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Gamma-Chain Heterogeneity of Fetal Hemoglobin in Nonblack β- and δβ-Thalassemia and HPFH Heterozygotes and Homozygotes

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The fetal hemoblobin (Hb F) of a few hundred nonblack patients with a heterozygosity or homozygosity for β-thalassemia (β-thal), δβ-thalassemia (δβ-thal), and some forms of the hereditary persistence of Hb F (HPFH) was isolated by DEAE-cellulose chromatography and further characterized by high-pressure liquid chromatography. Quantitative data for the three types of γ chain ($^{A}\gamma^{T}$, $^{A}\gamma^{I}$, and $^{G}\gamma$) were compared with those obtained for the Hb F from black patients with similar conditions.

The $^{G}\gamma$ chain levels in nonblack β-thal heterozygotes varied greatly and did not fall into two distinct groups with high or low levels, as has been observed in blacks. The level of the $^{A}\gamma^{T}$ chain in $^{A}\gamma^{T}$ heterozygotes did not differ significantly when this anomaly was in cis or in trans to the β-thal determinant. Beta-thalassemia homozygotes from Turkey and Yugoslavia, had $^{G}\gamma$ values varying between 40% and 80%. Only 13 of 34 patients carried the $^{A}\gamma^{T}$ gene. Nine were $^{A}\gamma^{T}$ heterozygotes with an $^{A}\gamma^{T}$/total $^{A}\gamma$ level averaging 39% and four were $^{A}\gamma^{T}$ homozygotes.

The $^{G}\gamma$ chain levels in nonblack δβ-thal heterozygotes varied between 28% and 46%. An additional $^{A}\gamma^{T}$ chain heterozygosity in cis to the δβ-thal determinant demonstrated that over 90% of the γ chains is produced by genes in cis to this anomaly. Analyses of members of two relatively large families with β-thal, δβ-thal, and the $^{A}\gamma^{T}$ chain heterozygosities and homozygosities occurring in different combinations allowed a more or less quantitative evaluation of the production of γ-chain genes in cis or in trans to either of the two types of thalassemia determinants. Such calculations were possible both in simple heterozygotes and in persons with the β-δβ-thalassemia condition.

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The Indian type of $^{G}\gamma^{A}\gamma$-HPFH heterozygosity was studied in four individuals of whom two were $^{A}\gamma^{T}$ heterozygotes. This condition differs from the black types in higher levels of $^{G}\gamma$ chain and in a considerable contribution of γ-chain genes in trans to the HPFH determinant. The Swiss type of HPFH heterozygosity was studied in 30 subjects belonging to six Yugoslavian families; some were $^{A}\gamma^{T}$ heterozygotes. The level of $^{G}\gamma$ chain averaged over 80% in nine members of one family; the anomaly in this family deserves further study.

Key words: β-thalassemia; $\delta\beta$-thalassemia; HPFH; HPL chromatography; Hb F; $^{A}\gamma^{T}$, $^{A}\gamma^{I}$, and $^{G}\gamma$ chains.

INTRODUCTION

The heterogeneity of the γ chain of human Hb F has been studied for several years. The variation at position 136 was discovered in 1968 [1], and the existence of two nonallelic γ-chain genes producing γ chains with either glycine ($^{G}\gamma$) or alanine ($^{A}\gamma$) in this position has been confirmed by structural DNA analyses [2–4]. Ricco et al [5] observed that the γ-chain variant Hb F-Sardinia [6] occurred at a high incidence in Italian thalassemia patients. This finding has been confirmed by others [7,8]. Hb F-Sardinia was found to be a variant of the $^{A}\gamma$ chain [9,10] and is presently known as the $^{A}\gamma^{T}$ chain because a threonyl residue replaces an isoleucyl residue at position 75 of the $^{A}\gamma$ chain. The occurrence of the $^{A}\gamma^{T}$ chain is widespread although its incidence varies greatly among the world population [8].

Recent advances in the methodology to separate and quantitate the three types of γ chain ($^{A}\gamma^{T}$, $^{A}\gamma^{I}$, $^{G}\gamma$) in small amounts of Hb F have greatly facilitated a (re)evaluation of the γ-chain heterogeneity in normal persons, newborn babies, and in patients with a variety of inherited disorders (for a review, see [11]). High-pressure liquid chromatography (HPLC) was particularly useful in the study of a large number of newborn black babies and adults [12]; of sickle cell anemia patients [13]; and of black patients with β-thalassemia, $^{G}\gamma$-$\delta\beta$-thalassemia, and some forms of the hereditary persistence of Hb F (HPFH) [14]. This paper describes the results of comparable studies for many nonblack patients with similar conditions. This large collection of data was only possible through the combined efforts of several investigators in different countries. Some of the data have been presented, and are published in the appropriate Proceedings [15].

MATERIALS AND METHODS

Subjects

Over 480 persons participated in this study. Many were members of families with distinct abnormalities who have been studied (often for several years) by hematologists in India, Mexico, Turkey and Yugoslavia. Others were (mainly black) subjects who participated in testing programs conducted by the Sickle Cell Center. Data on several of these persons have been published before [14] and these results are included in this study only for the purpose of comparison.

One hundred and four normal persons (both black and white), aged 1 to 78 years, served as controls. The β-thalassemia heterozygotes concerned 121 blacks and 72 subjects from Mexico, Turkey, and Yugoslavia. The β-thalassemia homozygotes (many producing Hb A) were six black Americans and 34 subjects from Turkey and Yugoslavia.

Patients with two forms of δβ-thalassemia were evaluated. One group of 13 blacks had the $^{G}\gamma$-δβ-thalassemia heterozygosity [14] while 15 subjects of Mediterranean descent were $^{G}\gamma^{A}\gamma$-δβ-thalassemia heterozygotes [16] and one was a homozygote. Moreover, six patients had the β^{0}-δβ-thalassemia condition. Family studies were required to support the diagnoses derived from clinical and hematological observations.

Four different types of HPFH were studied. The $^{G}\gamma^{A}\gamma$-HPFH heterozygosity was observed in 79 blacks from Georgia, while a comparable condition was present in four Indians (previously described in [17]). Nine black patients with the likely diagnosis of $^{G}\gamma$-β^{+}-HPFH trait were also included [14]. The Swiss type of HPFH trait (detailed in [18]) was present in 30 members of six families from Yugoslavia.

Blood samples (5 to 20 ml) were collected in vacutainers with EDTA as anticoagulant and were analyzed at the local institutions. Samples from overseas were airmailed to Augusta as washed packed red cells or as isolated Hb F. Informed consent was obtained.

Methods

Hematological data were collected with routine methods in use in the different hematological laboratories. None of these data will be presented in this paper; several families were studied in great detail and have been described elsewhere [8,17,19–21]. Hb A_2 was determined by microchromatography on DEAE-cellulose [22]; and Hb F (as F_{AD}) was quantified by an alkali denaturation procedure [23]. Starch gel electrophoresis [24] was used to exclude the presence of abnormal hemoglobins.

