‖ See (7, 8).
¶ See (9); two subjects were also heterozygous for Hb-S.

TABLE VI
Postnatal Change of the $^{G}\gamma$, $^{A}\gamma$, and $^{T}\gamma$-Chains in a $^{G}\gamma$-HPFH Heterozygote

Age of individual	Residues in position 136		$^{T}\gamma$-chains
	Gly	Ala*	
			%
Birth	0.86	0.14	17
56 d	0.90	0.13	15
112 d	0.95	0.08	8

* The residues of alanine in γCB-3 minus 2 because alanine is always present at positions 138 and 140.

The $^{T}\gamma$-chain in β-thalassemia and δβ-thalassemia. The data of Table VII show that Hb-F has been examined for the presence of the $^{T}\gamma$-chain in many individuals with β-thalassemia from a wide range of ethnic groups and geographical localities. Frequencies in individual groups appear meaningless because of limited data, but the results do suggest that the $^{T}\gamma$-chain is widespread in β-thalassemia as shown also by Ricco et al. (3). This is particularly true in ethnic groups surrounding the Mediterranean area (Italian-Greek-Yugoslavian) where, from the data of Table VII, the frequency approaches 0.16.

As previously noted (4) the $^{T}\gamma$-chain is not very evident in Blacks with β-thalassemia, and only 6 of 50 Black individuals with heterozygous or homozygous β-thalassemia were positive for the $^{T}\gamma$-chain.

TABLE VII
The Tγ-Chain in β- and δβ-Thalassemia

Population	Condition	Total No.	No. of positives
Blacks	β-Thal. Heterozygotes*	29	5‡
Blacks	β-Thal. Homozygotes	21	1
Non-Blacks	β-Thal. Heterozygotes§	35	9
Non-Blacks	β-Thal. Homozygotes‖	65	23
Non-Blacks	δβ-Thal. Heterozygotes¶	16	11
Non-Blacks	δβ-Thal. Homozygotes	7	0
Blacks	δβ-Thal. Heterozygotes ($^{G}\gamma$-type)	3	0
Yugoslavia	Lepore Heterozygotes	2	0
Yugoslavia	Lepore Homozygotes	2	0
Yugoslavia	Lepore β-Thal.	4	1

* Includes two persons with C-β Thalassemia and three with S-β Thalassemia.
‡ Three are members of the same family.
§ Includes four persons with E-β Thalassemia.
‖ 18 from Italy or Greece (12 pos.); 13 from Yugoslavia (5 pos.); 11 from Israel (1 pos.); 10 from Turkey and Iran (2 pos.); 5 from India (0 pos.); one from Malta (pos.); one from Holland (neg.); one Chinese (pos.); 4 from the Far East (1 pos.); one unknown (neg.).
¶ Many belong to one Yugoslavian family; see Fig. 5.

Fig. 2 shows the wide distribution from 19 to 70% $^{T}\gamma$-chain in the Hb-F of nine non-Black individuals with β-thalassemia trait who had Hb-F of the order of 2%; although one had only 0.3% and two had 5.5 and 10%. The meaning of this wide range is not apparent.

16 δβ-thalassemia heterozygotes and 7 δβ-thalassemia homozygotes have been studied. However, the large number of positive cases (11 heterozygotes) is not indicative for a high frequency of the $^{T}\gamma$-chain in this condition because 7 belong to the same family. Moreover, none of the seven homozygotes had the $^{T}\gamma$-chain. The percent $^{T}\gamma$-chain in the 11 δβ-thalassemia heterozygotes averaged ≅61% with a range of 51–71% (Fig. 2).

Family studies. Data on members of four families with β-thalassemia or δβ-thalassemia are presented in Figs. 3–5.

