A FOUR-BASE DELETION 5' TO THE \( ^\gamma \) GLOBIN GENE IS A COMMON POLYMORPHISM

To the Editor:

Approximately 60% of the \( \gamma \)-globin chains of adults are \( ^\gamma \)-globin chains. Reduced proportions of \( \gamma \)-chains have been reported in individuals with a 4-base deletion 5' to the \( ^\gamma \)-globin gene (−AGCA between −222 to −225). In Sardinians, this deletion was reported to be linked to \( \beta^s \)-thalassemia (\( \beta^39 \) C → T). It was suggested that the deletion might serve as a marker for this mutation. We detected this deletion in an individual who had an unusually high level of \( \gamma \) (82.1%). We found that this deletion was a common polymorphism in the American black and white populations. In all individuals examined, the proportion of \( ^\gamma \)-globin was normal. Furthermore, the deletion was linked to a normal \( \beta \)-globin gene in most individuals, but was linked to the \( \beta^s \)-globin gene in at least two subjects.

Hemoglobin (Hb) F was partially purified and the \( ^\gamma / \gamma \) ratio was obtained by reverse-phase high performance liquid chromatography. \( ^\gamma \) and \( ^\gamma \)-globin gene promoters were specifically amplified. For restriction digests, 10 \( \mu \)L of the amplified DNA was digested with \( Bbv \) I and subjected to electrophoresis on a 3% Nu Sieve GTG agarose, 0.8% agarose gel for 2 hours at 35 V. The stained gels were viewed under UV light. The amplified DNA was reamplified asymmetrically yielding single stranded template for dideoxy sequencing.

We detected the −222 to −225 4-base deletion in a black woman heterozygous for \( \beta^s \)-thalassemia caused by a 1.4-kb deletion involving the 5' portion of the \( \beta \)-globin gene. The deletion was in the \( ^\gamma \)-gene promoter region. The subject had 2.2% HbF and 82.1% \( ^\gamma \). The \( \gamma \)-globin genes in this patient were normal. Other than the 4-base deletion, no promoter mutations were found. Two children with HbS-\( \beta^s \)-thalassemia did not have the deletion, but it was found in a sib with sickle cell trait (0.1% HbF, 70.4% \( ^\gamma \)), linking the deletion to a normal \( \beta \)-globin allele in this family.

Because reports in the literature suggested this deletion was associated with low \( \gamma \), we decided to investigate further its prevalence and effect. We amplified the \( ^\gamma \) promoter region that resulted in a 734-bp fragment. DNA was digested with \( Bbv \) I as shown in the Fig 1. The single \( Bbv \) I site within this fragment produces fragments of 282 and 452 bp. When present, the 4-base deletion abolishes the \( Bbv \) I site, producing a single fragment of 730 bp. To corroborate these results, the presence of the deletion was confirmed by direct sequencing of the amplified fragment in some samples.

Of 55 individuals studied, we found 12 heterozygous for the
Fig 1. Agarose gel electrophoresis of polymerase chain reaction amplified DNA digested with Bbv I. Lanes 1, 2, and 3 (reading from the left) are samples from homozygotes for the deletion, lanes 4 and 5 are from individuals without the 4-base deletion, and lanes 6 and 7 are from individuals heterozygous for the deletion.

<table>
<thead>
<tr>
<th>Table 1. Frequency of Individuals With the 4-Base Deletion</th>
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<td>Population</td>
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<td>Black, HbAS</td>
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<tr>
<td>Black, HbAA</td>
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<tr>
<td>White, HbAA</td>
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*Percent in individuals with the 4-base deletion.

mutation (21.8%; gene frequency, 12.4%). There were two homozygotes for the mutation (3.6%; expected, 1.5%). Both homozygotes had sickle trait. The proportion of \( \gamma \)-globin was 53.3% and 67.0% in the homozygotes. Table 1 summarizes the results of our findings and demonstrates that the \( \gamma \) values for the affected individuals were normal.