Hb F was isolated by DEAE-cellulose chromatography and further purified as described in detail before [22,25,26]. All isolated hemoglobin samples contained at least 40% Hb F as judged by visual inspection of a starch gel. The three types of γ chain ($^{A}\gamma^{T}$, $^{A}\gamma^{I}$, and $^{G}\gamma$) in the Hb F were quantitated by HPL chromatography; details of this method have been presented on numerous occasions [12–14,27]. Quantitation of

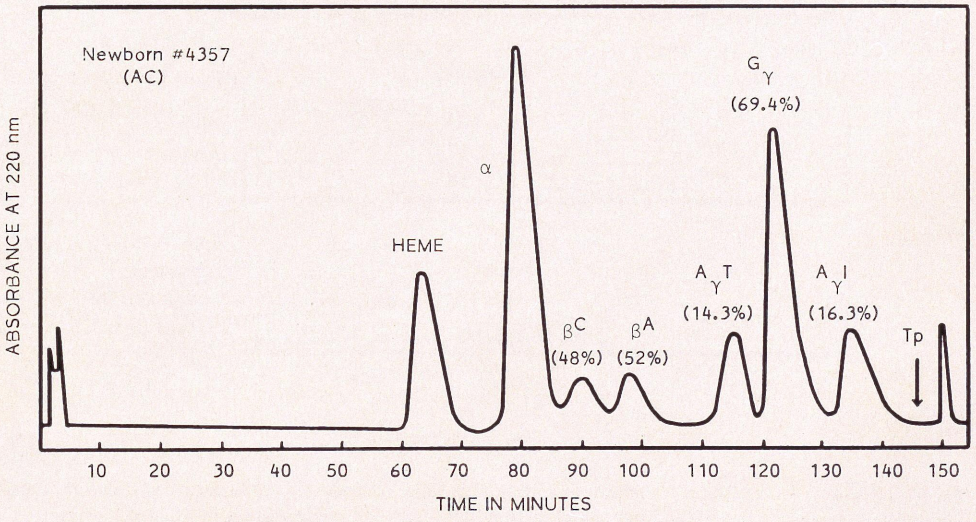

Fig. 1. The separation of heme, α chain, the two β chains, and the three γ chains from the hemoglobin of a newborn baby with Hb C trait and a $^{A}\gamma^{T}$ heterozygosity using an HPL chromatographic method.

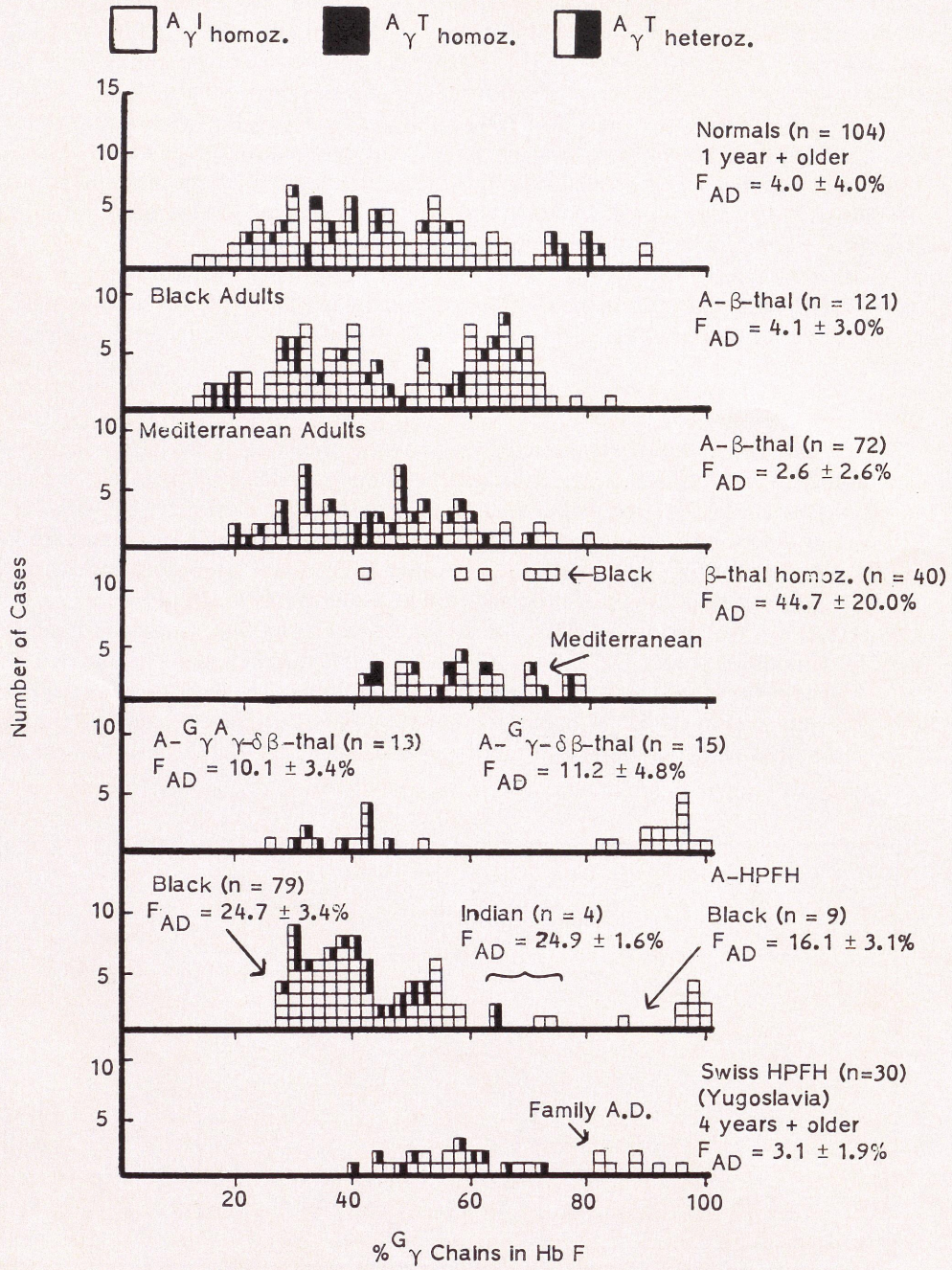

Fig. 2. The distribution of the $^G\gamma$ chain values among normal persons and patients with various inherited hemoglobin disorders. Several individuals have an additional $^A\gamma^T$ heterozygosity or homozygosity.

these chains was reproducible with a SD not exceeding 2%; values of the $^{A}\gamma^{T}$ or $^{A}\gamma^{I}$ chain below 2% were considered unreliable. Figure 1 presents a chromatogram illustrating the separation of the chains.