Family C. Hematological data about this family have been reported by Friedman et al. (10). Fig. 3 presents the relevant part of the pedigree with quantitative data of several types. The propositus, III-7, is homozygous for β-thalassemia and is at least heterozygous for the β^{+}-type. In addition, he has both Hb-A_2 and Hb-A_2'. In the mother, her sister, and two siblings of the propositus, Hb-A_2' is in cis to the β-thalassemia determinant, whereas the father and one sibling have Hb-A_2 in cis to the β-thalassemia determinant. The data show that $^{T}\gamma$-chain is not produced in cis to β-thalassemia and Hb-A_2' on the maternal side. On the other hand, the propositus, his father (II-15), and a sibling with Hb-A_2 (III-6) do produce $^{T}\gamma$-chains. The percentage of $^{T}\gamma$-chains in the Hb-F of the propositus and

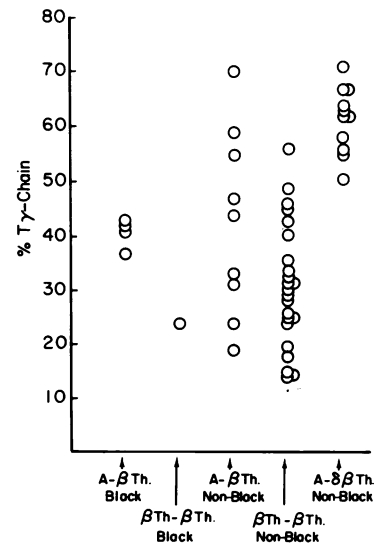

FIGURE 2 Distribution of the percentage of $^{T}\gamma$-chain in positive samples from adults with different types of thalassemia.

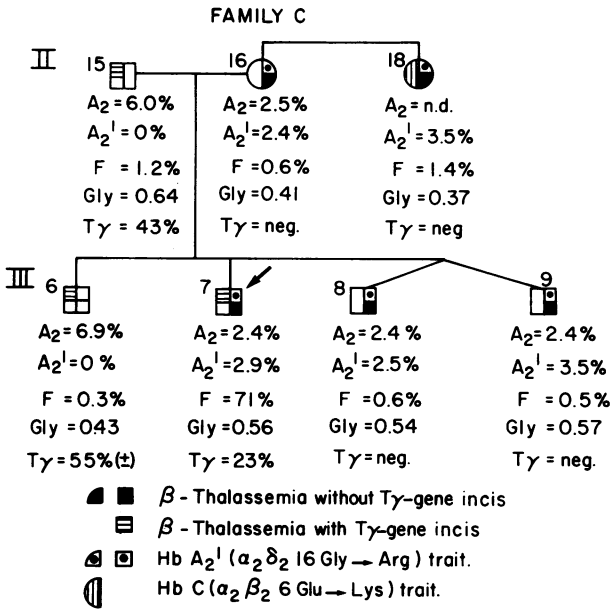

FIGURE 3 Partial pedigree of a family with β-thalassemia, Hb-A_2', and Hb-C.

his father could be accurately determined; however, that in the sibling III-6 was somewhat high because of technical problems. These data indicate that the propositus who must be heterozygous for the $^{T}\gamma$-determinant had about one-half the percentage of $^{T}\gamma$-chain found in the Hb-F of his father. Assuming that his Hb-F comes about equally from both maternal and paternal β-thalassemia determinants, Hb-F without $^{T}\gamma$-chain from the maternal determinant has diluted the Hb-F with the $^{T}\gamma$-chain from the paternal determinant.

In the Chinese Family W., the daughter II-1, who is heterozygous for β-thalassemia, inherited the $^{T}\gamma$-determinant from her mother and not from her father (Fig. 4). The sibling II-2, who is homozygous for β-thalassemia, is also heterozygous for the $^{T}\gamma$-determinant. The 70% $^{T}\gamma$-chains in the Hb-F of II-1 and one-half that percentage in II-2 suggest that, as in Family C., there is a dilution of $^{T}\gamma$-chains in this β-thalassemia homozygote.

The expression of the $^{T}\gamma$-chain in Family B. is also illustrated in Fig. 4. Because homozygote II-2 had no Hb-A at birth, nor did she produce any before her first transfusion at 112 d (11), β^{o}-thalassemia must be present. The percentages of $^{T}\gamma$-chain in the father, the mother, and one older sister, who also had a thalassemia homozygozity, are rather similar; thus, one might conclude that there is no dilution effect in these homozygotes who are also homozygous for the $^{T}\gamma$-chain.

