Further Studies of the Frequency and Significance of the
$\gamma$-Chain of Human Fetal Hemoglobin

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

The $\gamma$-chain is readily detectable in $\beta$-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 $\gamma$-gene. The $\gamma$-chain has not been
detected in the human fetal hemoglobin that is synthe-
sized in increased amounts in persons with the
hereditary persistence of fetal hemoglobin.

Although the $\gamma$-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 $\gamma$-chain to the sources of human fetal globulin from
determinants in cis and in trans, the conclusion has
been reached that the $\gamma$-chain is the product of a
mutant $\gamma$-locus which should be named the $\alpha\gamma$-chain.

INTRODUCTION

The $\gamma$-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 $\gamma$-chains; namely, one with glycine in position
136 (the $\gamma$-chain) and a second with alanine in this

$^1$ 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.

position (the $\gamma$-chain). The recent discovery of the
$\gamma$-chain by Ricco et al. (3) has added yet another facet
to the many ways in which the study of Hb-F has pro-
vided data about the genetics of this fetal protein. In the
$\gamma$-chain, the isoleucyl residue at position 75 of the
$\gamma$-chain is replaced by a threonyl residue. Although
Ricco et al. (3) particularly examined the frequency of
the $\gamma$-chain in $\beta$-thalassemia, they also detected its
presence in cord bloods. Our studies of the $\gamma$-chain
(4) substantiated their data in $\beta$-thalassemia of in-
dividuals of Mediterranean origin, but detected no
$\gamma$-chains in the $\beta$-thalassemia of Blacks. Moreover,
our data from cord blood suggested that the frequency of
the $\gamma$-chain might be related to ethnic source or
geographical locality, and quantitatively was present in
greater percentage than reported by Ricco et al. Be-
cause of the limited data, conclusions could only be
tentative, and statistical evaluation had limited mean-
ing. 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 in-
vestigations of the $\alpha$- to $\gamma$-ratio in Hb-F (1, 2). In most of
these, not only $\gamma$ and $\alpha$-chains, but considerable percentages
of $\beta$-chains were also present. For most samples, the $\alpha$- to
$\gamma$-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 $\gamma$-$\gamma$ 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-
ggested 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 $\gamma T$-9 peptides is pure as isolated, and the interpretation of the amino-acid analyses is subjective to a degree.

RESULTS

The $\gamma$-chain in cord bloods. Table II summarizes the results from the examination for $\gamma$-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 $\gamma$-gene in this group was 0.14. There are 149 negative results in the present 194 so that the $\gamma$-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 $\gamma$-chain is presented in Fig. 1. Two groups were observed: One group of four averaged 31% $\gamma$-chain, and the remaining 39 averaged 18% (17% for 23 Black babies and 19% for 16 Caucasian newborn).

The 31% $\gamma$-chain corresponds rather well with the average value of 29% $\gamma$-chain in the newborn babies (5). It had previously been suggested that one sample with 35% $\gamma$-chain (this is N.W. of Table II [4]) which now averages 33% in three determinations) represented a $\gamma$-homozygote. The detection of three others in this sample range strengthens this conclusion. One
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% $\gamma$-chain. Both parents had Hb-F levels of $<2\%$ and were positive for the $\gamma$-chain.

The $\gamma$-chain in sickle cell anemia. The Hb-F of 73 cases of sickle cell anemia (11 Black newborns, 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 $\gamma$-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 $\gamma$-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 $\gamma$-chain (M.P., to be discussed later) and all others (SS, sickle cell trait [AS], and SC) heterozygous for the $\gamma$-chain, the frequency of the $\gamma$-gene in sickle-cell disease approximates 0.06, which suggests that the occurrence of the $\gamma$-gene in combination with the $\beta^+$-gene is less than in combination with the $\beta^A$-gene.

The $\gamma$-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 $\alpha$-HPFH phenotype, all were negative for the $\gamma$-chain (Table V). These results do not exclude the presence of a $\gamma$-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

### Table III

**The $\gamma$ Chain in Persons* with Abnormal Hemoglobins**

<table>
<thead>
<tr>
<th>Population</th>
<th>Condition</th>
<th>Total No.</th>
<th>No. of positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>SS</td>
<td>51</td>
<td>5</td>
</tr>
<tr>
<td>Black</td>
<td>AS</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Black</td>
<td>AC</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Black</td>
<td>CC</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Black</td>
<td>SC</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>India</td>
<td>AS</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>India</td>
<td>SS</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Israel</td>
<td>SS</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* 11 months and older.