RESULTS

Figure 2 presents the distribution of the $^{G}\gamma$ chain percentages in the Hb F from numerous normal adults, from β-thal heterozygotes and homozygotes, from 28 $\delta\beta$-thalassemia heterozygotes, and from more than 120 individuals with one of many forms of HPFH. The presence of an $^{A}\gamma^{T}$ heterozygosity or homozygosity in these numerous samples is also indicated in Figure 2. Since results of family studies are included, the data cannot be used to calculate gene frequencies accurately.

β-Thalassemia

A surprisingly large variation in the $^{G}\gamma$ chain values was observed in the 104 normal adults including several younger children, 1–3 years old, with slightly elevated Hb F levels. Black β-thalassemia heterozygotes showed a similar variation although two distinct groups with high and low $^{G}\gamma$ chain levels (and identical Hb F percentages) were readily identified, confirming older data [14,28,29]. The results of Figure 2 show a similar wide variation in the $^{G}\gamma$ values of the β-thalassemia heterozygotes of Mediterranean origin but without recognizing distinct subgroups. The average Hb F level (% F_{AD}) in these persons was distinctly less than in the black β-thalassemia heterozygotes. Black and Mediterranean β-thalassemia homozygotes had $^{G}\gamma$ values between 40% and 80%, similar to earlier descriptions [21,29].

In a previous paper [14] we reported differences between the relative amounts of $^{A}\gamma^{T}$ chain (in percentage of total $^{A}\gamma$ chain) in the Hb Fs of black β-thalassemia heterozygotes who had this $^{A}\gamma^{T}$ heterozygosity either in cis or in trans to the β-thalassemia determinant. The same data are again presented in the left panel of Figure 3. Similar data for Mediterranean β-thalassemia heterozygotes show no such differences (right panel, Fig. 3) but the average relative percentage of $^{A}\gamma^{T}$ chain (as percentage of total $^{A}\gamma$ chain) of 34.6 $\pm$ 11.1% (SD) was about twice that of black β-thal traits with the $^{A}\gamma^{T}$ chain in trans (16.4 $\pm$ 7.3% [SD]) and about one-half that of black β-thal traits with the $^{A}\gamma^{T}$ chain in cis (65.7 $\pm$ 12.6% [SD]). This observation made it impossible to predict the relationship between the $^{A}\gamma^{T}$ chain and the β-thalassemia heterozygosity and only through careful analyses of pedigrees could an in cis or in trans assignment be made (Fig. 4 illustrates some useful pedigrees).

Figure 5 lists data on six black β^{+}-thal homozygotes and 34 β-thal homozygotes of Mediterranean origin (many patients produced small amounts of β chains; however, transfusion requirements often made a differentiation between the $\beta^{+}\beta^{+}$, $\beta^{+}\beta^{0}$, and $\beta^{0}\beta^{0}$ subclasses impossible). All blacks were negative for the $^{A}\gamma^{T}$ chain. Of the 34 white homozygotes, 21 (including one brother and sister) were $^{A}\gamma^{T}$ negative; nine (including two brothers) were $^{A}\gamma^{T}$ heterozygotes; and four (including one brother and sister) were $^{A}\gamma^{T}$ homozygotes. Thus, the incidence of the $^{A}\gamma^{T}$ chain (11 of 31 cases) was considerably lower in our patient population than that reported by Ricco et al [5] who found that 39 of 42 of their homozygous patients were carriers of the $^{A}\gamma^{T}$ chain. The average $^{A}\gamma^{T}$ value (as percentage of the total $^{A}\gamma$ chain) in the nine $^{A}\gamma^{T}$ heterozygotes was 39%, which is not too different from the average value of 45% ($\pm$ 4.8% [SD]) found for normal newborn babies with a similar $^{A}\gamma^{T}$ heterozygosity (see also [12]).

