**Italian Type of Deletional Hereditary Persistence of Fetal Hemoglobin**


We report a new type of deletion of the β globin gene cluster in the Italian population that confers a phenotype of hereditary persistence of fetal hemoglobin (HPFH) to the carriers. This deletion begins ~5 kilobases (kb) 5' to the δ globin gene and ends ~30 kb 3' to the β globin gene, in close proximity to the 3' end of an Indian HPFH. In all four previously described HPFH types, a repetitive Alu I region 5' to the δ globin gene is largely or completely deleted; the 5' end of the new HPFH is consistent with this common feature. In addition, the finding that Italian and Indian HPFHs, as well as reported for other groups of deletions, have very close 3' ends, strengthens the idea that common mechanisms may operate in generating these deletions. Finally, we show that, in spite of similar 5' breakpoints, the deletion of Spanish δβ-thalassemia is at least 8 kb longer than that of Negro HPFH type I, thus ruling out the hypothesis that the overall extent of the deletion might influence the level of γ globin chain synthesis.

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HEREDITARY persistence of fetal hemoglobin (HPFH) and δβ-thalassemia are inherited disorders characterized by the persistent synthesis of fetal hemoglobin (α2γ2) during adult life. Patients with HPFH show a higher level of γ chain synthesis, an almost balanced ratio of α/ non-α globin chain synthesis, and only slight red cell abnormalities; on the contrary, δβ-thalassemia patients have a lower level of γ globin synthesis and a more pronounced thalassemic phenotype. In the last years, these rare conditions have been extensively investigated at the molecular level to provide better understanding of the physiology of the hemoglobin switch.

A large group of HPFH and δβ-thalassemias is characterized by extensive deletions in the β-like globin gene cluster. Molecular studies of these conditions have led to two main groups of hypotheses to explain the different level of γ chain synthesis observed in these syndromes. First, γ gene expression may be influenced by the overall extent of the deletion; larger deletions may generate HPFH, and smaller ones may generate δβ-thalassemia by disrupting the normal chromatin structure in different ways. Second, a deletion may be effective either by removing DNA regions or by bringing new sequences to the globin gene region. The loss or addition of specific sequences in the non-α globin gene cluster may be relevant to the level of expression of γ globin genes. For example, in Sicilian δβ-thalassemia, ~6 kilobase (kb) pairs from the β globin gene and 3' to it are additionally lost in comparison with the deletion causing β0-thalassemia, resulting in higher expressions of γ globin genes. On the other hand, a small region containing a pair of inverted Alu I repeats located 5' to the δ globin gene area is consistently deleted in HPFH but not in GA, δβ-thalassemia (Spanish type). These data could suggest that loss of specific DNA regions either 3' or 5' to the β globin cluster may influence the level of γ globin gene expression; on the other hand, these same deletions have the potential of bringing "enhancer" sequences, originally situated at some distance, in proximity to the γ globin genes. We have identified a new deletion generating HPFH in three clearly unrelated Italian families. The 5' endpoint of this deletion maps in close proximity to the Alu I region 5' to the δ globin gene, where two different deletions causing Negro HPFH type I and Spanish δβ-thalassemia begin. On the contrary, the 3' endpoint of the new deletion is closer to the β globin gene than are those observed in the above two conditions and is very similar to that observed in an Indian HPFH.

MATERIALS AND METHODS

Nine subjects belonging to three different families from Southern Italy were studied. Hematological data and RBC indices were obtained by standard procedures. Hb A2 levels, Hb F percentage, globin chain synthesis, and the relative proportion of G, and A, chains in the Hb F were determined as previously described. DNA was obtained from subjects A-I, B-I, and C-II (see Table 1). DNA preparation and digestion, electrophoretic separation of DNA fragments, transfer to nitrocellulose filters, hybridization, and washing procedures were as previously described. The following probes were used: genomic fragments corresponding to the β, δ, and γ globin genes; the RIH probe, a 0.5 kb genomic fragment ~4 kb 5' to the δ globin gene; the RK 29 probe, a 1.2 kb fragment and the 3' endpoint of the Negro HPFH type I deletion; the 5' probe, a 0.8 kb fragment and the 3' endpoint of the Negro HPFH type I deletion; the 7.6 probe, a 0.8 kb fragment and the 3' endpoint of the Negro HPFH type I deletion; a 0.5 Hind III unique fragment ~25 kb 5' to the 3' endpoint of Negro HPFH type I deletion, and a 0.75 kb HinI/EcoRI fragment derived from a plasmid containing a 1.1 kb Bam HI/Bgl II genomic fragment, obtained from the p3'N1OR plasmid (D. Mager, personal communication, 1986) and a 0.75 kb Hind III/EcoRI fragment derived from a plasmid containing a 1.1 kb Bam HI/Bgl II genomic fragment, obtained from the 3' end of an Indian HPFH deletion and mapping at ~30 kb 3' to the β globin gene (Fig. 1).