† The $\gamma$-chain values of the five positive SS patients are listed in Table IV; percent $\gamma$ in one AS person was 41%, and 17% in one SC individual.

### Table IV

**The $\gamma$-Chain in SS**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>$\gamma$</th>
<th>Gly in $\gamma$CB-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.C.</td>
<td>Adult 29*, 23*</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>M.P.</td>
<td>Adult 27*, 36*</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>L.P.</td>
<td>Adult 28</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>T.A. t</td>
<td>28 mo</td>
<td>22</td>
<td>0.50</td>
</tr>
<tr>
<td>31 mo</td>
<td>18</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>34 mo</td>
<td>25</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>F.N.</td>
<td>Cord 19</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>6 wk</td>
<td>16</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>14 mo</td>
<td>29</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>19 mo</td>
<td>22</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>C.N.</td>
<td>Cord 19</td>
<td>0.75</td>
<td></td>
</tr>
</tbody>
</table>

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

The number of samples. The data from a newborn infant with the $\gamma$-HPFH heterozygosity (N. O. C. [6]) suggest that the $\gamma$-chain can indeed be present in trans to the HPFH determinant (Table VI). At birth, 17% $\gamma$-chain and $\geq14\%$ $\beta^A$-chain were detected. Both values decreased with time, which suggests that cessation of $\gamma$-chain synthesis by a locus in trans to the $\alpha$-HPFH determinant parallels the decrease of the $\gamma$-chain production.

### Table V

**The $\gamma$-Chain in Adults with HPFH**

<table>
<thead>
<tr>
<th>Population</th>
<th>Condition</th>
<th>Total No.</th>
<th>No. of positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>$\alpha$-heterozygote*</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Black</td>
<td>$\alpha$-homozygote</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>India</td>
<td>$\alpha$-heterozygote</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>India</td>
<td>$\alpha$-heterozygote†</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>India</td>
<td>$\alpha$-homozygote</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Black</td>
<td>$\alpha$-heterozygote§</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Caucasian</td>
<td>$\alpha$-heterozygote§</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Black</td>
<td>$\alpha$-heterozygote with Hb-Kenya</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

* 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 6γ, 7γ, and 7γ-Chains in a 6γ-HPFH Heterozygote

<table>
<thead>
<tr>
<th>Age of individual</th>
<th>Residues in position 136</th>
<th>7γ-chains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gly</td>
<td>Ala*</td>
</tr>
<tr>
<td>Birth</td>
<td>0.86</td>
<td>0.14</td>
</tr>
<tr>
<td>56 d</td>
<td>0.90</td>
<td>0.13</td>
</tr>
<tr>
<td>112 d</td>
<td>0.95</td>
<td>0.08</td>
</tr>
</tbody>
</table>

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

The 7γ-chain in β-thalassemia and δβ-thalassemia. The data of Table VII show that Hb-F has been examined for the presence of the 7γ-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 7γ-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 7γ-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 7γ-chain.

Fig. 2 shows the wide distribution from 19 to 70% 7γ-chain in the Hb-F of 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 7γ-chain in this condition because 7 belong to the same family. Moreover, none of the seven homozygotes had the 7γ-chain. The percent 7γ-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-A2 and Hb-A2; in the mother, her sister, and two siblings of the propositus, Hb-A2 is in cis to the β-thalassemia determinant, whereas the father and one sibling have Hb-A2 in cis to the β-thalassemia determinant. The data show that 7γ-chain is not produced in cis to β-thalassemia and Hb-A2 on the maternal side. On the other hand, the propositus, his father (II-15), and a sibling with Hb-A2 (III-6) do produce 7γ-chains. The percentage of 7γ-chains in the Hb-F of the propositus and

