Observations on the Levels of Hb A2 in Patients With Different δ-Thalassemia Mutations and a δ Chain Variant

By J.F. Codrington, H-W. Li, F. Kutlar, L-H. Gu, M. Ramachandran, and T.H.J. Huisman

Hb A2 and its variant B2 (αδ16(A13)Gly → Arg) were quantitated in the blood of subjects with three different types of δ-thalassemia and with the δ-B2 anomaly in cis or in trans to the β-thalassemia determinant. In one family, the δ-B2 mutation was in cis to a newly discovered codon 47 (+A) frameshift. The levels of Hbs A2 and B2 were nearly the same and approximately 70% higher than those in simple Hb B2 heterozygotes. In two additional families, the δ-B2 variant was in trans to either a deletional δ-thalassemia (1,393 bp) involving part of the β-globin gene and part of the δ-globin gene promoter, or to the −88 C → T promoter mutation. In both instances, the Hb B2 level was increased by approximately 80%, but the Hb A2 level was increased by approximately 270% and 200%, respectively. These data indicate two mechanisms that will cause an increase in δ chain production. One is consistent with a general mechanism concerning the relative excess of α chains in β chain deficiencies which will combine with δ chains to form variable levels of Hb A2, dependent on the severity of the β chain deficiency. The second concerns the loss of β-globin gene promoter activity, perhaps by an absence of (or decreased) binding of specific protein(s) to this segment of DNA and a concomitant increase in δ-globin gene promoter activity in cis.

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THE INCREASE in the level of Hb A2 in β-thalassemia (thal) heterozygotes was discovered in 1957 when the percentage of Hb A2 was found to be approximately twice that in normal adults. More recent studies have indicated that the relative increase depends on the type of β-thal allele that is present. For instance, carriers of some mild types of β-thal, such as those caused by the C → T mutation at IVS-I-6 or by A → G mutations in the polyadenylation site, have only slight increases in their Hb A2 percentages, while heterozygotes for a silent type of β-thal due to the C → T mutation at nucleotide (nt) −101 of the β-globin gene promoter have nearly normal Hb A2 levels. These observations are consistent with the concept that α chains combine much more readily with the β chain of Hb A than with the δ chain of Hb A, and that in conditions with variable β chain deficiencies, the increase in Hb A2 level should vary accordingly. However, there are exceptions to this rule; for instance, persons with a heterozygosity for either one of two mild promoter mutations (namely, C → T at position −88 or A → G at position −29, which are common among blacks) have Hb A2 values at least as high as those observed among heterozygotes for the more severe β-thal alleles, like the C → T mutation at codon 39 or the G → A mutation at IVS-I-110 in Mediterranean patients. Furthermore, β-thal heterozygotes with a δ chain variant like δ-B, or δ16(A13)Gly → Arg in cis or in trans to the thalassemia mutation have similar amounts of Hb A2 and the Hb A2 variant, indicating that the increased Hb A2 synthesis in β-thal is derived from δ genes both in cis and in trans to the β-thal mutation.

Unusually high Hb A2 levels of 7% to 9% have been found in β-thal heterozygotes with a deletional defect that involves the 5’ segment of the β-globin gene and part of the β-globin gene promoter. The deletions concern a 292-bp deletion observed in a Turkish family, a 1,393-bp deletion observed among blacks, and the much larger 4,237-bp deletion seen in members of a Czechoslovakian family, all types share the loss of the 5’β promoter. We recently detected a β-thal heterozygote from Surinam who had the 1,393-bp deletion and a Hb B2 heterozygosity and who exhibited disproportionate levels of Hb A2 and Hb B2. This observation prompted us to reevaluate some members of two families with both β-thal and Hb B2 heterozygosities who were described over 25 years ago.

MATERIALS AND METHODS

Blood samples. Samples from three members of family K and from four members of a second black family (family M) were collected in The Netherlands (two samples) and Surinam (five samples) and shipped by air to Augusta, GA. Blood samples from one black male of family K, his 6-year-old twin daughters, and from a fourth, unrelated adult black female were obtained locally. Collection was in vacutainers with ethylenediamine tetraacetate as anticoagulant. Informed consent was obtained.

Methods. Hematologic data were collected with an automated cell counter. Hb F was quantitated by cation exchange high performance liquid chromatography (HPLC) using the PolyCAT (Columbia, MD) column system. Hb A2 and Hb B2 were quantitated by the same PolyCAT HPLC procedure and by the analytic diethylaminoethyl (DEAE)-cellulose procedure with Tris-KCN-HCl developers and a pH gradient; a similar method was used in the earlier publications describing some of these individuals. DNA was isolated from white blood cells with the method described by Poncz et al. Identification of the B2 variant (δ16(A13)Gly → Arg) was by hybridization of amplified DNA18 with probes specific for a G → C mutation in codon 16 of the δ-globin gene. A similar procedure was used to identify one of the δ-thal mutations; this methodology has been described in previous publications. The approximately 1.4-kb deletion present in the third family was detected by gene mapping as shown earlier. The mutation in the third family was new and was identified through sequencing of amplified DNA.11 It was confirmed by hybridization of amplified DNA with a specific, radiolabeled oligonucleotide probe.

