The British Form of Hereditary Persistence of Fetal Hemoglobin Results From a Single Base Mutation Adjacent to an S1 Hypersensitive Site 5' to the ^ \gamma \gamma \ globin Gene

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The ^ \gamma \gamma \ and ^ \gamma \gamma \ genes of an individual homozygous for the British form of ^ \gamma \gamma \ nondeletion hereditary persistence of fetal hemoglobin have been cloned and partially sequenced. The ^ \gamma \gamma \ gene was normal, but the ^ \gamma \gamma \ gene was found to have a single base change (T -> C) at -198 bp relative to the cap site. Supercoiled plasmids containing normal ^ \gamma \gamma \-genes or the mutant ^ \gamma \gamma \-gene displayed an S1-hypersensitive site immediately 5' to the base change.

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RESULTS

Full details of the phenotypic effects of British HPFH have been described previously.9,10 Homozygotes are clinically healthy and have high levels of fetal hemoglobin (Hb F) in their blood, reaching up to 50% in some cases. The main feature of the disease is that Hb F is produced throughout life in non-deletion HPFH. The ^ \gamma \gamma \ gene is expressed in all tissues, while the ^ \gamma \gamma \ gene is expressed only in hematopoietic cells. The ^ \gamma \gamma \ gene contains a deletion that results in the production of a functional ^ \gamma \gamma \-chain, which is not produced in ^ \gamma \gamma \-homozygotes. The deletion is present in all bands of the ^ \gamma \gamma \-gene and is flanked by a 3-kb S1 nuclease-sensitive site (HSS) that is not present in the ^ \gamma \gamma \-gene.

CONCLUSIONS

The British form of HPFH is due to a single base change in the ^ \gamma \gamma \-gene, which results in the expression of a functional ^ \gamma \gamma \-chain. This finding has important implications for the understanding of the molecular basis of HPFH and other genetic disorders of the globin genes. Further studies are needed to understand the mechanism of transcriptional regulation of the ^ \gamma \gamma \-gene in non-deletion HPFH and to explore the role of the HSS in regulating gene expression.

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cally and hematologically normal except for increased Hb F levels of 18% to 21%, in which the proportion of $\gamma$-chains is 90% to 95%. Levels of Hb F in heterozygotes are lower, 3.5% to 10%, with a similar $\delta$-$\gamma$ ratio but a markedly uneven intercellular distribution. Restriction enzyme mapping of the homozygotes has shown no abnormalities within the $\beta$-globin gene cluster\textsuperscript{14}; haplotype analysis showed homozygosity at all the polymorphic restriction enzyme sites in the $\beta$-globin gene cluster\textsuperscript{15}.

**Sequence of the $\delta$-$\gamma$-gene.** The subcloned $\delta$-$\gamma$-gene was sequenced from position $-325$ bp to $+503$ bp relative to the cap site (+1). The sequence was almost identical to that described previously as the normal chromosome B allele (J. Slightom, personal communication, 1985)\textsuperscript{16}; the only differences were a $T \rightarrow C$ transition at $-158$ and a $C \rightarrow T$ transition at $+210$, both of which have been reported in other normal B-type alleles.\textsuperscript{5}

**Sequence of the $\delta$-$\gamma$-gene.** A total of 673 bp of the $\delta$-$\gamma$ gene were sequenced, from $-383$ bp to $+290$ bp, and the $\delta$-$\gamma$ sequence was also that of a typical B-type chromosome, but including the $-158$ $T \rightarrow C$ and $+210$ $C \rightarrow T$ changes noted in the $\delta$-$\gamma$-gene. One additional change was also observed, a $T \rightarrow C$ transition at position $-198$ (Fig 1), in a similar but different position to those seen in other cases of nondeletion HPFH.

**S1 nuclease sensitivity.** Among the changes known to be associated with gene expression is an increased susceptibility of chromatin at the 5' end of the gene to digestion by a variety of nucleases. In some cases these regions may also be

![Fig 1](image-url)  
*Fig 1.* The DNA sequencing gel of part of the British HPFH $\delta$-$\gamma$-gene. The base change at position $-198$ is indicated.

