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, 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 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 (αγγγ) during adult life. Patients with HPFH show a higher level of γ chain synthesis, an almost balanced ratio of α/γ 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.1 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.2-9 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.10 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 Hb Lepore, resulting in higher expressions of γ globin genes.11-12 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 HPFH10 but not in G,A,δβ-thalassemia (Spanish type).9 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.21 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 I1 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.22

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 Gγ and A2, chains in the Hb F were determined as previously described.23 DNA was obtained from subjects A II-1, B I-1, and C II-2 (see Table 1). DNA preparation and digestion, electrophoretic separation of DNA fragments, transfer to nitrocellulose filters, hybridization, and washing procedures were as previously described.11 The following probes were used: genomic fragments corresponding to the β, δ, and γβ globin genes; the RH probe, a 0.5-kb genomic fragment ~4 kb 5' to the δ globin gene24; the RK 29 probe, a 1.2-kb fragment ~18 kb from the 3' side of the β globin gene;25 the HPFH-3D and HPFH-3E probes, two genomic fragments from the 3' endpoint of the Negro HPFH type I deletion26; the 7.6 probe, a 0.8-kb fragment ~6 kb 3' to the deletion endpoint of Negro HPFH type I27; a 0.5 Hind III unique fragment ~25 kb 5' to the 3' endpoint of Negro HPFH type I, obtained from the p35N10R plasmid (D. Mager, personal communication, 1986) and a 0.75 kb Hind I-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 gene27 (Fig 1).

RESULTS

Italian patients with HPFH have almost normal Hb, MCV, and MCH values (Table 1). Except for patient B I-1, who showed 14% Hb F, all the other subjects had Hb F values ranging between 21 and 30% with a pancellular distribution and Gγ/Gδ + A2 ratio of 0.31 to 0.39 (mean 0.35). Hb A2 was slightly decreased in all cases. Globin chain synthesis always gave unbalanced β/α ratios, but almost
Fig 1. Comparison of the extent of several deletions in the β globin gene cluster causing G. A, hereditary persistence of fetal hemoglobin (HPFH) and δβ-thalassemias. The restriction map of the region 3′ to the β globin gene cluster was derived from refs. 12 and 19. Probes used in this study are indicated above the DNA map. Short arrows indicate the polarity of the Alu I repeats. The region indicated by a question mark is of unknown length. Extensions of the different deletions are indicated by black bars. Shaded areas indicate uncertainties about the precise location of the breakpoints. φ, Bam HI; †, EcoRI; Δ, Hpa I restriction sites. The Hpa I and Bam HI sites marked by asterisks are polymorphic in normal DNA.
### Table 1. Hematological Data of Carriers of Italian HPFH

<table>
<thead>
<tr>
<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 A1 (%)</th>
<th>Hb F (%)</th>
<th>G-γ/γ + A, (cpm)</th>
<th>β + γ/γ + A, (cpm)</th>
<th>G,-/G, + A, + A,</th>
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<tbody>
<tr>
<td>Family A</td>
<td></td>
<td></td>
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<tr>
<td>I.1</td>
<td>F</td>
<td>30</td>
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<td>25</td>
<td>1.8</td>
<td>21</td>
<td>0.63</td>
<td>0.86</td>
<td>0.31</td>
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<tr>
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<td>F</td>
<td>29</td>
<td>13.7</td>
<td>79</td>
<td>25</td>
<td>1.8</td>
<td>14</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>II.1</td>
<td>F</td>
<td>5</td>
<td>12.2</td>
<td>76</td>
<td>24</td>
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<td>F</td>
<td>3</td>
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<td>25</td>
<td>1.9</td>
<td>22</td>
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<td>—</td>
<td>0.39</td>
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<tr>
<td>I.1</td>
<td>M</td>
<td>52</td>
<td>13.4</td>
<td>74</td>
<td>28</td>
<td>1.9</td>
<td>30</td>
<td>0.59</td>
<td>0.90</td>
<td>—</td>
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<tr>
<td>I.3</td>
<td>F</td>
<td>48</td>
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<td>84</td>
<td>27</td>
<td>1.8</td>
<td>30</td>
<td>—</td>
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</tbody>
</table>

*G,-/G, + A, + A, are obtained by isoelectric focusing separation.*

HPFH, hereditary persistence of fetal hemoglobin.

**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 $\delta^S$-thalassemia: on the other hand, the low intensity of the normal bands suggested complete deletion of the $\delta$ 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 $\psi_b$ probe, although in this case the intensity of the abnormal band was comparable to that of the normal one (not shown). In addition, the $\psi_b$ probe demonstrated an abnormally high-mol-wt EcoRI fragment that we were unable to detect clearly with RIH (Fig 2B and Table 2). These data suggest that the EcoRI 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 EcoRI 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 (pRK29, 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 EcoRI, Hpa I, Hinc II, Xba I, and Bgl II digest of HPFH DNA (Fig 2C and Table 2). Rehybridization

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**Table 2. DNA From Italian HPFH Carriers and Normal Controls Digested With Different Restriction Enzymes and Hybridized With RIH, $\psi_b$ and 0.75-kb Probes**