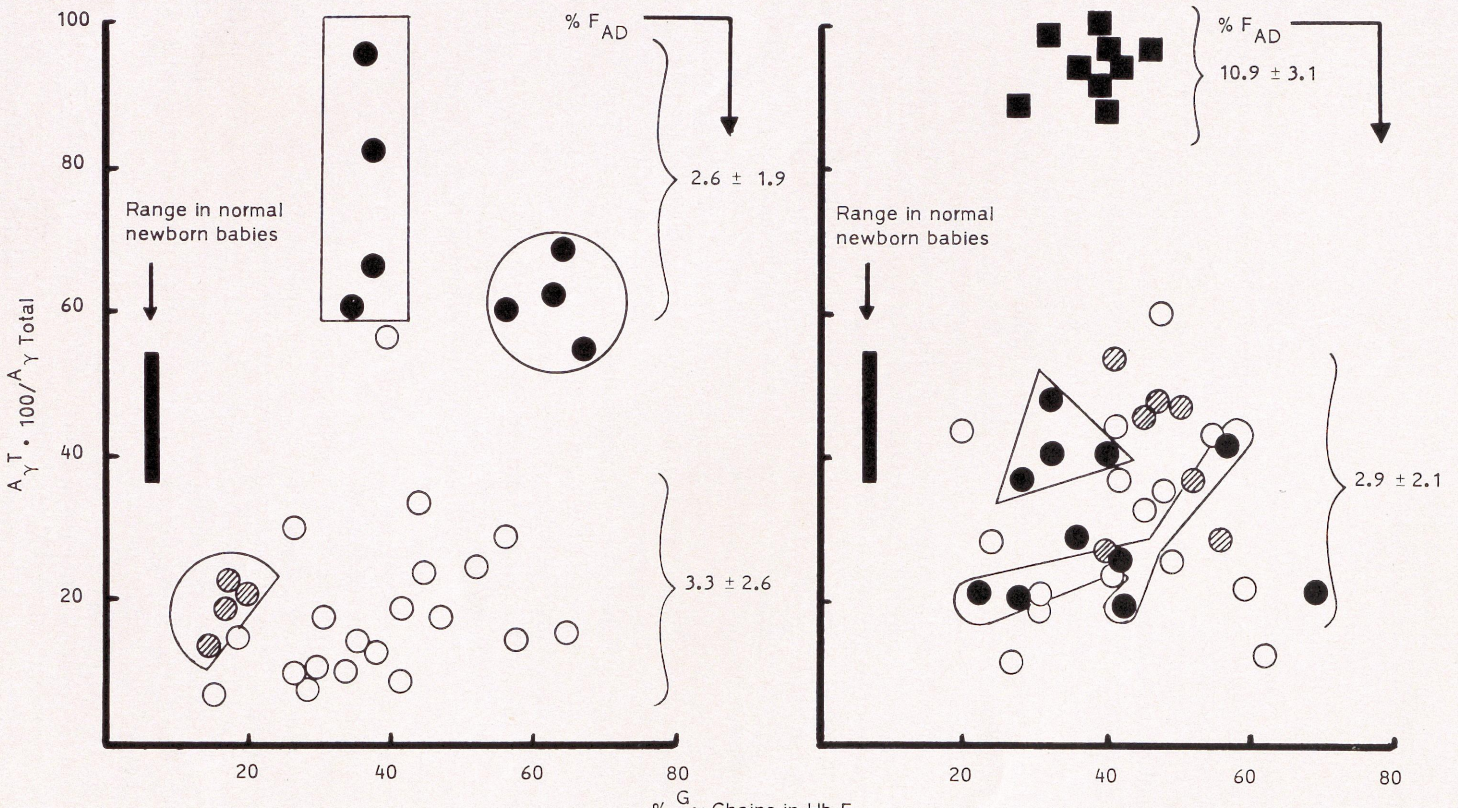

Fig. 3. The percent $^{A}\gamma^{T}$ chain (as percentage of total $^{A}\gamma$ chain) plotted against the percent $^{G}\gamma$ chain in black β-thalassemia heterozygotes (left panel) and in β-thalassemia and $\delta\beta$-thalassemia heterozygotes of Mediterranean origin (right panel). ● $^{A}\gamma^{T}$ in cis; ▨ $^{A}\gamma^{T}$ in trans; ○ relation of $^{A}\gamma^{T}$ to β-thal is undetermined. Boxed-in values are from families. ■ nine $\delta\beta$ traits with an $^{A}\gamma^{T}$ heterozygosity in cis.

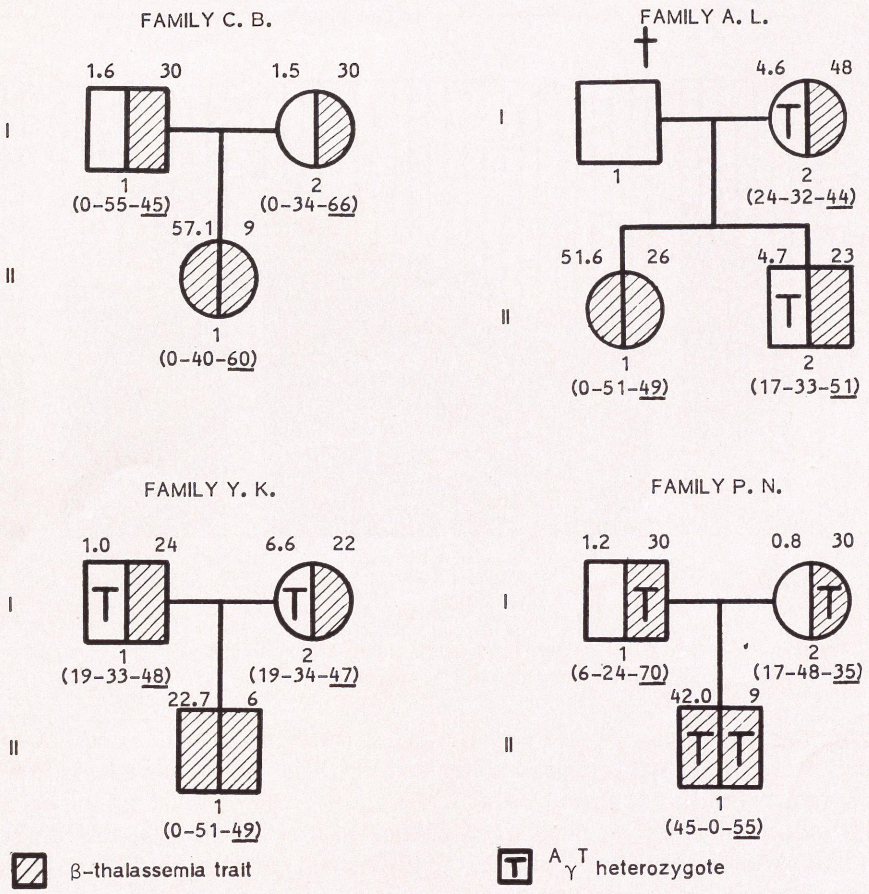

Fig. 4. Pedigrees of four families with β-thalassemia from Yugoslavia and Turkey, useful in evaluating the in cis or in trans location of the $^{A}\gamma^{T}$-chain anomaly. The percent F_{AD} is listed at the upper left hand corner and the age at the upper right hand corner; (30-30-40) indicates percentages of the $^{A}\gamma^{T}$, $^{A}\gamma^{I}$, and $^{G}\gamma$ chains.

δβ-Thalassemia

The appropriate panel of Figure 2 again demonstrates the striking differences between the $^{G}\gamma$-δβ-thalassemia observed in blacks and the $^{G}\gamma^{A}\gamma$-δβ-thalassemia most commonly detected in the Mediterranean population. Nine of the 13 $^{G}\gamma^{A}\gamma$-δβ-thal heterozygotes also were heterozygous for the $^{A}\gamma^{T}$ chain. The average percentage of the $^{A}\gamma^{T}$ chain (94.4 ± 2.9% [SD]) (Fig. 3) suggests that over 90% of the γ chains produced in this condition originate from the γ chain genes in cis to the δβ-thal determinant (confirming data reported previously [14,16]). Data from studies of two relatively large families are most illustrative (Fig. 6). Four members of the Mexican family O (see [20]) were β-thal heterozygotes with the $^{A}\gamma^{T}$ chain in cis to the β-thal determinant because the normal mother (case I-1) of two heterozygotes was $^{A}\gamma^{T}$ negative. All three children of one of two $^{A}\gamma^{T}$ positive β-thal heterozygous brothers and an $^{A}\gamma^{T}$ positive δβ-thalasse-

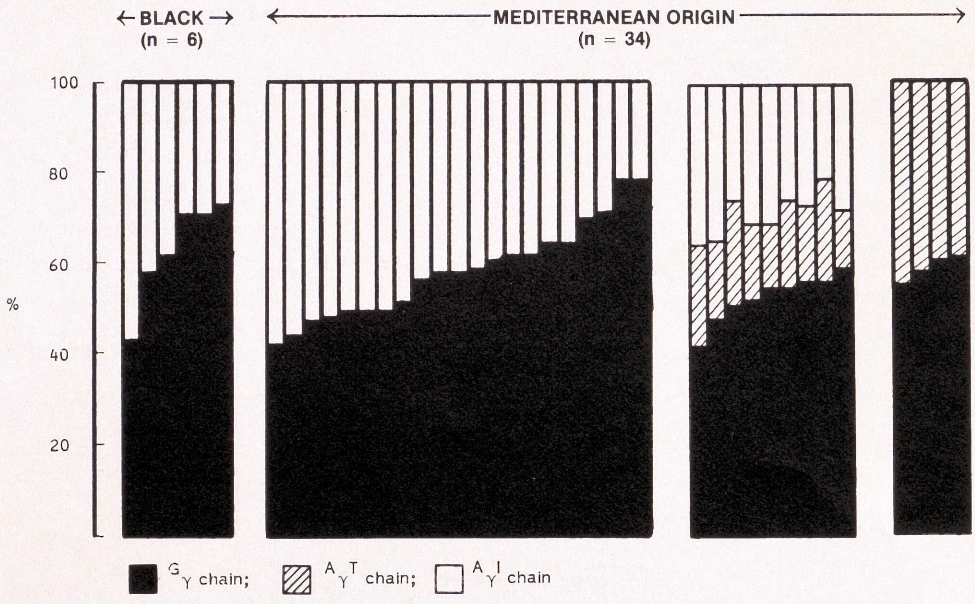

Fig. 5. The relative amounts of the $^{G}\gamma$, $^{A}\gamma^{T}$ and $^{A}\gamma^{I}$ chains in the Hb F of 40 β-thalassemia homozygotes. The $^{A}\gamma^{T} \cdot 100$/total $^{A}\gamma$ ratio in the nine $^{A}\gamma^{T}$ heterozygotes averaged 39%.