The relationships in Family D., which have been described in other respects (12), are given in Fig. 5. The proposita (III-4), who has both δβ-thalassemia and β-thalassemia, received the β-thalassemia determinant from her mother (II-8) who is negative for the $^{T}\gamma$-chain.

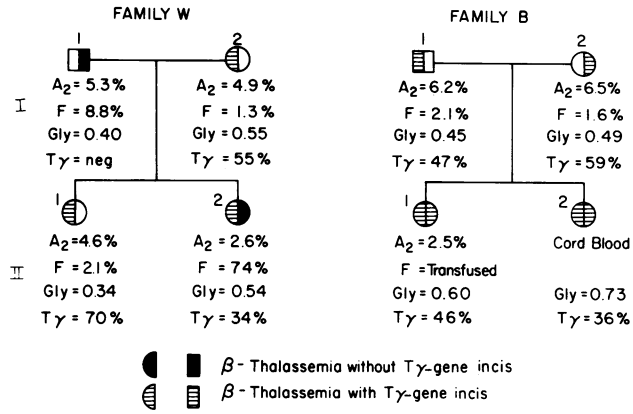

FIGURE 4 Partial pedigrees of two families with β-thalassemia.

On the paternal side, the percentage of the $^{T}\gamma$-chain has been determined in the Hb-F of the father (II-7) and three of his siblings as well as in the grandfather and two cousins of the proposita. The father of the proposita and the other affected relatives have the $^{T}\gamma$-gene in cis to the δβ-thalassemia determinant and are heterozygous for both. The 35% $^{T}\gamma$-chains in the Hb-F of the proposita, in contrast to the average of 62% in the seven δβ-thalassemia heterozygotes, again reflects the dilution effect from the non-$^{T}\gamma$-containing Hb-F of the β-thalassemia determinant.

The $^{T}\gamma$-chain in miscellaneous conditions. Table VIII lists a variety of miscellaneous conditions whose Hb-F has been examined for the presence of $^{T}\gamma$-chains. The frequency of the $^{T}\gamma$-gene in this very heterogeneous collection of conditions is 0.09 and does not differ significantly from the frequency in all cord bloods (*vide supra*). Consequently, it seems probable that the

TABLE VIII

The $^{T}\gamma$-Chain in Persons with Some Acquired Hematological Conditions and in Normal Individuals

Condition	Total No.	No. of positives
JCML*	7	3
Erythroleukemia	3	0
Leukemia‡	8	2
Fanconi's Anemia	14	1
Blackfan-Diamond Anemia	3	1
Aplastic Anemia	7	3
D-trisomy	9	2
Normals	8	3
Miscellaneous§	35	2

* Juvenile chronic myelogenous leukemia.

‡ Various forms.

§ Undiagnosed cases of which many had normal hematology, but all had elevated levels of Hb-F to some degree.

presence of the $^{T}\gamma$-chain in the Hb-F of these individuals simply reflects the appropriate component in their genetic makeup and is unrelated to the specific condition.

DISCUSSION

The incidence of the $^{T}\gamma$-chain. The $^{T}\gamma$-chain occurs widely among the human races and various ethnic groups. Although the available data are still limited, it appears that its incidence is the lowest in the Black race. Moreover, no $^{T}\gamma$-chain was found in the Hb-F of persons with the HPFH condition and only in a few Black patients with sickle cell anemia and homozygous β-thalassemia. The absence of the $^{T}\gamma$-chain in the Hb-F of HPFH heterozygotes does not exclude its presence in trans to the HPFH determinant. The observations made in the newborn $^{G}\gamma$-HPFH heterozygote (Table VI) show that the $^{T}\gamma$-chain gene may be present in trans, but that with the decrease in the activity of the $^{G}\gamma$- and $^{A}\gamma$-genes in trans, the relative production of the $^{T}\gamma$-chain decreases accordingly to a level at which it will not be detectable any longer. The frequency of $^{T}\gamma$-chain in Caucasian β-thalassemia homozygotes (23 of 65 cases studied, which corresponds to a frequency of 0.18) is less than that reported by Ricco et al. (3) (39 of 42 cases studied, corresponding to a frequency of 0.46) and is not significantly different from that of newborn (Table II). It appears desirable to analyze the Hb-F from newborn of different racial and ethnic backgrounds to evaluate the world-wide occurrence of the $^{T}\gamma$-chain.