<table>
<thead>
<tr>
<th>Population</th>
<th>Condition</th>
<th>Total No.</th>
<th>No. of positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blacks</td>
<td>β-Thal. Heterozygotes*</td>
<td>29</td>
<td>51</td>
</tr>
<tr>
<td>Blacks</td>
<td>β-Thal. Homozygotes</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>Non-Blacks</td>
<td>β-Thal. Heterozygotes</td>
<td>35</td>
<td>9</td>
</tr>
<tr>
<td>Non-Blacks</td>
<td>β-Thal. Homozygotes†</td>
<td>65</td>
<td>23</td>
</tr>
<tr>
<td>Non-Blacks</td>
<td>δβ-Thal. Heterozygotes§</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>Non-Blacks</td>
<td>δβ-Thal. Homozygotes</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Blacks</td>
<td>δβ-Thal. Heterozygotes (7γ-type)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Yugoslavia</td>
<td>Lepore Heterozygotes</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Yugoslavia</td>
<td>Lepore Homozygotes</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Yugoslavia</td>
<td>Lepore β-Thal.</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

* 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.

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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 propositor who must be heterozygous for the \( ^{\tau} \gamma \)-determinant had about one-half the percentage of \( ^{\tau} \gamma \)-chain found in the Hb-F of his father. Assuming that his Hb-F comes about equally from both maternal and paternal \( \beta \)-thalassemia determinants, Hb-F without \( ^{\tau} \gamma \)-chain from the maternal determinant has diluted the Hb-F with the \( ^{\tau} \gamma \)-chain from the paternal determinant.

In the Chinese Family W., the daughter II-1, who is heterozygous for \( \beta \)-thalassemia, inherited the \( ^{\tau} \gamma \)-determinant from her mother and not from her father (Fig. 4). The sibling II-2, who is homozygous for \( \beta \)-thalassemia, is also heterozygous for the \( ^{\tau} \gamma \)-determinant. The 70% \( ^{\tau} \gamma \)-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 \( ^{\tau} \gamma \)-chains in this \( \beta \)-thalassemia homogygote.

The expression of the \( ^{\tau} \gamma \)-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), \( \beta ^{\tau} \)-thalassemia must be present. The percentages of \( ^{\tau} \gamma \)-chain in the father, the mother, and one older sister, who also had a thalassemia homogygosity, are rather similar; thus, one might conclude that there is no dilution effect in these homogygotes who are also homozygous for the \( ^{\tau} \gamma \)-chain.

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

On the paternal side, the percentage of the \( ^{\tau} \gamma \)-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 propositor. The father of the propositor and the other affected relatives have the \( ^{\tau} \gamma \)-gene in cis to the \( \delta \beta \)-thalassemia determinant and are heterozygous for both. The 35% \( ^{\tau} \gamma \)-chains in the Hb-F of the propositor, in contrast to the average of 62% in the seven \( \delta \beta \)-thalassemia heterozygotes, again reflects the dilution effect from the non-\( ^{\tau} \gamma \)-containing Hb-F of the \( \beta \)-thalassemia determinant.

The \( ^{\tau} \gamma \)-chain in miscellaneous conditions. Table VIII lists a variety of miscellaneous conditions whose Hb-F has been examined for the presence of \( ^{\tau} \gamma \)-chains. The frequency of the \( ^{\tau} \gamma \)-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 \( ^{\tau} \gamma \)-Chain in Persons with Some Acquired Hematological Conditions and in Normal Individuals

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total No.</th>
<th>No. of positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>JCML*</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Erythroleukemia</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Leukemia†</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Fanconi’s Anemia</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Blackfan-Diamond Anemia</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Aplastic Anemia</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>D-trisomy</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Normals</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Miscellaneous§</td>
<td>35</td>
<td>2</td>
</tr>
</tbody>
</table>

* 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.
The presence of the $\gamma$-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 $\gamma$-chain. The $\gamma$-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 $\gamma$-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 $\beta$-thalassemia. The absence of the $\gamma$-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 $\gamma$-HPFH heterozygote (Table VI) show that the $\gamma$-chain gene may be present in trans, but that with the decrease in the activity of the $\gamma$- and $\delta$-genes in trans, the relative production of the $\gamma$-chain decreases accordingly to a level at which it will not be detectable any longer. The frequency of $\gamma$-chain in Caucasian $\beta$-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 $\gamma$-chain.