mia heterozygote were $^{A}\gamma^{T}$ positive. The oldest son had the same condition as his mother, whereas the two younger children had the β^{0}-$\delta\beta$-thalassemia condition with a homozygosity for the $^{A}\gamma^{T}$ chain. A similar $^{A}\gamma^{T}$ positive $^{G}\gamma^{A}\gamma$-$\delta\beta$-thalassemia heterozygosity was present in several members of the family D from Yugoslavia (this family was discussed in detail before [8]); however, the 10-year-old girl with the β-$\delta\beta$-combination had an $^{A}\gamma^{T}$ chain heterozygosity.

The relative percentages of the three types of γ chain in six patients with β-$\delta\beta$-thalassemia (three from the above two families, one additional patient from Turkey, and two patients from Yugoslavia) and in one $\delta\beta$-thal homozygote are shown in Figure 7. Although low $^{G}\gamma$ chain percentages are observed in $\delta\beta$-thal heterozygotes (Fig. 2) and in the $\delta\beta$-thal homozygote (see also [16]), increased levels of $^{G}\gamma$ chains (average: 61%) were observed in all six β-$\delta\beta$-thalassemia patients. This is rather surprising because the high relative percentage of $^{A}\gamma^{T}$ chains in the two β-$\delta\beta$-thalassemia patients with an $^{A}\gamma^{T}$ heterozygosity (76% of the total $^{A}\gamma$ chains) would suggest that the γ-chain genes in cis to the $\delta\beta$-thalassemia determinant contributed most to the level of Hb F in these children.

Only one $^{G}\gamma^{A}\gamma$-$\delta\beta$-thal heterozygote with an $^{A}\gamma^{T}$ heterozygosity in trans of the $\delta\beta$-thal determinant has been discovered; this 43-year-old female was a member of a Yugoslavian family in which both β-thal and $\delta\beta$-thal were present (Fig. 8). The $^{A}\gamma^{T}$ level was low (17% of the total $^{A}\gamma$ level), again indicating the large contribution of the two γ-chain genes in cis to the $\delta\beta$-thal determinant.

The HPFH Conditions

The appropriate data presented in Figure 2 greatly expand results summarized in a previous study [14]. Blood samples from 79 black $^{G}\gamma^{A}\gamma$-HPFH heterozygotes were

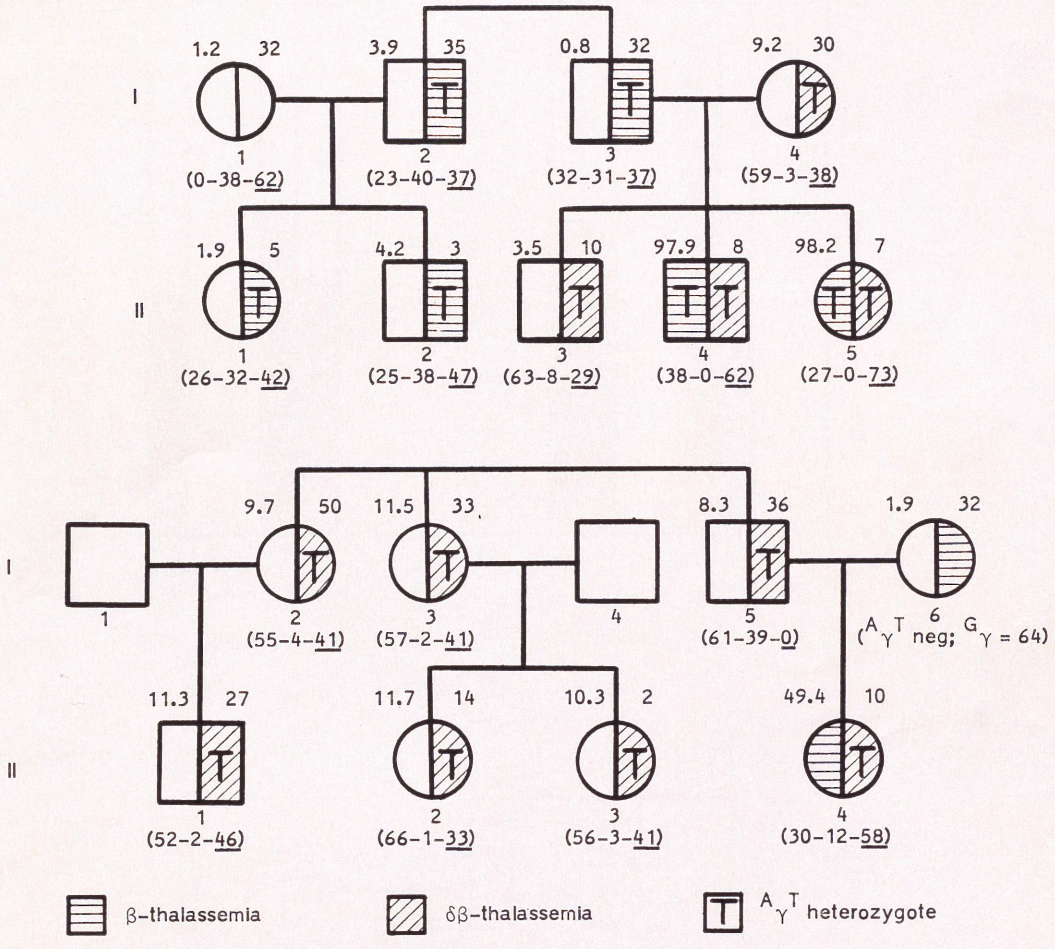

Fig. 6. Two families with δβ-thalassemia, β-thalassemia, and the $^{A}\gamma^{T}$ chain anomaly. Top: Family O from Mexico (published previously [20]). Bottom: Family D from Yugoslavia, described in an earlier publication [8]. The families are discussed in detail in the text. The percent F_{AD} is listed at the upper left hand corner and the age at the upper right hand corner; (30-30-40) indicates percentages of the $^{A}\gamma^{T}$, $^{A}\gamma^{I}$, and $^{G}\gamma$ chains.

available; several belonged to large families [19]. It is obvious that two subgroups have been recognized, confirming earlier data [30,31]. One group has an average $^{G}\gamma$ value of 36%, whereas the second has a $^{G}\gamma$ value averaging 52%. Low and high values are probably genetically controlled, ie, high values are confined to specific families. The $^{A}\gamma^{T}$ chain has been found in both subgroups, which is not surprising considering that this $^{A}\gamma^{T}$ variant is in trans to the HPFH determinant.