The $^{T}\gamma$-chain in relation to the $^{G}\gamma$- and $^{A}\gamma$-chains. Because all samples of Hb-F have either $^{G}\gamma$- or $^{A}\gamma$-chains or both, that is, either glycine or alanine or both at position 136, it follows that the $^{T}\gamma$-chain must have one or both. Ricco et al. (3) concluded from indirect evidence that the $^{T}\gamma$-chain "certainly has glycine in position 136" and might also have alanine. In their investigation of Hb-F-Sardinia (which is another example of the $^{T}\gamma$-chain in a thalassemia individual), Grifoni et al. (13) came to the same conclusion. However, when in previous studies the fraction of $^{T}\gamma$-chain was plotted against the fraction of γ-chains that are $^{G}\gamma$-chains, a rather ill-defined inverse correlation appeared to exist (4); conversely, this would indicate a direct correlation with the fraction of $^{A}\gamma$-chains in Hb-F. Such a plot, however, is too simple and ignores completely the source of Hb-F and the possibility that the $^{T}\gamma$-chain gene is in cis or in trans (or both) of the primary genetic determinant of the condition involved. The data given in Fig. 6 again compare the fraction of $^{G}\gamma$-chains with that of the $^{T}\gamma$-chain. (A comparison of the percent $^{T}\gamma$-chain with the percent $^{A}\gamma$-chain is avoided because the accuracy of the percent $^{A}\gamma$-chain might be somewhat less than that of the $^{G}\gamma$-chain as a result of the presence

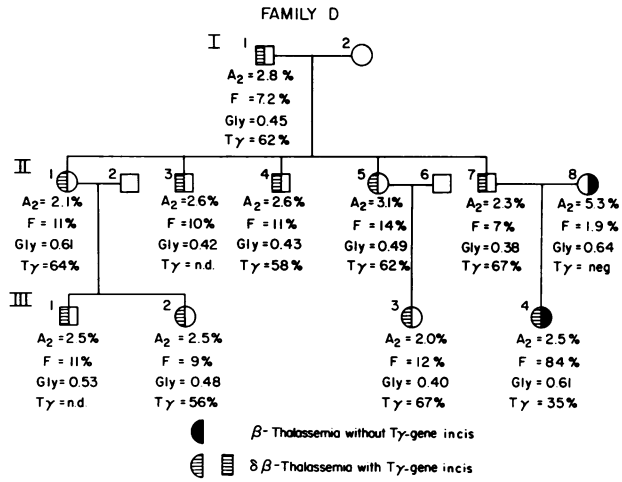

FIGURE 5 Pedigree of Family D. with $\delta\beta$-thalassemia and β-thalassemia.

of two additional alanyl residues in the γCB-3 peptide.) Assessment of these data relies heavily upon the accuracy and precision of the quantitative results and the agreement that may be expected from determinations in two laboratories (Table I). The data of Fig. 6 show a wide scattering. However, one group of data suggests, with a considerable degree of confidence, an inverse correlation between the percent $^{T}\gamma$-chains and the percent $^{G}\gamma$-chain (represented by the solid line); these results are from all $\beta\delta$-thalassemia heterozygotes,