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RESULTS

The three families and their \( \beta \)-thal types. Family K is of Dutch-Surinam origin and was first described in 1961. Family members with a \( \beta \)-thal heterozygosity also had a heterozygosity for Hb B, or \( \alpha_2 \beta_2 \)A1(A13)Gly \( \rightarrow \) Arg; no normal members carried the Hb B, variant and no \( \beta \)-thal heterozygote was without it. This observation was explained by assuming that the two abnormalities were linked and occurred on the same chromosome. The average values for Hb B, (2.0%) and Hb A, (2.2%) for 12 normal family members (measured by carboxyl-methyl cellulose chromatography) were found to have a simple Hb B, heterozygosity. The Hb A, (2.0%) and Hb B, (1.9%) were only slightly less than the average Hb A, value for some samples in the two groups differ considerably, the relative quantities for Hb B, and Hb A, (listed as 100.B2/total) were similar.

The percentages of Hb A, and Hb B, were determined with both procedures are listed in Table 1; although the absolute values given for some samples in the two groups differ considerably, the relative quantities for Hb B, and Hb A, (listed as 100.B2/total) were similar.

Table 1. The Levels of Hb A, and Hb B, in Hb B, Heterozygotes With and Without an Additional \( \beta \)-Thal Heterozygosity

<table>
<thead>
<tr>
<th>Subject*</th>
<th>Sex/Age (y)</th>
<th>DEAE-Cellulose Chromatography</th>
<th>PolyCAT HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B, (%)</td>
<td>A, (%) Total (%)</td>
<td>B, (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family K (Hb B, in cis, ( \beta )-thal mutation, codon 47 [+A])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.K. (IV-1)</td>
<td>F/36</td>
<td>1.98 2.51 4.49 44.1</td>
<td>2.55 2.60 5.15 49.5</td>
</tr>
<tr>
<td>H.R. (III-4)</td>
<td>M/64</td>
<td>2.52 2.72 5.24 48.1</td>
<td>2.36 2.45 4.81 49.1</td>
</tr>
<tr>
<td>E.C.R. (III-2)</td>
<td>F/60</td>
<td>1.71 2.36 4.07 42.0</td>
<td>2.32 2.41 4.73 49.0</td>
</tr>
<tr>
<td>Average</td>
<td>2.07 2.53 4.60 45.0</td>
<td>2.41 2.49 4.90 49.2</td>
<td></td>
</tr>
<tr>
<td>Family R (Hb B, in trans, ( \beta )-thal mutation, -88 [C ( \rightarrow ) T])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.R. (II-2)</td>
<td>M/39</td>
<td>2.17 4.39 6.56 33.1</td>
<td>2.52 4.49 7.01 35.9</td>
</tr>
<tr>
<td>Second collection</td>
<td>2.48 4.75 7.21 34.1</td>
<td>2.57 4.47 7.04 36.5</td>
<td></td>
</tr>
<tr>
<td>Family M: (Hb B, in trans, ( \beta )-thal mutation, 1,393-bp deletion)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L.M.</td>
<td>M/54</td>
<td>1.77 4.75 6.52 37.3</td>
<td>2.51 5.47 7.98 31.5</td>
</tr>
<tr>
<td>Controls ( \beta )-thal absent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.B.</td>
<td>F/6</td>
<td>1.36 1.60 2.96 45.9</td>
<td>1.24 1.47 2.81 47.7</td>
</tr>
<tr>
<td>L.B.</td>
<td>F/6</td>
<td>1.29 1.50 2.79 46.2</td>
<td>1.45 1.50 2.95 49.2</td>
</tr>
<tr>
<td>D.C.</td>
<td>F/37</td>
<td>1.24 1.49 2.73 45.4</td>
<td>ND ND ND ND</td>
</tr>
<tr>
<td>Average</td>
<td>1.30 1.53 2.83 45.9</td>
<td>1.40 1.49 2.89 48.4</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: ND, not determined.

*Numbers between parentheses identify the numbers in the pedigrees as published in 1961 and 1963. The controls S.B. and L.B. are the twin daughters of E.R. of family R. The control D.M., who is the daughter of L.M., has an additional Hb S heterozygosity; the value for Hb A, with the polyCAT HPLC procedure may be slightly too high because of contamination with a minute quantity of a modified Hb S.
\(\beta\)-thal heterozygotes with the same mutation was 5.4% \pm 0.4%, with a range of 4.5% to 6.6% (unpublished data). The level of Hb \(B_2\) in L.M. of family M, with the 1,392-bp deletion, was 2.51% and that of Hb \(A_2\) was 5.47%, an increase of approximately 80% and of 270% over the values for Hb \(B_2\) and Hb \(A_2\), respectively, in the Hb \(B_2\) heterozygotes. The level of 7.98% for \(\delta\) chain containing Hb types is similar to values observed for other patients with the same deletion.\(^6\)\(^,\)\(^7\)\(^,\)\(^9\) The values for Hb \(A_2\) and Hb \(B_2\) in the one nonthalassemic member of this family who also has an Hb S heterozygosity were nearly the same.