<table>
<thead>
<tr>
<th>Probe</th>
<th>Enzyme</th>
<th>DNA Fragment Size, kb</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIH</td>
<td>Pst I</td>
<td>3.2  4.6</td>
</tr>
<tr>
<td></td>
<td>Bgl II</td>
<td>4.2  2.7</td>
</tr>
<tr>
<td></td>
<td>Hinc II</td>
<td>12.0 7.6†</td>
</tr>
<tr>
<td></td>
<td>Bam HI</td>
<td>14.0 15.0</td>
</tr>
<tr>
<td></td>
<td>Hind III</td>
<td>9.0 17.0</td>
</tr>
<tr>
<td>$\psi_b$</td>
<td>Eco RI</td>
<td>13.6 7.2</td>
</tr>
<tr>
<td></td>
<td>Hpa I</td>
<td>17.6 14.0</td>
</tr>
<tr>
<td>0.75</td>
<td>Eco RI</td>
<td>13.6 9.0</td>
</tr>
<tr>
<td></td>
<td>Hpa I</td>
<td>17.6 26.0</td>
</tr>
<tr>
<td></td>
<td>Hinc II</td>
<td>12.0 11.5</td>
</tr>
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<td></td>
<td>Xba I</td>
<td>8.0 10.5</td>
</tr>
<tr>
<td></td>
<td>Bgl II</td>
<td>4.2 6.2</td>
</tr>
</tbody>
</table>

*Only the size of the abnormal fragments is reported.
†The two Hinc II sites 5' to the Alu region are polymorphic in normal DNA, generating with the RIH probe fragments of different length (1.6, 4.6, and 7.6 kb, respectively).

HPFH, hereditary persistence of fetal hemoglobin.

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![Fig 3. Map of the regions corresponding to the 5' and 3' breakpoints of the Italian hereditary persistence of fetal hemoglobin (HPFH) deletion, in normal (a) and in HPFH (b) chromosomes. The position of the restriction sites of the region corresponding to the 0.75-kb probe was derived partially from ref. 22 (Eco RI, Bam HI, and Hpa I sites). Ba, Bam HI; Hp, Hpa I; Hd, Hind III; H, Hinc II; B, Bgl II; E, EcoRI; P, Pst I; X, Xba I. The Pst I abnormal site marked with a double asterisk (detected by the RIH and not visible with the 0.75-kb probe) was precisely mapped by Bgl II-Pst I and Xba I-Pst I double digestions (data not shown). The Hinc II sites indicated by a single asterisk are polymorphic in normal DNA. The position of the 3' Hinc II site on the HPFH chromosome was obtained by Bam HI–Hinc II double digestion and hybridization with the 0.75-kb probe; the 5' Hinc II site was deduced on the basis of the length of the Hinc II abnormal fragment. Data are consistent with the absence of both Hinc II polymorphic sites in the HPFH DNA.](https://www.bloodjournal.org/content/649/3/1099/F2.large.jpg)
tion of the same filters with the ψβ 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 RIH probe. However, as already reported for the EcoRI digestion, with the RIH 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 RIH 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 δβ-thalassemia is close to 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 δβ-thalassemia does not hybridize to probes HPFH 3D and to probe 7.6, although a ψβ 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 δβ-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 δβ-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 δβ-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 δβ-thalassemia DNA. Unfortunately, a DNA fragment derived from the 3' site of the breakpoint in Spanish δβ-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 γ chain synthesis in HPFH and δβ-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 δ gene, ending ~30 kb 3' to the β 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 δβ-thalassemias, clearly indicates that the overall extent of the deletion is not related to the phenotype, as deletions causing δβ-thalassemia may be both longer (Spanish δβ-thalassemia, present article) and shorter (Sicilian δβ-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 β globin gene region, may be brought into the γ globin region by the deletion.13 Any such sequence existing 3' to the Negro HPFH type I breakpoint would have the potential to stimulate γ globin gene expression in Negro HPFH type I and II and would be lost in Spanish δβ-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 β globin cluster, an Alu region ~4 kb 5' to the δ globin gene. Although it is clear that persistent expression of γ globin genes does not require deletion of this region (as in various δβ-thalassemias), it has been proposed that the additional loss of these sequences may raise γ globin expression to the high levels observed in HPFH.9,10 This idea is not contradicted by our new HPFH; however, two recently reported cases15,16 show that certain deletions including the Alu region do not necessarily increase γ globin expression to very high levels. A Japanese δβ-thalassemia was recently reported in which Hb F production was low (5% to 12%) in spite of the deletion of

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Fig 4. Comparison of the restriction enzyme sites 3' to the breakpoint in Negro hereditary persistence of fetal hemoglobin (HPFH) type I and Spanish δβ-thalassemia. The map for HPFH was derived from the normal and HPFH DNA clones in ref. 12. The δβ-thalassemia map is taken from refs. 9 and 11. Probes used in the present experiment are indicated. Alu I sequences in Spanish δβ-thalassemia DNA are indicated by a solid square and rectangle (5' and 3' Alu repeat), respectively. B, Bgl II: X, Xba I; E, EcoRI; Ba, Bam HI; H, Hind III; P, Pst I.

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ITALIAN DELETIONAL HPFH

the Alu sequences.16 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.17

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 colleagues18 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.

ACKNOWLEDGMENT

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Italian type of deletional hereditary persistence of fetal hemoglobin

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