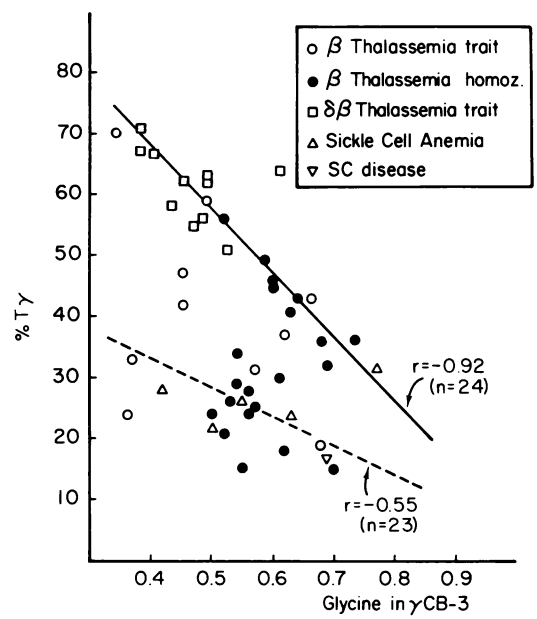

FIGURE 6 Relationship between the percentage of $^{T}\gamma$-chain and the fraction of $^{G}\gamma$-chain in the Hb-F from persons with different forms of thalassemia, and with SS and SC diseases. See text for details.

from several (but not all) β-thalassemia heterozygotes, from slightly <50% of the $^{T}\gamma$-chain positive β-thalassemia homozygotes, and from one of the five $^{T}\gamma$-chain positive SS patients. The others form a group in which relation between the percent $^{T}\gamma$-chain and the percent $^{G}\gamma$-chain is much less clear (represented by the broken line).

Thus, the first set of data suggests an inverse correlation between the percent $^{T}\gamma$-chains and the fraction of $^{G}\gamma$-chains (which is similar to a direct correlation with the fraction of $^{A}\gamma$-chains). Individuals belonging to this group may be either homozygous for the $^{T}\gamma$-gene and produce Hb-F equally in cis and in trans (examples are several homozygous β-thalassemia patients and the one SS patient), or are heterozygous for the $^{T}\gamma$-gene and produce Hb-F only in cis (examples are several β-thalassemia traits and all βδ-thalassemia traits). However, if an individual is heterozygous for the $^{T}\gamma$-gene and produces Hb-F equally in cis and in trans (examples are most β-thalassemia homozygotes, the four SS patients, and perhaps a few β-thalassemia traits) a dilution effect is evident, and the percent $^{T}\gamma$-chain is reduced considerably.

Some of the available data of Fig. 6 are difficult to explain because it cannot be determined with certainty into which category they belong. It is noteworthy, however, that the three β-thalassemia homozygotes of Fig. 4 fall in the expected categories, namely II-2 of Family W. with a $^{T}\gamma$-chain heterozygosity in the category of decreased percent $^{T}\gamma$-chain, and cases II-1 and II-2 with a $^{T}\gamma$-chain-homozygosity in the category in which the correlation between the percentage of $^{T}\gamma$- and $^{A}\gamma$-chain is clearly evident.

Fig. 7 gives similar data for individuals without hemoglobinopathies, and, although the data scatter considerably, an inverse correlation between the percentages of $^{T}\gamma$- and $^{G}\gamma$-chains appears to exist. A comparison of these data with those of Fig. 6 suggests that all persons are heterozygous for the $^{T}\gamma$-chain.

Thus, the data demonstrate a distinct inverse relationship between the $^{T}\gamma$- and the $^{G}\gamma$-chains: as the $^{G}\gamma$-chain decreases in a sample, the $^{T}\gamma$-chain increases. We conclude, therefore, that the $^{T}\gamma$-chain is an $^{A}\gamma$-chain: that is, has an alanyl residue in position 136 and should be designated $^{TA}\gamma$-chain. This conclusion is given support by the fact that none of the samples of Hb-F that has exclusively $^{G}\gamma$-chains also has $^{T}\gamma$-chains. It is also supported by the cord-blood data because the value of 31% $^{T}\gamma$-chain in the four presumed homozygotes correlates well with the 29% $^{A}\gamma$-chain found in newborn babies (5). Furthermore, the data listed in Table VI show that in this baby, who is positive for the $^{T}\gamma$-chain and heterozygous for the $^{G}\gamma$-HPFH condition, the production of both the $^{A}\gamma$-chain and the $^{T}\gamma$-chain decreases simultaneously and to the same extent.