RESULTS

Italian patients with HPFH have almost normal Hb, MCV, and MCH values (Table 1). Except for patient B-I, who showed 14% Hb F, all the other subjects had Hb F values ranging between 21 and 30% with a pancellular distribution and G,/G, + A, ratios of 0.31 to 0.39 (mean 0.35). Hb A, was slightly decreased in all cases. Globin chain synthesis always gave unbalanced β/α ratios, but almost...
Fig. 1. Comparison of the extent of specific deletions in the $\beta$-globin gene cluster causing $G_{\alpha}$ (a hereditary persistence of fetal hemoglobin [HPFH]) and $\delta$-thalassemia. The restriction maps are derived from refs. 12 and 19. Probes used in this study are indicated above the DNA map. Short arrows indicate polarity of the $\delta$ 2 primer site, the $\delta$ 1 site is marked by a blank bar. Shaded areas indicate uncertainties about the precise location of the breakpoints. $\bullet$ Bam H1; $\Delta$ Ap I; Hpa I restriction sites. The $\delta$ 2 and $\delta$ 3 sites are polymorphic in normal DNA.
### Table 1. Hematological Data of Carriers of Italian HPFH

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<th>Case</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Hb (g/dl)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>Hb A₂ (%)</th>
<th>Hb F (%)</th>
<th>β/α (cpm)</th>
<th>β + γ/α (cpm)</th>
<th>G₆/G₄ + A₄*</th>
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</table>

*G₆/G₄ + A₄* are obtained by isoelectric focusing separation.

HPFH, hereditary persistence of fetal hemoglobin.

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**Fig 2.** Analysis of the DNA restriction fragments in an Italian hereditary persistence of fetal hemoglobin (HPFH) heterozygous carrier (H) and a normal control (C) with the (A) RIH, (B) ψβ, and (C) 0.75-kb probes.
normal $\beta + \gamma/\alpha$ ratios, due to the high level of $\gamma$ chain production.

Individuals from all three families were studied by Southern blot techniques. Digestion with several restriction enzymes and hybridization to the $\beta$ and $\delta$ probes did not show any abnormal fragments, ruling out the previously described Sicilian $\delta^\alpha$-thalassemia\(^5\); on the other hand, the low intensity of the normal bands suggested complete deletion of the $\delta^\beta$ globin cluster on the HPFH chromosome (not shown).

Using the RIH probe, digestion with $Pst$ I, $Bgl$ II, $Hinc$ II, $Bam$ HI, and $Hind$ III (Fig 2A and Table 2), in addition to normal fragments, gave abnormal bands usually of lower intensity as compared with the normal ones. The same abnormal fragments (with the exception of the $Bgl$ II digest) could be visualized using a 4.2-kb $Bgl$ II $\psi\delta$ probe, although in this case the intensity of the abnormal band was comparable to that of the normal one (not shown). In addition, the $\psi\beta$ probe demonstrated an abnormally high-mol-wt $Eco$RI fragment that we were unable to detect clearly with RIH (Fig 2B and Table 2). These data suggest that the $Eco$RI site immediately 3' to the RIH region may be missing, and that the deletion begins somewhere within the 500-bp RIH sequence, thus explaining the faintness of the abnormal fragments obtained with this probe. As will be shown later, the same abnormal $Eco$RI fragment is detectable with a probe mapping to the 3' end point of the deletion.

To map the 3' endpoint of the deletion, six probes located at different distances from the 3' side of the $\beta$ globin gene were used ($p$RK29, 0.5 $Hind$ III derived from the p3'Nior, 0.75-kb probe, HPFH 3E, HPFH 3D, and 7.6). The 0.75-kb fragment excised from the plasmid $Bam$ HI-$Bgl$ II 1.1, mapping immediately 3' to the deletion endpoint of an Indian HPFH\(^22\) ~30 kb 3' to the $\beta$ globin gene, demonstrated abnormal bands in $Eco$RI, $Hpa$ I, $Hinc$ II, $Xba$ I, and $Bgl$ II digest of HPFH DNA (Fig 2C and Table 2). Rehybridization...
tion of the same filters with the \( \psi \beta \) probe identified, as expected, the EcoRI, Hpa I, and Hinc II abnormal fragments with those observed with the 0.75-kb probe. The Bgl II abnormal fragment comigrated with the one detected by the RHI probe. However, as already reported for the EcoRI digestion, with the RHI probe we were unable to find the abnormal Xba I fragment clearly detectable with the 0.75-kb probe.

Figure 3 shows restriction sites detectable in normal DNA with the 0.75-kb probe, and compares them with the map for the HPFH DNA. Assuming that the 5' end of the deletion lies somewhere within the 500 bp of the RHI region, the length of the abnormal Hind III, Eco RI and Bgl II abnormal fragments places the 3' end of the deletion at ~2 kb 5' to the 0.75-kb probe.

