Linkage Analysis of Nondeletion Hereditary Persistence of Fetal Hemoglobin

Abstract. *Nondeletion forms of hereditary persistence of fetal hemoglobin may result from regulatory disorders of globin gene expression. The defects in two such conditions were localized by demonstrating a tight genetic linkage between the disorders and polymorphic restriction endonuclease sites within the β-like globin gene complex. In one instance, the defect probably occurred outside the region of DNA between the ε- and β-globin genes.*

The genes that determine the structure of the non-α-like chains of human embryonic, fetal, and adult hemoglobin lie in a linked cluster on chromosome 11 in the order 5′-ε-^{G}γ-^{A}γ-δ-β-3′ (*1*). To learn more about the regulation of their expression during development, the molecular basis for the group of conditions called hereditary persistence of fetal hemoglobin (HPFH) in which hemoglobin F (Hb F:$\alpha_2{}^{G}\gamma_2$, $\alpha_2{}^{A}\gamma_2$) synthesis persists into adult life (*2*) has been examined. Some forms of HPFH result from deletions within the γ-δ-β globin gene complex, but so far their size and position have shown no obvious correlation with persistent γ-globin gene activity (*3–5*). In the homozygous state, these forms of HPFH are characterized by poorly hemoglobinized red cells and imbalanced globin chain synthesis (*6*), indicating that they are of limited value as models for studying the physiological regulation of Hb F production.

However, there are other forms of HPFH in which there are no detectable gene deletions, the hematological findings are completely normal, and, as in normal development, γ- and β-globin chain production is regulated so that there is no globin chain imbalance (*2*). Progress in defining the molecular basis of these nondeletion forms of HPFH has been hampered by the lack of information about the chromosomal location of their determinants in relation to the γ-δ-β globin gene complex. By using restriction endonuclease site polymorphisms around and within this complex, we demonstrated linkage between the determinants for two different forms of nondeletion HPFH and the β-like globin gene family and in one case showed that it probably lies outside of the region between the ε- and β-globin genes.

The human γ-globin genes are polymorphic for a single Hind III restriction enzyme site in the large intervening sequence of each gene (*7*). Either the ^{G}γ- or ^{A}γ-globin genes, or both, may contain the cleavage site, generating four different chromosomal combinations of polymorphic sites in the linked ^{G}γ- and ^{A}γ-globin genes. If the presence of the site is denoted by + and its absence by −, the four combinations are ++, −−, +−, and −+, although the fourth combination has not been observed to date. These haplotypes, if different, can be used as markers to differentiate the two homologous chromosomes carrying the β-globin genes. A third polymorphism occurs at a Bam HI cleavage site 8.3 kilobase pairs (kbp) to the 3′ side of the β-globin gene (*8*), and a fourth at a Hinc II site 0.5 kbp to the 5′ side of the ε-globin gene (*9*). These restriction enzyme site polymorphisms were studied in two families with nondeletion HPFH.

Several varieties of nondeletion HPFH have been defined by the level and structure of the Hb F in heterozygotes. One family, with "British type" of HPFH, has homozygotes for heterocellular HPFH; each has approximately 20 percent Hb F (containing 10 percent ^{G}γ- and 90 percent ^{A}γ-globin chains), the remaining hemoglobins consisting of A and A_2 (*10*). Thus ^{G}γ-, ^{A}γ-, δ-, and β-globin genes are all expressed in the red cells of these adults. Their normal red cell indices and balanced synthesis of α- and non-α-globin chains indicate that the overall output directed by the β-like globin gene complex is normal and that the increased γ-globin chain production is balanced by a reciprocal decrease in β-globin chain synthesis (*10*). Restriction enzyme analysis has revealed a completely normal organization of this gene complex (*11*). There are no globin chain markers in this family to determine whether the HPFH phenotype results from a determinant linked to the globin genes. However, Hind III digests of DNA from 16 family members indicated that three different haplotypes were segregating. In 12 individuals, the haplotypes could be deduced unequivocally from the restriction fragment pattern (Fig. 1). In the other four individuals, the observed pattern of G+−/A+− could have resulted from either a (++/−−) or (+−/−+) combination. Inspection of the pedigree allowed the former combination to be inferred in two cases. The HPFH homozygotes all had the (−−/−−) pattern, and each of the heterozygotes carried a (−,−) haplotype, including the parents (the father's combination −−/+− could be inferred from the pedigree). The complete matching of the HPFH phenotype with the (−−) Hind III haplotype is strong evidence that the two are linked. Indeed, subjecting the pedigree to linkage analysis [using the LIPED