Recent studies of the fetal hemoglobins of five

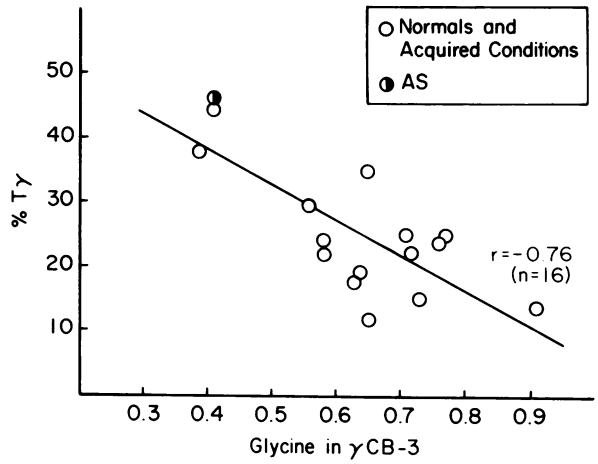

FIGURE 7 Relationship between the percentage of $^{T}\gamma$-chain and the fraction of $^{G}\gamma$-chain in the Hb-F from normal persons and from individuals with acquired hematological disorders.

orangutans have shown that each of these samples have a $^{V}\gamma$-chain, i.e., a chain with a valyl residue in position 75, in addition to the $^{I}\gamma$-chain which has an isoleucyl residue in position 75 (14). This heterogeneity is in addition to the one in position 135 which is occupied by either a threonyl residue (the $^{T}\gamma$-chain) or an alanyl residue (the $^{A}\gamma$-chain) (15). The present information suggests that Ile-75 and Thr-135 are in one chain (the $^{IT}\gamma$-chain) and Val-75 and Ala-135 in the other chain (the $^{VA}\gamma$-chain). Thus, this type of heterogeneity is similar to that of the human fetal hemoglobins, although the incidence of the human $^{TA}\gamma$-chain appears to be considerably less than that of the $^{VA}\gamma$-chain of the orangutan.

Some genetic considerations. Is the human $^{TA}\gamma$-gene that produces the $^{TA}\gamma$-chain a mutant of the "normal" $^{A}\gamma$-gene or genes, or is it a duplication? We have presented evidence (16, 17) for pairs of nonallelic $^{G}\gamma$- and $^{A}\gamma$-genes which are arranged in the order G:g:A:a and which produce γ-chains in the ratio of 4:2:2:1; for simplicity, they are abbreviated *G*, *g*, *A*, and *a* where upper case letters denote the more active gene and lower case the less active gene. Hybridization experiments (18, 19) do not agree with these ideas; however, their accuracy may not distinguish between models with three or four genes per haploid chromosome. Although some lines of evidence point to a pair of nonallelic $^{G}\gamma$-genes (17, 20, 21), the evidence for two nonallelic $^{A}\gamma$-genes is much less direct. The linear correlation between the percentages of $^{T}\gamma$- and $^{A}\gamma$-chains that is illustrated in Figs. 6 and 7, and the nearly identical $^{T}\gamma$-chain and $^{A}\gamma$-chain values in the four newborn with a $^{T}\gamma$-homozygosity, suggest that these chains are the product of a single $^{A}\gamma$-gene which is also a $^{T}\gamma$-gene; because of the relatively low general frequency of $^{T}\gamma$-genes, it seems unlikely that the $^{T}\gamma$-mutation would be present both in *A* and *a* genes. All evidence, there-

fore, implies a single $^{A}\gamma$-gene. Furthermore, the linear correlation points to the $^{TA}\gamma$-gene as a mutant of the normal $^{A}\gamma$-gene rather than as a duplication such that nonallelic $^{A}\gamma$- and $^{TA}\gamma$-genes are present on each haploid chromosome.

ACKNOWLEDGMENTS

This work was supported in part by U. S. Public Health Service grants HL-02558 and HL-05168. This is Contribution 5847 from the Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, Calif.

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