Four HPFH heterozygotes from India, studied previously [17], were available for further analyses; two of these were $^{A}\gamma^{T}$ heterozygotes. Family studies indicated that the $^{A}\gamma^{T}$ chain variant was in trans to the HPFH determinant (Fig. 9). Surprisingly, the $^{G}\gamma$-chain value was considerably higher in these heterozygotes than in the blacks with a

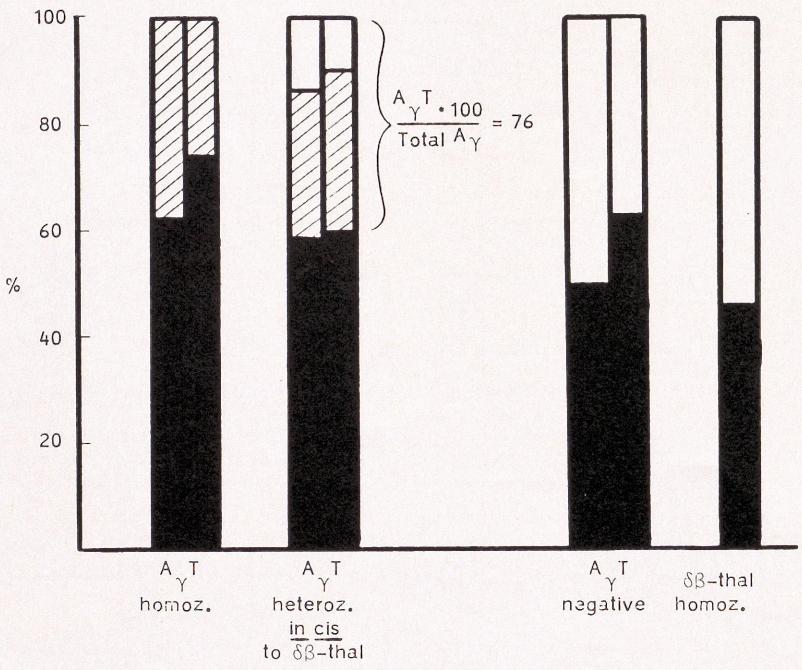

Fig. 7. The relative quantities of the $^{G}\gamma$ (■), $^{A}\gamma^{T}$ (▨), and $^{A}\gamma^{I}$ (□) chains in six patients with β^{0}-$\delta\beta$-thalassemia and one patient with a $\delta\beta$-thalassemia homozygosity.

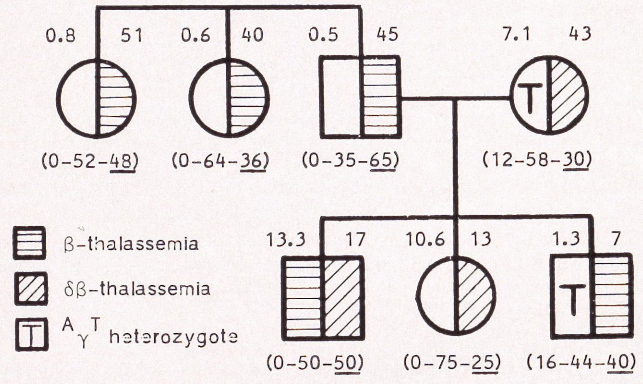

Fig. 8. Family M from Yugoslavia with a $^{G}\gamma^{A}\gamma$-$\delta\beta$-thalassemia heterozygote having $^{A}\gamma^{T}$ heterozygosity in trans. The percentage F_{AD} is listed at the upper left hand corner and the age at the upper right hand corner: (30-30-40) indicates percentages of the $^{A}\gamma^{T}$, $^{A}\gamma^{I}$, and $^{G}\gamma$ chains.

comparable anomaly (see also [14]) but the $^{G}\gamma$ value was distinctly lower than that in the nine black $^{G}\gamma$-$\delta^{+}\beta^{+}$-HPFH heterozygotes, which averaged 97% (Fig. 2).

The ill-defined Swiss type of HPFH heterozygosity was observed in 30 members of six families from Yugoslavia. In several, the $^{G}\gamma$ value was varying between 40% and 70%, but numerous members of one family had values in excess of 80% (Fig. 10). Pedigree analyses of this family showed an average Hb F_{AD} value of 5.1% in nine