Gilman et al. reported reduced \( \gamma \) (40%) in a black patient heterozygous for \( \beta \)-thalassemia and HbC. The 4-base deletion in this patient was present in cis to the \( \beta \)-thalassemia allele. The reduced proportion of \( \gamma \)-globin was ascribed to the 4-base deletion. Manca et al. found the same deletion in Sardinians with \( \beta \)-thalassemia and unusually low \( \gamma \) values. Seventy percent of the chromosomes with the \( \beta \)9 mutation also had the \( \gamma \)T-globin gene polymorphism. The simple heterozygote for \( \beta \)-thalassemia, who was also heterozygous for the deletion, had unusually low levels of \( \gamma \)T-globin when compared with \( \gamma \). It was assumed that the deletion was located in cis to the \( \gamma \)T-globin gene.

On the basis of our work, the −222 to −225 4-base deletion appears to be a common polymorphism. Why our results differ from those cited above is unclear. One possible explanation is that our subjects came from a multiracial heterogeneous population.

REFERENCES

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3. Manca L, Gallisai D, Masala B, Gilman JG: Sardinian \( \beta / \beta \)-thalassemia with reduced \( \gamma \) globin has a four base-pair deletion in the \( \gamma \)-gene promoter. Blood 72:66a, 1988 (abstr)

RESPONSE

In response to the letter of Coleman et al, our recent data on northern Sardinian \( \beta \)-thalassemia and Cameroons sickle cell anemia patients have shown that \( \gamma \)1\T genes are consistently associated with the 4-base deletion, and that less \( \gamma \) than normal \( \gamma \)1\T was found in the fetal hemoglobin of \( \gamma \)1\T/\( \gamma \)1\T heterozygotes. However, \( \beta \)-thal and SS cases differed in results for \( \gamma \)1/\( \gamma \)1 and \( \gamma \)1/\( \gamma \)1\T homozygotes.

For \( \beta \)-thal, 6 haplotype I/1 had 53% ± 3% \( \gamma \) and 47% ± 3% \( \gamma \)1\T, while 25 haplotype II/II had 62% ± 4% \( \gamma \) and 38% ± 4% \( \gamma \)1\T (P < .001). This agrees with our original observations on a beta-thal-kindred. For Cameroons SS, our data have shown that 17 Benin/Benin, 13 Benin/Eton, and 4 Eton/Eton haplotypes had equal ratios of \( \gamma \)1\T/\( \gamma \)1, \( \gamma \)1\T/\( \gamma \)1\T, and \( \gamma \)1/\( \gamma \)1\T respectively. \( \gamma \)1\T/\( \gamma \)1\T was 2:3 in Benin/Eton; \( \gamma \)1\T but not \( \gamma \)1 was associated with the 4-base deletion in Cameroons SS patients.

Therefore, our data have shown that the 4-base deletion was correlated with low \( \gamma \)1\T in both \( \beta \)-thal and SS \( \gamma \)1/\( \gamma \)1\T. However, the \( \gamma \)1/\( \gamma \)1\T Eton/SS did not show low percent \( \gamma \)1 compared with percent \( \gamma \)1\T of the \( \gamma \)1/\( \gamma \)1\T Benin/Benin SS. This is difficult to explain by any simple hypothesis designed to account for lower \( \gamma \)1\T than \( \gamma \)1 in the \( \gamma \)1/\( \gamma \)1 Benin/Eton. It may be that the 4-base deletion decreases \( \gamma \)1\T, and lowers \( \gamma \)1 in cis to the SS cases. It will be necessary to study expression in model systems such as transgenic mice to determine whether low \( \gamma \)1\T expression is due to the 4-base deletion.

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1. Manca L, Cocco E, Gallisai D, Masala B, Gilman JG: Diminished \( \gamma \)1\T fetal globin levels in Sardinian haplotype II \( \beta \)-thalassemia patients are associated with the four base pair deletion in the \( \gamma \)1\T promoter, Br J Haematol 78:105, 1991
A four-base deletion 5' to the A gamma globin gene is a common polymorphism [letter]

MB Coleman, MH Steinberg and JG 3d Adams