![Fig 2](image-url)  
*Fig 2.* (A) Restriction enzyme sites used in mapping the S1 nuclease hypersensitive site (S1 HSS) in the $\delta$-$\gamma$-gene. The shaded boxes are the coding regions and the triangle marks the poly A addition site. The sense or antisense strands were labeled at the $Stu1$ site, after S1 digestion, to enable position determination of the HSS, as detailed in Materials and Methods. (B) Major S1 hypersensitive sites in plasmids containing normal (lanes 1 and 3) and British HPFH (lanes 2 and 4) $\delta$-$\gamma$-genes, labeled on antisense (lanes 1 and 2) and sense (lanes 3 and 4) strands. Lanes 5, 6, 7, 8 are G, A, T, C sequencing products of British HPFH $\delta$-$\gamma$-gene labeled on the sense strand at the $Stu1$ site.
demonstrable as S 1 nuclease HSS in supercoiled plasmid DNA. Therefore, the S 1 nuclease HSS in plasmids containing normal $^6\gamma$- and $^8\gamma$-genes were mapped and the results compared with the $^8\gamma$-gene isolated from the British HPFH homozygote.

Figure 2A shows the key sites used in the S 1 nuclease analysis, while Fig 2B shows that the strongest S 1 HSS upstream of the $\gamma$-promoter lies between nucleotides −204 and −220, immediately to the S' side of the region in which the mutations in the British HPFH, and other forms of nondeletion HPFH, occur. There is no difference in the position or architecture of this S 1 HSS between the normal and British HPFH $^8\gamma$-genes. The only change that has been noted is that plasmids containing the British HPFH promoter regions appear to be converted to nicked circles by S 1 at approximately twice the rate of the normal control (Fig 3). The S 1 HSS is essentially symmetrical on sense and antisense strands and appears to show two hypersensitive regions within the overall HSS. This is shown diagrammatically in Fig 4. Plasmids containing the $^6\gamma$-promoter region show an identical S 1 HSS, whereas $^6\gamma$- or $^8\gamma$-containing plasmids, linearized before S 1 treatment, do not show sites of S 1 sensitivity (data not shown). Therefore, S 1 hypersensitivity is a property of DNA structure and requires torsional stress for its induction. There are additional, much weaker, sites of S 1 hypersensitivity, on the antisense strand only and centering around nucleotides −190, −184, and −124 found in both the normal and the British HPFH $^8\gamma$-genes (Fig 2B), as well as one site at −420 on the $^8\gamma$-genes but not on $^6\gamma$ (data not shown).

**DISCUSSION**

Cloning and sequencing of the $\gamma$-genes from a homozygote for British HPFH has demonstrated, as the only unusual feature, a single base substitution (T → C) at position −198, 5' to the cap site of the overexpressed $^8\gamma$-gene. This observation, together with three single base substitutions described recently in similar disorders (at $^8\gamma$ − 117 in Greek HPFH, at $^8\gamma$ − 196 in an Italian case, and at $^6\gamma$ − 202 in $^6\gamma2$’ HPFH) and the lack of similar substitutions in the normal $\gamma$-genes, makes a causal relationship between the substitution and the phenotypic effect of increased $\gamma$-chain production in adult life very likely, even if it has yet to be demonstrated directly.

The region of DNA 5' to the $\gamma$-genes has been implicated in the regulation of $\gamma$-gene expression by several lines of evidence. Deletions that remove sequences between −260 and −104 cause a marked reduction of $\gamma$-gene transcription in a transient expression assay. The region is marked by upstream transcription initiation sites, although the significance of such sites has not yet to be established. Digestion of intact nuclei with DNase I has revealed a DNase I HSS 5' to the expressed $\gamma$-genes at −100 in K 562 erythroleukemia cells and in human fetal erythroid cells when $\gamma$-chain production is fully active, but not in adult cells, where they are largely repressed. Such sites appear, therefore, to play a developmentally related role in regulation in vivo.

Of the nondeletion HPFH disorders, the British family is the best characterized at the phenotypic level. Thus, at birth, British HPFH heterozygotes have normal Hb F levels and a normal $^6\gamma$-$^8\gamma$ chain ratio; were the affected chromosome producing $\gamma$-chains at the same ratio as in adult life (>90% $^8\gamma$), it would have been readily detectable in the newborn period. Adult homozygotes for British HPFH show no hematologic abnormalities and, in particular, no evidence of imbalanced production of $\alpha$- versus $\gamma + \beta$-chains. In other words, $\gamma$-chain production in these cells does not appear to be additional to a normal level of $\beta$-chain production, but to be

![Figure 3](image-url)

**Fig 3.** Time course of conversion of plasmids containing normal (A) and British HPFH (B) $^8\gamma$-genes from supercoils (sc) to open circles (oc) or linear (l) molecules.