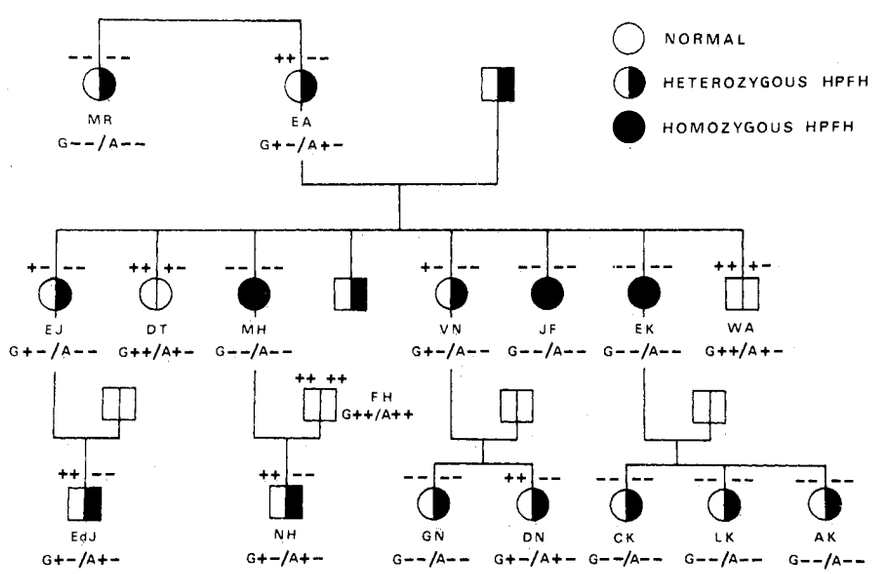

Fig. 1. Pedigree of the family with the British type of nondeletion HPFH. DNA was isolated from peripheral blood samples by phenol extraction (*17*) and digested with the restriction endonuclease Hind III. Digested DNA was fractionated by agarose gel electrophoresis, transferred to a nitrocellulose filter, hybridized to a ^{32}P-labeled γ-globin complementary DNA (cDNA) plasmid JW151 (*18*) and subjected to autoradiography (*19*). Hind III digestion of DNA-generated ^{G}γ-globin fragments of either 8.0 kbp (G−) or 7.2 kbp (G+) and ^{A}γ-globin fragments of 3.5 kbp (A−) or 2.7 kbp (A+). The observed Hind III fragment pattern is detailed below each family member and the Hind III polymorphism haplotypes deduced from each pattern are detailed above each family member.

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program (*12*)] produced an lod score of 2.28 at a recombination fraction (θ) of 0.06, indicating tight linkage.

In the second family (Indian), segregation of the genes for hemoglobin S (Hb S) (β^S) and β-thalassemia as well as a form of nondeletion HPFH ("Swiss type") associated with small increases in Hb F (containing both $^G\gamma$ and $^A\gamma$ chains) suggested that the HPFH determinant might be linked to the β^S-globin gene (*13*). Of the nine offspring, one (KU), an apparent recombinant, had inherited the β^S-globin gene without the HPFH phenotype. Restriction enzyme analysis with Hind III (Fig. 2) in this family, in which the parents had different haplotypes, confirmed the genetic interpretation obtained by hemoglobin analysis. The father was also heterozygous for both the Bam HI and the Hinc II polymorphisms (Fig. 2). Inspection of the pedigree showed that the Bam HI site was absent (−) and the Hinc II site present (+) in the chromosome carrying the β^S-globin gene. Thus, in the father, four independent markers around the globin gene complex span a distance of approximately 50 kbp. Their arrangement on the two chromosomes is $Hinc^+$-$^A\gamma^H$-β^S-Bam^- and $Hinc^-$-$^A\gamma^{H+}$-β^A-Bam^+. If the HPFH gene, which appears to be linked to the β^S-globin gene in this family, lies within the area of DNA spanned by these markers, recombination between this gene and the β^S-globin gene would produce a chromosome with a new arrangement of the markers. But analysis of KU showed that he had the paternal combination of $Hinc^+$-$^A\gamma^H$-β^S-Bam^-; that is, this portion of the chromosome was inherited intact (*14*). The possibility that recombination occurred between the $^A\gamma$- and β-globin loci in both maternal and paternal meioses to generate the observed pattern is excluded by the presence of the polymorphic Hind III site in both $^G\gamma$-globin genes in this individual. Thus the linked HPFH gene must lie either on the 5′ side of the ε-globin gene or on the 3′ side of the polymorphic Bam HI site. This type of analysis cannot exclude the possibility that KU has retained the HPFH determinant, but that its expression has been modified or totally repressed. However, the stability of the phenotype in KU over several years, as measured by alkali denaturation and F cell numbers, suggests that the loss of expression was not a transient alteration or due to analytical error. Similar analyses of other families with apparent recombinants may help to further clarify this situation.