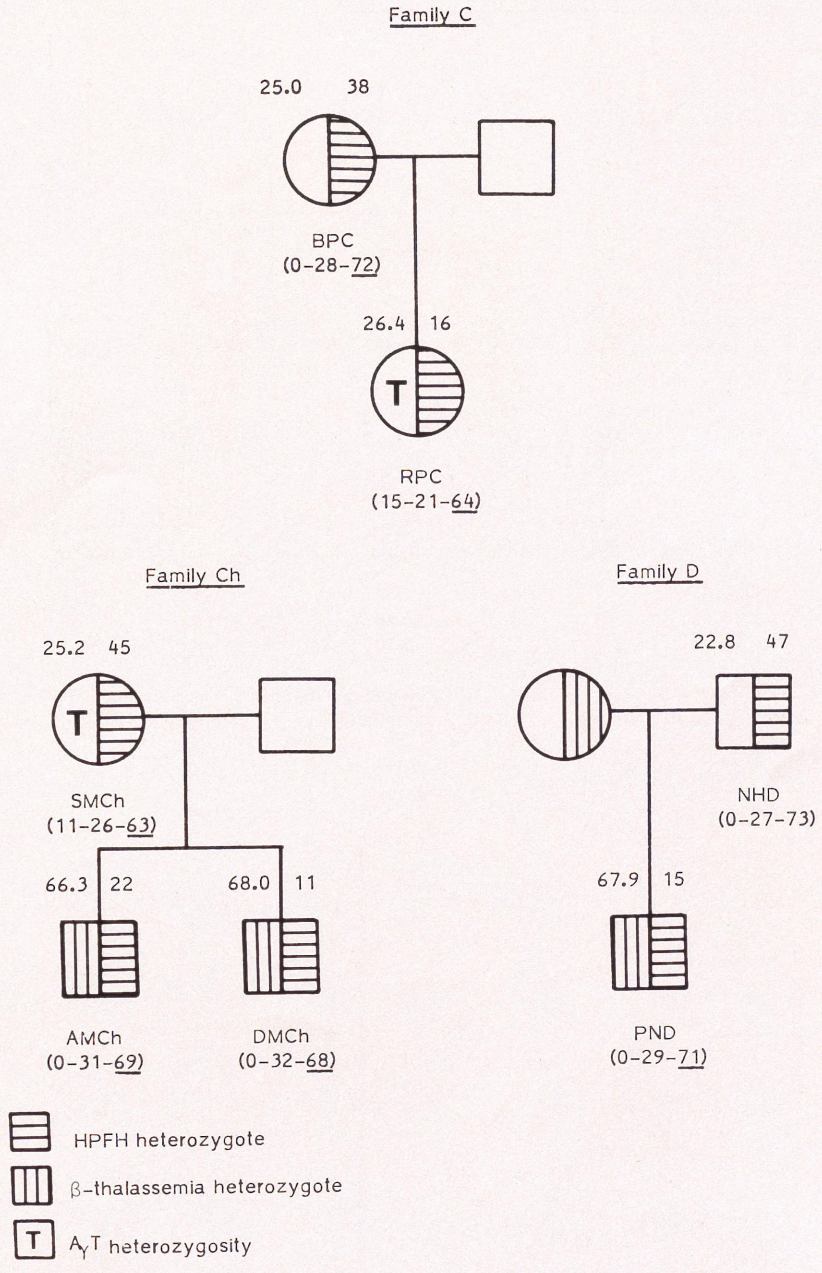

Fig. 9. Three families with $^{G}\gamma\,^{A}\gamma$-HPFH and β-thalassemia from India (see text and [17] for details). The percentage F_{AD} is listed at the upper left hand corner and the age at the upper right hand corner; the values (30-30-40) indicate the percentages of the $^{A}\gamma^{T}$, $^{A}\gamma^{I}$, and $^{G}\gamma$ chains.

heterozygotes with an average $^{G}\gamma$ value of 82%. These data suggest the presence of a $^{G}\gamma$ type of HPFH in this white family (a similar type of HPFH has been observed in black families [29,31,32]) with only a limited contribution to the total γ-chain production by the γ-chain genes in trans to the HPFH determinant.

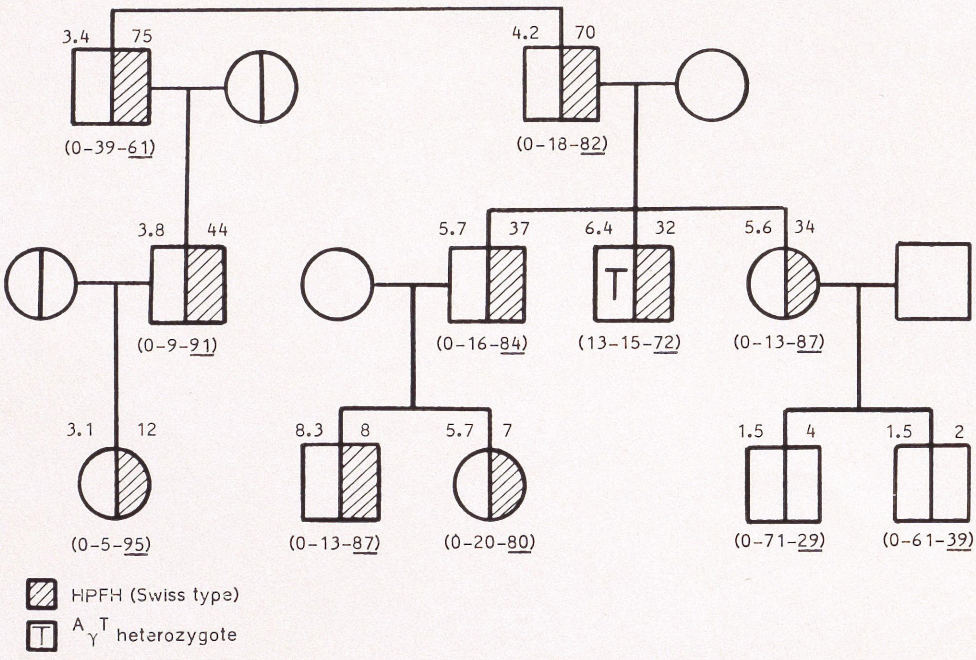

Fig. 10. Family A.D. with the Swiss type of HPFH living in Yugoslavia. The percentage F_{AD} is listed at the upper left hand corner and the age at the upper right hand corner; (30-30-40) indicates percentages of the $^{A}\gamma^{T}$, $^{A}\gamma^{I}$, and $^{G}\gamma$ chains.

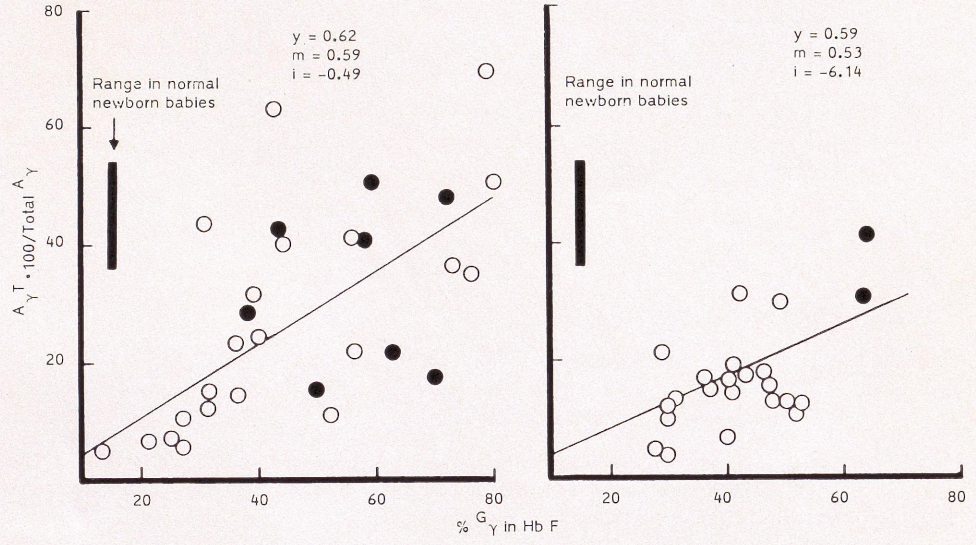

Fig. 11. Left panel: The percent $^{A}\gamma^{T}$ chain (as percent of total $^{A}\gamma$ chain) plotted against the percent $^{G}\gamma$ chain in normal persons, 1 year and older (○, Hb F_{AD} = 3.6 ± 3.5%), and in eight subjects with a Swiss type of HPFH heterozygosity (●, Hb F_{AD} = 3.1 ± 1.4%). Right panel: The percent $^{A}\gamma^{T}$ chain (as percent of total $^{A}\gamma$ chain) plotted against the percent $^{G}\gamma$ chain in $^{G}\gamma^{A}\gamma$-HPFH heterozygotes. ○:blacks; ●: Indians.

Numerous normal adults, Swiss type of HPFH heterozygotes, and black and Indian HPFH heterozygotes were also $^{A}\gamma^{T}$ heterozygotes; the $^{A}\gamma^{T}$ mutation was located in trans to the appropriate anomaly when such a condition was observed. Figure 11 presents plots of the total $^{A}\gamma^{T}$ percentages (as percentages of total $^{A}\gamma$ chain production) versus the percent $^{G}\gamma$ chain. The data showed a wide scattering; however, a significant relationship was evident indicating an increase in the $^{A}\gamma^{T}/^{A}\gamma$ total ratio with increasing $^{G}\gamma$-chain values. Thus, increased $^{G}\gamma$-chain values in HPFH heterozygotes (black, Indian, and Swiss types) are primarily due to an increased production of γ chains in trans of the HPFH determinant. Also, in normal adults the $^{A}\gamma^{T}$ heterozygosity allows the identification of the γ-chain genes on the chromosome that are the major contributors to the production of Hb F.