![Figure 4](image-url)

**Fig 4.** Diagrammatic representation of the S 1 HSS 5' to the promoter of the $^8\gamma$-gene. The sizes of the arrows represent the frequency with which a particular site is cut. *T → C transition in British HPFH.
associated with a reciprocal reduction in β-chain output. Furthermore, the intercellular distribution of Hb F is clearly heterogeneous, and in heterozygotes, many cells do not contain detectable amounts of Hb F.  

If the single base substitutions upstream from the γ-genes are responsible for the increased Hb F production in these disorders, does the phenotypic information provide any clues or place any constraints on the mechanism? The most likely explanation is that the region upstream of the affected gene is involved in the interaction with a trans-acting factor and that the effect of the substitution is to increase the affinity of this sequence for a factor that promotes γ-gene expression or, alternatively, to decrease its affinity for a repressor molecule. It is clear from the deletion HPFH conditions and other disorders that all of the trans-acting factors required for γ-gene expression are present in adult cells. Furthermore, there is evidence that all adult erythroid cells have the potential for Hb F production and that the low level of γ-gene expression in a small proportion of adult red cells (F cells) appears to be controlled stochastically. It is quite plausible, therefore, that the effect of the single base substitution, by altering the affinity for a putative trans-acting factor, would be to increase the normal propensity of adult cells to make a little Hb F rather than by activating a dormant gene in a novel manner. This notion is consistent with the variable Hb F levels in heterozygotes and the heterocellular distribution. One would not expect, therefore, any effect during fetal life when γ-gene expression is maximal anyway, presumably as a result of a more profound change in chromatin structure of the globin gene complex. This again is consistent with the observations on the phenotype in the cord blood of heterozygotes.

The reciprocal relationship between γ- and β-chain synthesis in the affected cells is likely to be a direct corollary of γ-gene transcription. Although we have no evidence in the British HPFH family that the reciprocity is a cis-active effect, that does appear to be the case in Oγβ* HPFH when there is a β' or β̂ marker on the other chromosome: any competition between γ- and β-genes for trans-acting factors should affect both β-genes equally. Models in which reciprocity in γ- and β-gene expression could occur as a result of a 5′ → 3′ polarity in the propagation of altered chromatin structure during erythroid cell maturation have been discussed previously. A delay in this progression as a result of an increased propensity to transcribe the affected γ-gene in adult cells could result in reduced β-gene transcription.

The four single base substitutions associated with nondeletion HPFH (Fig 5) could represent a single regulatory region spanning at least from −117 to −202, or two separate regions, one based on −117 and the other including −196 through −202. The −117 site lies within the upstream member of a duplicated region of 16 nucleotides, which includes the CCAAT box, a region of known regulatory importance. The sites based around −200 are adjacent to a GC-rich region with similarities to regulatory sequences associated with the 21 bp repeat of SV40 virus, herpes virus TK, and an African green monkey gene. These sequences are known to be associated with the binding of a specific protein, SP1. This is also the region that we have demonstrated to contain the S1 HSS site in supercoiled plasmids containing the normal Oγ- and β-genes.

An S1 HSS has been mapped to a string of G residues (−196 through −180) in the chick adult β-globin gene, although cutting is unilateral in this case, and to a homopurine stretch in the region −199 through −207 in human β-globin genes. The γ-globin gene HSS is less homogeneous in that it contains a block of seven pyrimidine residues, followed by five consecutive purine residues, and is bilateral. It will not form any apparent cruciform structure, which could account for its nuclease sensitivity, and therefore the sequence requirement for its structure is unclear. Although the S1 HSS was unaffected by the base substitution at position −198 in the British HPFH γ-gene, it is possible that a trans-acting regulatory element interacting with the S1 HSS site might have further contacts in the −200 region that are altered by the substitution. Although we can only speculate about such mechanisms at the moment, it should soon be possible to approach the question of specific protein binding to the region immediately upstream of the γ-genes, greatly facilitated by the availability of cloned material from four different but similar natural mutants.

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The British form of hereditary persistence of fetal hemoglobin results from a single base mutation adjacent to an S1 hypersensitive site 5' to the A gamma globin gene

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