Provisional genetic studies had suggested a recombination rate between the β-globin locus and the Swiss HPFH determinant of about 10 percent (*15*), which corresponds to locations approximately 10^7 bases apart in molecular terms. That analysis was based on phenotypes and may have included mixed genotypes, some of which were linked and some unlinked to the globin genes. However, other evidence supports the linkage of determinants of Hb F levels to the β-globin locus (*16*). Our findings provide unequivocal evidence for the linkage of two different nondeletion HPFH determinants to the β-like globin gene cluster. The application of these techniques to other families, together with the discovery of further restriction enzyme site polymorphisms around this cluster, should allow a more precise localization of the HPFH determinants and lead to the understanding of their molecular basis. The persistent synthesis of even the small amount of Hb F that occurs in the forms of HPFH we described is sufficient to alleviate both sickle cell anemia and homozygous β-thalassemia (*13*).

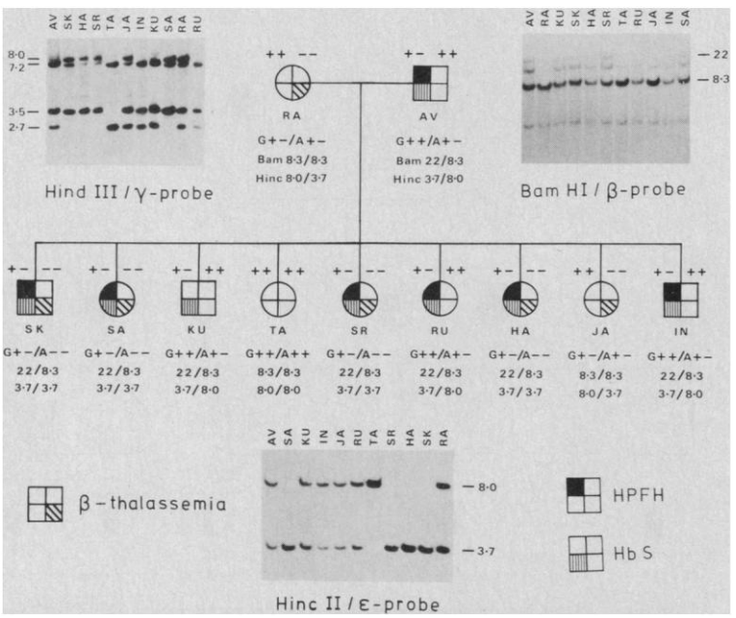

Fig. 2. Pedigree of the family with the Swiss type of nondeletion HPFH, Hb S, and β-thalassemia. Hind III–digested DNA was hybridized to a γ-cDNA probe (JW151), Bam HI–digested DNA to a β probe (a subcloned Pst I β-genomic fragment in the plasmid pBR322), and Hinc II–digested DNA to an ε probe (pε1.3, a subcloned Bam HI Eco RI ε-genomic fragment in pBR322), as described in Fig. 1. The autoradiograph of each digest is shown. Hinc II digestion of DNA-generated ε-globin gene fragments of 8.0 kbp (−) or 3.7 kbp (+) and Bam HI digestion generated β-globin gene fragments of 1.8 kbp (not shown) and either 8.3 (+) or 22 kbp (−). The β probe also cross-hybridized to δ-globin gene fragments of 15.5 and 4.4 kbp.

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14. This was confirmed further by the mapping of two newly discovered Hinc II polymorphisms around the ψβ1 gene (*9*). The β^S chromosome of KU was ++, the same as the HPFH chromosome of AV. The normal chromosome of AV was −+; the normal chromosome of RA was −+ and the β-thalassemia chromosome of RA was −−. Thus the final haplotypes for the polymorphisms on the 5′ side of the β gene (ε-$^G\gamma$-$^A\gamma$-ψβ-3′ψβ) for AV were ++−++β^S and −+++−+β^A; and for RA were +−−−−β-thalassemia and −+++−+B^A.
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