Upstream Promoter Mutation Associated With a Modest Elevation of Fetal Hemoglobin Expression in Human Adults


In hereditary persistence of fetal hemoglobin, Hb F (α2γ2) is elevated after birth. Screening of sickle cell patients has revealed a family with elevated Hb F and high αglobin values. The propositus was a sickle cell patient with ~25% Hb F and 68.4% αγ. He was heterozygous for the Benin (#19) and Mor αβ haplotypes. Five AS relatives with the Mor haplotype had 2.5% ± 0.9% fetal hemoglobin and 92.8% ± 2.8% αγ, whereas two with the Benin haplotype had normal fetal hemoglobin (0.6%). The Mor haplotype is thus associated with the elevated Hb F in this family. The 13-kilobase (kb) BgIII fragment containing the γ and αγ genes of the Mor haplotype was cloned, and the αγ and αγ promoters were sequenced from ~383 to beyond the Cap sites. The Mor αγ gene was normal, but the αγ gene had a unique C → T mutation at ~202. A different mutation at ~202 of αγ (C → G) was previously detected by other researchers in association with considerably higher Hb F in AS cases (15% to 25%). These data suggest either that ~202 mutations affect the αγ and αγ promoters differently or that different nucleotide substitutions at ~202 have divergent effects. Alternatively, additional unknown mutations could cause the differences in gene expression.

Fetal Hemoglobin or Hb F (α2γ2) is predominant in RBCs of the human fetus and newborn baby. Within 6 months after birth, almost all fetal γ chain is replaced by adult β chain. In normal adults, Hb F is <1% of total hemoglobin, and the typical adult ratio of αγ to γ globins is 4:6.1

In hereditary persistence of fetal hemoglobin (HPFH), Hb F remains high in nonanemic adults. Several mutations of the Cap sites of the γ globin genes are associated with elevated Hb F containing predominantly one type of γ chain. The first such mutation (C → G) was found at 202 base pairs (bp) 5′ of the 6γ Cap site (~202) in association with 15% to 25% Hb F (α2γ2) in heterozygotes.2

A recent study of sickle cell anemia patients demonstrated an unusual case with elevated Hb F and a low (3:7) ratio of αγ to γ globins.3 The propositus was heterozygous for haplotype #19 (Benin) and γ2 (G) was found at 202 base pairs (bp) 5′ of the 6γ Cap site (~202) in association with 15% to 25% Hb F (α2γ2) in heterozygotes.2

The percentage of A2 (%A2) was determined by microcolumn high-performance liquid chromatography (HPLC)1 or %FαA2, as indicated1 and αγ-γ ratios3 have been described in detail previously.

The BgIII fragment containing both γ genes was cloned into λ phage vector EMBL3.10 Screening11 used the γIVSII probe. Because only one chromosome was of interest, it was necessary to select the appropriate clone. The Mor haplotype of M.N., associated with high Hb F and high αγ, was — at the PvuII site 3′ to the γ gene. This permitted its identification, since the other haplotype (#19 or Benin) was +.3 The difference was detectable by PvuII digestion and ethidium bromide staining.

Portions of the cloned DNA were subcloned into Bluescript plasmid (Stratagene Cloning Systems, La Jolla, CA). For the γ2 gene, two subclones were generated: One contained a Stul-BamHI insert beginning at ~383, and the other contained an Apal-BamHI insert beginning at ~205; both inserts extended 3′ close to IVSII. For the γ1 gene, only the former plasmid was made, because the C → T mutation at ~202 (discussed in the Results section) eliminated the Apal site.

These plasmids permitted the sequencing of the 5′ flanking regions of the γ1 and γ2 genes from ~383 to 30 bp 3′ of the Cap site. Sequencing by the dyeoxy procedure12 used 3P-dATP, the Sequenase kit of United States Biochemical Corporation (Cleveland), T3 or reverse-sequencing primer (Stratagene), and the reverse transcriptase sequencing kit of Stratagene.13

RESULTS

M.N. and his family were found during a screening of SS patients in the Southeastern United States.3 His Hb F was very high (~25%), and his αγ value of 68.4% was higher than that observed in most Black SS patients.13 He was heterozygous for haplotype #19 (Benin) (−−−−−−−−−−−−−−++++) and for an unusual haplotype called Mor (+−−−−−−−−−−+).13 The AS family members with the Mor haplotype had slightly elevated levels of Hb F (mean SD = 2.5% ± 0.9%, range 1.6% to 3.9%) and high αγ values (92.8% ± 2.8%, 89.1% to 95.9%). Table 1 lists hematologic values and the haplotypes for M.N. and his family.

The BgIII fragment containing the γ1 and γ2 genes was cloned for the Mor haplotype. DNA sequencing of the γ1 promoter from ~383 to the Cap site showed C at ~158, consistent with low α2 values,3 and otherwise no differences from published data.14 The αγ gene had G instead of C at ~369 and G instead of A at +25 (data not shown), which are known polymorphisms.2 In addition, the αγ gene had a

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unique C → T mutation at -202, as shown by DNA sequence data of Fig 1.

**DISCUSSION**

Hb F is normally <1% of total hemoglobin in human adults. In nondeleterious HPFH cases, however, production of Hb F increases and γ globin is predominantly either Gγ or Aγ.

