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 α₂γ values. The propositus was a sickle cell patient with ~25% Hb F and 68.4% α₂γ. He was heterozygous for the Benin (#19) and Mor β₀ haplotypes. Five AS relatives with the Mor haplotype had 2.5% ± 0.9% fetal hemoglobin and 92.8% ± 2.8% α₃γ, 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) BglII fragment containing the α₂γ and α₃γ genes of the Mor haplotype was cloned, and the α₂γ and α₃γ promoters were sequenced from −383 to beyond the Cap sites. The Mor α₂γ gene was normal, but the α₃γ gene had a unique C → T mutation at −202. A different mutation at −202 of α₃γ (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 α₂γ and α₃γ 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 α₂γ to α₃γ globins is 4:6.

In hereditary persistence of fetal hemoglobin (HPFH), Hb F remains high in nonanemic adults. Several mutations 5’ 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 α₀γ Cap site (−202) in association with 15% to 25% Hb F (α₀γ₂γ₂) in heterozygotes. A recent study of sickle cell anemia patients demonstrated an unusual case with elevated Hb F and a low (3:7) ratio of α₂γ to α₃γ. We now report DNA sequence analyses of this case that demonstrate a C to T mutation at −202 of the α₃γ gene.

MATERIALS AND METHODS

Blood collection, DNA preparation, restriction enzyme digestion, and blot hybridization have been described previously. Haplotypes were determined for eight restriction fragment length polymorphisms: The HincII site 5’ of the α gene, the XmnI site 5’ of the α₂γ gene, HindIII sites in the α₀γ and α₃γ genes, HincII sites in the β gene and 3’ to it, the AvaI site in the β gene, and the BamHI site 3’ to β. The percentage of A₅ (γ/α) was determined by microcolumn chromatography. Methods for determination of Hb F [by cation-exchange high-performance liquid chromatography (HPLC)] or %F₅₀,₆₀ as indicated] and α₂γ/α₃γ ratios have been described in detail previously.

The BglII fragment containing both γ genes was cloned into λ phage vector EMBL3. Screening1 used the γIVSI 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 α₂γ, was − at the PvuII site 3’ to the α₃γ gene. This permitted its identification, since the other haplotype (#19 or Benin) was +. 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 α₂γ 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 IVSI. For the α₃γ 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 α₂γ and α₃γ genes from −383 to 30 bp 3’ of the Cap site. Sequencing by the dideoxy procedure used P-dATP, the Sequencing kit of United States Biochemical Corporation (Cleveland), T3 or reverse-sequencing primer (Stratagene), and the reverse transcriptase sequencing kit of Stratagene.

RESULTS

M.N. and his family were found during a screening of SS patients in the Southeastern United States. His Hb F was very high (~25%), and his α₂γ value of 68.4% was higher than that observed in most Black SS patients. He was heterozygous for haplotype #19 (Benin) (−−−−+++++) and for an unusual haplotype called Mor (+−−−+++). 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 α₂γ 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 BglII fragment containing the α₂γ and α₃γ genes was cloned for the Mor haplotype. DNA sequencing of the α₂γ promoter from −383 to the Cap site showed C at −158, consistent with low α₂γ values, and otherwise no differences from published data. The α₃γ gene had G instead of C at −369 and G instead of A at +25 (data not shown), which are known polymorphisms. In addition, the α₃γ gene had a
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 nondeletion 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)21 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.21,22

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 α7 haplotypes #19 (Benin) (- - - - - - - +) and Mor (+ - - - - - - +). The Benin haplotype came from his AS father (case 11-1), 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 βS (#3) haplotype (- - - - - - + + +)27 and the Asian βS (#31) haplotype (++ + + + + +).28,29 Populations with the Asian βS haplotype may also have additional uncharacterized determinants of elevated Hb F28,30 that lie outside of the promoter region.26 It is notable that heterozygosity for the high Hb F Mor βS haplotype leads to Hb F values comparable to the highest seen in Asian βS homozygotes.28,31

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 \( \alpha_y \) gene (Table 1) had five to ten times less Hb F than those with the −202 C → G mutation of the \( \alpha_y \) gene.\(^2\) 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 \( \alpha_y \) and \( \alpha_y \) promoters, or to other unknown causes.

Figure 2 summarizes the mutations associated with increased expression of the \( \alpha_y \) and \( \alpha_y \) 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\(^19\) is near the −158 C → T mutation\(^2\) 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,\(^2\) 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\(^11\) and promoter.\(^34\)

The region from −198 to −192 (TCCCCAC), in which two mutations occur, has a sequence similar to the TC motif (TCCCCAG) of the SV40 viral enhancer.\(^35\) Collins and colleagues\(^2\) 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.\(^36\) 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 \( \gamma \)-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.

REFERENCES

1. Gilman JG, Huisman THJ: Two independent genetic factors in the \( \beta \)-globin gene cluster are associated with high \( \alpha \) levels in the Hb F of SS patients. Blood 64:452, 1984
2. Collins FS, Stoeckert CJ Jr, Serjeant GR, Forget BG, Weissman SM: \( \alpha \beta^+ \) Hereditary persistence of fetal hemoglobin: Cosmid cloning and identification of a specific mutation 5' to the \( \alpha_y \) gene. Proc Natl Acad Sci USA 81:4894, 1984
18. Tate VE, Wood WG, Weatherall D: The British form of hereditary persistence of fetal hemoglobin. Results from a single base mutation adjacent to an Sl hypersensitive site 5' to the \( \gamma \) globin gene. Blood 68:1389, 1986
20. Month S, Deligrosio K, Orchowski P, Rappaport E, Malladi P, Schwartz E, Surrey S: Analysis of the region 5' to the \( \gamma \) gene in a patient with \( \gamma \) \( \beta \)-HPFH/\( \beta \). Blood 68:76a, 1986
26. Miller BA, Oliveri N, Salameh M, Ahmed M, Antognetti G,


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