DISCUSSION

The newly developed (micro)chromatographic procedures allow the rapid collection of a large body of data concerning the heterogeneity of Hb F in various hematological disorders. Many observations reported here confirm results that have been published before [8, 12–14], but some of the data require additional comments.

Normal Adults

The results were obtained for normal persons, 1 year and older, many having slight elevations in their Hb F level. The wide variation in the $^{G}\gamma$ chain values (14%–90%) is puzzling and cannot be explained by technical complications alone. Thus, F cells with either high or low levels of $^{G}\gamma$ chains or with varying $^{G}\gamma$-to-$^{A}\gamma$ ratios are produced and often a mixture of "newborn type" and "adult type" of F cells appears to be present. There is some indication for a direct relationship between the level of $^{A}\gamma^{T}$ chain (as percentage of total $^{A}\gamma$ chain) and the percent $^{G}\gamma$ chain (Fig. 11), but the significance of this observation cannot be assessed without additional (mainly longitudinal) studies of selected cases. Perhaps continued analyses of the so-called Swiss type of HPFH condition might be helpful; the $^{G}\gamma$ chain level in affected members of such families appears about constant, ie, it may be high, as in family A.D. of Figure 10, or intermediate, as in the five additional families.

β-Thalassemia

Analyses of the Hb F from additional black β-thalassemia heterozygotes confirm earlier data [14,28] that two main subclasses are to be recognized, being characterized by low or high $^{G}\gamma$-chain levels (Fig. 2). Considerable variation is observed in the $^{G}\gamma$-chain percentages of the six black homozygotes; this variation is not dependent upon the amount of Hb F that is present. When an $^{A}\gamma^{T}$ heterozygosity is also present, its in cis or in trans location is helpful in evaluating the contribution of the γ-chain genes on the two chromosomes; the γ-chain production by genes in cis to the β-thal determinant is about three times that by γ-chain genes located on the opposite chromosome (Fig. 3).

The data for the Mediterranean β-thalassemia heterozygotes are somewhat different. No distinct subclasses were observed for the $^{G}\gamma$ values of the Hb F of 72 individuals with this condition, but a variation in $^{G}\gamma$ chain values similar to that in normal adults was present (Fig. 2). Surprisingly, no difference was seen in the contribution by the γ-chain genes either in cis or in trans to the β-thalassemia determinant (Fig. 3) suggesting that the elevated level of Hb F in this condition (which is somewhat lower than

that in black β-thal heterozygotes) is caused by an equal increase in activity of γ-chain genes on both chromosomes. The $^{G}\gamma$-chain values in the Hb F of the 34 Mediterranean β-thal homozygotes did not differ from those of blacks with a similar condition. The incidence of the $^{A}\gamma^{T}$ chain in Turkish and Yugoslavian β-thalassemia homozygotes was relatively low (11 out of 31 patients). The average value of 39% $^{A}\gamma^{T}$ chain (as percentage of total $^{A}\gamma$ chain) in the nine β-thal homozygotes who also had an $^{A}\gamma^{T}$ heterozygosity was comparable to that observed in newborn $^{A}\gamma^{T}$ heterozygotes, which suggests an equal contribution of the γ-chain genes located on both chromosomes. It will be of interest to extend these data and include particularly β-thal homozygotes both to $^{A}\gamma^{T}$ positive β-thal heterozygotes with distinctly different $^{G}\gamma$-chain values.

δβ-Thalassemia

The increased γ-chain production in heterozygotes of both types of δβ-thalassemia is caused by the increased output by γ-chain genes in cis to these determinants. This is clearly demonstrated by the data from $^{G}\gamma^{A}\gamma$-δβ-thalassemia heterozygotes with an $^{A}\gamma^{T}$ heterozygosity in cis; over 90% of the $^{A}\gamma$ chain was of the $^{A}\gamma^{T}$ type (Fig. 3). Most helpful were the data of the many members of the two families for which pedigrees are presented in Figure 6. Even in patients with a combination of β-thalassemia and $^{G}\gamma^{A}\gamma$-δβ-thalassemia, 75% of the γ-chain production probably originates by γ-chain genes in cis to the δβ-thal determinant (Fig. 7).

All white δβ-thalassemia heterozygotes and homozygotes studied thus far (Figs. 2, 3, and 7; see also [14,16,29]) have $^{G}\gamma$ values below 50%. However, considerably higher $^{G}\gamma$-chain values are observed when a β-thalassemia heterozygosity is also present. Typical examples are the two children of family O (Fig. 6) with this combination, who had $^{G}\gamma$ chain values above 60%. Apparently, a preferential activation of the $^{G}\gamma$ chain gene in cis to the β-thal determinant occurred, resulting in this value, which is significantly higher than that observed in a $^{G}\gamma^{A}\gamma$-δβ-thal homozygote (Fig. 7).

The HPFH Condition

Various types of HPFH have been discovered, both in black and white populations. None of the data given in Figure 2 are greatly different from those presented and reviewed in earlier publications [14,17,29–32]. The observed distinction between the two types of black $^{G}\gamma^{A}\gamma$-HPFH heterozygotes is more evident than seen in earlier studies. The pedigrees of the three Indian families with the HPFH condition are useful because the relatively high contribution of the γ-chain genes in trans to the HPFH determinant is clearly demonstrated (Figs. 9,11). This observation is rather surprising and supports the suggestion that this type of HPFH is distinctly different from that found among blacks [17,20].

The data on the Swiss type of HPFH are the first to show a considerable heterogeneity in this type of abnormality. Extension of these data through analyses of critical families is needed.

The structural analyses of the Hb F of patients with various conditions as listed in Figure 2 are of considerable help in further defining specific abnormalities. Moreover, the $^{A}\gamma^{T}$ heterozygosity can serve as an excellent marker for an evaluation of the contribution of γ-chain synthesis by genes in cis or in trans to specific determinants. It is hoped that continued analyses of this type together with detailed DNA analyses of selected patients may result in further definition of the abnormalities involved.

The data in Figure 2 are not extremely suitable for a calculation of the $^{A}\gamma^{T}$ frequencies among the various groups mainly because data on members of families are included. There seems to be little doubt, however, that the $^{A}\gamma^{T}$ frequency in Mediterranean thalassemia patients is about twice that observed in blacks. The $^{A}\gamma^{T}$ frequencies (not adjusted for family data) are 0.12 (normal adults), 0.10 (black A-β-thal), 0.20 (Mediterranean A-β-thal), and 0.22 (Mediterranean β-thal homozygotes); these values are lower than those calculated from data described by Ricco et al [5] and Saglio et al [10].

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