In three high Aγ types of HPFH, mutations were found in the 5′ flanking region of the Aγ gene at -117 (G → A),15,16 -196 (C → T),17 and -198 (T → C).18 In three types of high Gγ HPFH, mutations were seen at -161 (A → G),19 -175 (T → C),20 and -202 (C → G) of the Gγ gene. Transient expression studies in cultured erythroid cells have shown that mutant promoters (for -117, -158, -196, and -202) had increased expression as compared with the normal promoter.21-23

We have now found a new C → T mutation at -202 of the Aγ gene associated with elevated Hb F. The propositus (M.N.) was an SS patient with very high Hb F (-25%) and high Aγ (68%) levels (Table 1). He was heterozygous for βγ haplotypes #19 (Benin) (− − − − + + + +) and Mor (+ − − − − − − +). The Benin haplotype came from his AS father (case II-1), who had low Hb F (0.5%). An AS sister with the Benin haplotype (case III-2) also had low Hb F, whereas the five AS relatives with the Mor haplotype had elevated Hb F (2.5% ± 0.9%) and very high Aγ (92.8% ± 2.8%) (Table 1). Thus, the Mor haplotype with the C → T mutation at -202 of Aγ is likely to be responsible for the elevated Hb F of SS patient M.N.

High Hb F levels in SS patients have heretofore been associated with T at -158 of the Gγ gene.24-26 on the Senegal βγ (#3) haplotype (− + + + + + + +)27 and the Asian βγ (#31) haplotype (+++++++−−−)28,29 Populations with the Asian βγ haplotype may also have additional uncharacterized determinants of elevated Hb F that lie outside of the promoter region.26 It is notable that heterozygosity for the high Hb F Mor βγ haplotype leads to Hb F values comparable to the highest seen in Asian βγ homozygotes.26,31

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**Table 1. Hematologic Data for Selected Members of the Family of Propositus M.N.**

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex/Age</th>
<th>D.</th>
<th>Relationship</th>
<th>Haplotype*</th>
<th>Hb g/dL</th>
<th>PCV (L/L)</th>
<th>RBC (10^12/L)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
<th>Aγ (%)</th>
<th>Fαγt (%)</th>
<th>(%)</th>
<th>(%)</th>
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<tr>
<td>I-1</td>
<td>F/56</td>
<td>AS</td>
<td>Maternal grandmother</td>
<td>X/Mor</td>
<td>12.9</td>
<td>4.70</td>
<td>81</td>
<td>27.4</td>
<td>33.9</td>
<td>2.8</td>
<td>1.6</td>
<td>4.1</td>
<td>95.9</td>
<td>95.9</td>
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<tr>
<td>II-1</td>
<td>M/46</td>
<td>AS</td>
<td>Father</td>
<td>X/19</td>
<td>13.2</td>
<td>4.60</td>
<td>80</td>
<td>28.2</td>
<td>35.2</td>
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<td>0.5</td>
<td>18.9</td>
<td>81.1</td>
<td>81.1</td>
</tr>
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<td>Mother</td>
<td>X/Mor</td>
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<td>3.20</td>
<td>84</td>
<td>30.0</td>
<td>35.6</td>
<td>2.6</td>
<td>2.7</td>
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<tr>
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<td>AS</td>
<td>Aunt</td>
<td>X/Mor</td>
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<td>3.55</td>
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<tr>
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<tr>
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<td>X/Mor</td>
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<td>3.60</td>
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<td>ND</td>
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<tr>
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<td>M/17</td>
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<td>Propositus</td>
<td>19/Mor</td>
<td>11.1</td>
<td>3.20</td>
<td>88</td>
<td>33.2</td>
<td>37.6</td>
<td>3.0</td>
<td>28.6</td>
<td>31.6</td>
<td>68.4</td>
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</tr>
</tbody>
</table>

The pediography of this family with haplotype, Fαγt and Gγ data were previously published.3

*Haplotypes: Mor or (+ − − − + + + +); 19 or Benin or (− − − − + + + +); X − others.
†By microcolumn chromatography.6
‡By alkali denaturation.8
§By reverse-phase HPLC.9
||By cation-exchange HPLC.7

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**Fig 1.** DNA sequence data for the Mor haplotype of M.N. showing the −202 C → T mutation of the Aγ globin gene. Details given in text.

**Fig 2.** Comparison of nucleotide substitutions associated with elevation of Hb F production in human adults.
AS individuals with the C → T mutation at -202 of the θγ gene (Table 1) had five to ten times less Hb F than those with the -202 C → G mutation of the θγ gene. This difference in gene expression may result from a stronger effect of the C → G than the C → T mutation, to their different locations on θγ and θγ promoters, or to other unknown causes.

Figure 2 summarizes the mutations associated with increased expression of the θγ and γγ genes. A certain clustering is apparent. Several mutations, including the -202 C → T mutation of this report, occur in the GC-rich region of DNA between -208 and -192. The G → A mutation at -161 is near the -158 C → T mutation, and both have smaller effects on Hb F expression than do the other known mutations.

Two other mutations do not fit into clusters, but they occur within sequence motifs known to bind regulatory proteins in other genes: The -117 G → A mutation is at the CCAAT box, which is a protein-binding site, whereas the -175 T → C mutation is in an "octamer motif" ATGCAAT (-182 to -175), which binds a regulatory protein in the immunoglobulin heavy chain enhancer and promoter.

The region from -198 to -192 (TCCCCAG), in which two mutations occur, has a sequence similar to the TC motif (TCCCCAG) of the SV40 viral enhancer. Collins and colleagues noted that the -202 mutation is in a region similar to a binding site for the promoter-specific factor Sp1, which activates transcription in SV40 virus. It is therefore likely that the mutations shown in Fig 2 occur in DNA-sequence motifs that bind regulatory proteins. The mutations at -161 and -158, with minor effects on γγ-gene expression, may then lie either on the fringe of the octamer protein-binding site or in a region of DNA whose protein-binding sequence is not yet known.

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