The 5' endpoint of a deletion found in Spanish \( \delta \beta \)-thalassemia is close to the one observed in our HPFH and Negro HPFH type I; it was of interest to investigate if the 3' endpoint in this condition was in close proximity to that found in these HPFHs. DNA from a homozygous patient with Spanish \( \delta \beta \)-thalassemia does not hybridize to probes HPFH 3D and to probe 7.6, although a \( \psi \beta \) probe clearly detects the region 5' to the breakpoint when hybridized to the same filter (data not shown).

A comparison of the restriction enzyme map of Spanish \( \delta \beta \)-thalassemia with the map of cloned DNA from the region corresponding to the 3' breakpoint in Negro HPFH type I indicates that the deletion in Spanish \( \delta \beta \)-thalassemia must extend at least 2.0 kb 3' to the 7.6 probe, as shown by the lack of concordance between Pst I and Bgl II sites 3' to the 7.6 in normal DNA and sites 3' to the breakpoint in Spanish \( \delta \beta \)-thalassemia DNA (Fig 4). We cannot exclude however, that the Hind III and Bgl II sites at the 3' end of the normal map may correspond to similar sites in Spanish \( \delta \beta \)-thalassemia DNA. Unfortunately, a DNA fragment derived from the 3' site of the breakpoint in Spanish \( \delta \beta \)-thalassemia shows the presence of repetitive sequences, which precludes its use in genomic blotting studies.

**DISCUSSION**

The data reported in this paper are relevant to models proposed to interpret different levels of \( \gamma \) chain synthesis in HPFH and \( \delta \beta \)-thalassemia on the basis of different deletions. Analysis of the DNA from Italian HPFH carriers reveals that the deletion begins immediately 5' to the two Alu I repeats upstream of the \( \delta \) gene, ending ~30 kb 3' to the \( \beta \) globin gene.

Of the three hypotheses outlined in this report, at least one can now be ruled out by our data; Fig 1, which compares several deletions causing G,A, HPFH and \( \delta \beta \)-thalassemias, clearly indicates that the overall extent of the deletion is not related to the phenotype, as deletions causing \( \delta \beta \)-thalassemia may be both longer (Spanish \( \delta \beta \)-thalassemia, present article) and shorter (Sicilian \( \delta \beta \)-thalassemia) than are several HPFHs.

An additional hypothesis suggests that an enhancer sequence (or any other elements capable of modifying gene expression), originally located at some distance 3' to the \( \beta \) globin gene region, may be brought into the \( \gamma \) globin region by the deletion. Any such sequence existing 3' to the Negro HPFH type I breakpoint would have the potential to stimulate \( \gamma \) globin gene expression in Negro HPFH type I and II and would be lost in Spanish \( \delta \beta \)-thalassemia. It is difficult to conceive, however, that the same element would be responsible for the HPFH phenotype in our case as well as in Indian HPFH, because their 3' breakpoints are >30 kb away from the region that might contain this element. Nevertheless, the remarkable coincidence of pairs of 3' endpoints of different HPFH (Indian and Italian, HPFH I and II) may suggest that an additional element capable of modifying gene expression could exist immediately 3' to the Indian–Italian HPFH endpoint. In conclusion, plural mechanisms rather than a single mechanism may be required to explain all the different phenotypes.

An alternative hypothesis focuses attention on the 5' endpoint of the deletions. With the present case, five different deletion-type HPFH syndromes are now known (four G,A, HPFH plus Hb Kenya), all of which largely or completely remove, in addition to parts of the \( \beta \) globin cluster, an Alu region ~4 kb 5' to the \( \delta \) globin gene. Although it is clear that persistent expression of \( \gamma \) globin genes does not require deletion of this region (as in various \( \delta \beta \)-thalassemias), it has been proposed that the additional loss of these sequences may raise \( \gamma \) globin expression to the high levels observed in HPFH. This idea is not contradicted by our new HPFH; however, two recently reported cases show that certain deletions including the Alu region do not necessarily increase \( \gamma \) globin expression to very high levels. A Japanese \( \delta \beta \)-thalassemia was recently reported in which Hb F production was low (5% to 12%) in spite of the deletion of
ITALIAN DELETIONAL HPFH

the Alu sequences. It was remarked, however, that in this case the deletion extended in close proximity to the γ globin gene domain, possibly affecting its expression, particularly that of the Aγ globin gene. In addition, it is now clear that the simple deletion of the Alu region not including the whole δβ globin cluster in a type of δβ-thalassemia does not in itself significantly increase the Hb F level, at least in the heterozygotes; a homozygote for this type of δβ-thalassemia shows an exceptionally high level of Hb F production, however.13

Both the overall lengths of the deletion in Italian and Indian HPFH are very similar, with their 5' and 3' ends being roughly in the same regions. A similar situation has been described for the Negro HPFH I and II and on the basis of these and other similar findings, Vanin and colleagues proposed a model for explaining the mechanism of these deletions, suggesting that distant sequences may be brought into proximity during DNA replication, generating a loop that occasionally may be excised. Our findings further support the idea that common mechanisms may operate in generating specific classes of deletions.

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