**DISCUSSION**

The results obtained in this study, summarized in Table 1, suggest the following. First, simple Hb \(B_2\) heterozygotes have nearly equal levels of Hb \(A_2\) and Hb \(B_2\), indicating that the \(G \rightarrow C\) substitution at codon 16 of the \(\delta\) gene (Gly \(\rightarrow\) Arg in the \(\delta\) chain) does not affect the relative synthesis of this variant chain and, thus, the presence of this \(\delta\) gene marker offers an opportunity to study the relative formation of Hb \(A_2\) derived from \(\delta\) chains in cis or in trans to a \(\beta\)-thal mutation. The one child of family M with Hb \(B_2\) and Hb S heterozygosities also had closely similar Hb \(A_2\) and Hb \(B_2\) values. Second, the nearly equal values for Hb \(A_2\) and Hb \(B_2\) in family K with the frameshift at codon 47 (+A) confirm earlier data\(^6\) and suggest that mutations within the \(\beta\)-globin gene do not influence the relative synthesis of \(\delta\) chains derived from \(\delta\) genes in cis or in trans to the \(\beta\)-thal gene but only the total Hb \(A_2\) formation. Third, the high level of Hb \(A_2\) of nearly 5.5% in a subject with the deletional \(\beta\)-thal who has a \(\delta\)-B mutation in trans and a Hb \(B_2\) level of 2.5% indicates that the excess Hb \(A_2\) is derived from \(\delta\) chains in cis to the \(\beta\)-thal deletion. Finally, the high level of Hb \(A_2\) (nearly 4.5%) in a patient who had the \(-88C \rightarrow T\) mutation and the Hb \(B_2\) mutation in trans (the Hb \(B_2\) value was 2.5%) showed that this mutation in the CACC box of the \(5'\beta\) promoter increases Hb \(A_2\) formation from \(\delta\) chains in cis to this \(\beta\)-thal mutation. There are considerable differences between the Hb \(A_2\) and Hb \(B_2\) percentages found for this person; in 1963, at the age of 11 years, the values were Hb \(A_2\) 2.70% and Hb \(B_2\) 1.80% and, in 1990, at the age of 39 years, the average Hb \(A_2\) and Hb \(B_2\) values by DEAE-cellulose chromatography were 4.57% and 2.32%, respectively. The considerably lower values found in the earlier study might be due to a coexisting iron deficiency, although this possibility was not evaluated at that time.

These data suggest that at least two mechanisms can be responsible for higher levels of Hb \(A_2\) in \(\beta\)-thal heterozygotes. One general mechanism concerns the relative excess of \(\alpha\) chains in \(\beta\) chain deficiencies that is present in all types of \(\beta\)-thal, albeit at different levels dependent on the type of \(\beta\)-thal. The second mechanism appears to be loss of \(\beta\)-globin gene promoter activity. Popovich et al\(^12\) were the first to suggest that the transcription of the \(\delta\)-globin gene is affected by loss of the \(5'\beta\) promoter, perhaps because the \(5'\beta\) and \(5'\delta\) promoters are influenced by the same \(3'\beta\) enhancer. The data presented here support this hypothesis and suggest that a mutation in the proximal CACC has a nearly similar effect as the loss of the entire \(5'\delta\) promoter. It may be that loss of (or decreased) binding of a specific protein or a complex of proteins to this segment of DNA results in a decrease in or absence of \(\delta\)mRNA formation and, indirectly, in an increase in \(\delta\)mRNA formation because protein binding at the CACC box of the \(5'\delta\) promoter is not affected. Such a difference would not exist when the \(\beta\)-thal mutation is outside this promoter region and the formation of \(\beta\)mRNA (although ineffective) is not influenced by this mutation. Another mechanism explaining the high \(\delta\) chain production is that the deletion of the \(\beta\)-globin gene promoter or the mutations in the TATA \((-29,A \rightarrow G)\) or CACC \((-88,C \rightarrow T)\) boxes.

- **Fig 1.** Identification of the frameshift at codon 47 (+A) of the \(\beta\) chain leading to a \(\beta\)-thal. (Left) Part of a sequencing gel locating the frameshift at codon 47. (Right) Confirmation of the codon 47 (+A) frameshift by hybridization of the amplified DNA with \(^{32}\)P-labeled probes.

- **Table 1.** Relative synthesis of \(\beta\) and \(\delta\) chain deficiencies that is present in all types of \(\beta\)-thal, albeit at different levels dependent on the type of \(\beta\)-thal.
partially removes competition for limiting transcription factors, which makes these more readily available to the δ-globin gene promoter. However, this would affect equally the promoters of both δ-globin genes and not only the δ-globin gene in cis to the β-thal allele.

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