The $\gamma$-chain in relation to the $\delta$- and $\gamma$-chains. Because all samples of Hb-F have either $\delta$- or $\gamma$-chains or both, that is, either glycine or alanine or both at position 136, it follows that the $\gamma$-chain must have one or both. Ricco et al. (3) concluded from indirect evidence that the $\gamma$-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 $\gamma$-chain in a thalassemia individual), Grifoni et al. (13) came to the same conclusion. However, when in previous studies the fraction of $\gamma$-chain was plotted against the fraction of $\gamma$-chains that are $\delta$-chains, a rather ill-defined inverse correlation appeared to exist (4); conversely, this would indicate a direct correlation with the fraction of $\gamma$-chains in Hb-F. Such a plot, however, is too simple and ignores completely the source of Hb-F and the possibility that the $\gamma$-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 $\gamma$-chains with that of the $\gamma$-chain. (A comparison of the percent $\gamma$-chain with the percent $\gamma$-chain is avoided because the accuracy of the percent $\gamma$-chain might be somewhat less than that of the $\gamma$-chain as a result of the presence of two additional alanyl residues in the $\gamma$CB-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 $\gamma$-chains and the percent $\gamma$-chain (represented by the solid line); these results are from all $\beta$-thalassemia heterozygotes.

![Figure 5](image_url)  
**Figure 5** Pedigree of Family D. with $\delta$-thalassemia and $\beta$-thalassemia.

![Figure 6](image_url)  
**Figure 6** Relationship between the percentage of $\gamma$-chain and the fraction of $\gamma$-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 Ty-chain positive β-thalassemia homozygotes, and from one of the five Ty-chain positive SS patients. The others form a group in which relation between the percent Ty-chain and the percent Gγ-chain is much less clear (represented by the broken line).

Thus, the first set of data suggests an inverse correlation between the percent Ty-chains and the fraction of Gγ-chains (which is similar to a direct correlation with the fraction of γγ-chains). Individuals belonging to this group may be either homozygous for the Ty-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 Ty-gene and produce Hb-F only in cis (examples are several β-thalassemia traits and all ββ-thalassemia traits). However, if an individual is heterozygous for the Ty-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 Ty-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 Ty-chain heterozygosity in the category of decreased percent Ty-chain, and cases II-1 and II-2 with a γγ-chain-homozygosity in the category in which the correlation between the percentage of Ty- and γγ-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 Ty- and Gγ-chains appears to exist. A comparison of these data with those of Fig. 6 suggests that all persons are heterozygous for the Ty-chain.

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

Recent studies of the fetal hemoglobin of five orangutans have shown that each of these samples have a γγ-chain, i.e., a chain with a valyl residue in position 75, in addition to the Ty-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 Ty-chain) or an alanyl residue (the γγ-chain) (15). The present information suggests that Ile-75 and Thr-135 are in one chain (the Ty-chain) and Val-75 and Ala-135 in the other chain (the γγ-chain). Thus, this type of heterogeneity is similar to that of the human fetal hemoglobin, although the incidence of the human Tyγγ-chain appears to be considerably less than that of the γγ-chain of the orangutan.

Some genetic considerations. Is the human Tγγ-gene that produces the Tyγγ-chain a mutant of the "normal" Ty-gene or genes, or is it a duplication? We have presented evidence (16, 17) for pairs of nonallelic Gγ- and γγ-genes which are arranged in the order G:γ/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 γγ-genes (17, 20, 21), the evidence for two nonallelic γγ-genes is much less direct. The linear correlation between the percentages of Ty- and γγ-chains that is illustrated in Figs. 6 and 7, and the nearly identical γγ-chain and Ty-chain values in the four newborn with a Ty-hemoglobin, suggest that these chains are the product of a single γγ-gene which is also a Ty-gene; because of the relatively low general frequency of γγ-genes, it seems unlikely that the Ty-mutation would be present both in A and a genes. All evidence, there-
fore, implies a single \( \gamma \)-gene. Furthermore, the linear correlation points to the \( \tau \gamma \)-gene as a mutant of the normal \( \gamma \)-gene rather than as a duplication such that nonallelic \( \gamma \)-and \( \tau \gamma \)-genes are present on each haploid chromosome.

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**REFERENCES**


