**Rapid Detection of Spanish (δβ)\(^{-}\)-Thalassemia Deletion by Polymerase Chain Reaction**

By J.L. Vives-Corrons, M.A. Pujades, A. Miguel-García, A. Miguel-Sosa, and S. Cambiazzo

δβ-Thalassemia and hereditary persistence of fetal hemoglobin (HPFH) are inherited disorders characterized by the persistent synthesis of fetal hemoglobin (HbF) during adult life. The Spanish type of δβ-thalassemia is a mild thalassemic condition due to a large deletion starting at the Alu I repeat between the A\(_{\text{δ}}\) and δ-globin genes immediately 3’ to the RIH probe and extending 11 and 17 kb downstream of the 3’ endpoints of HPFH 1 and HPFH 2, respectively. Using probes from the Spanish (δβ)\(^{-}\)-thalassemic DNA, the 3’ breakpoint region has been mapped to a point approximately 8.5 to 9.0 kb downstream from that of HPFH type 1 and, as we know the restriction sites 3’ to this breakpoint, the presence of the deletion can be identified with the polymerase chain reaction (PCR). In the present study, a PCR method using three specific oligonucleotides has been developed for the identification of the Spanish (δβ)\(^{-}\)-thalassemia in 100 patients with δβ-thalassemia (99 heterozygotes with mild anemia, decreased mean corpuscular volume, and 5% to 15% HbF, and one homozygote with 100% HbF and thalassemia intermedia phenotype). We conclude that the finding of the Spanish type of (δβ)\(^{-}\)-thalassemia in all the patients studied here suggests Spain as the most probable origin of this thalassemic phenotype. Moreover, the amplification of the fragment encompassing the deletion junction and normal sequence is useful for the rapid molecular detection of Spanish (δβ)\(^{-}\)-thalassemia. © 1992 by The American Society of Hematology.

**MATERIAL AND METHODS**

Ninety-nine δβ-thalassemia carriers belonging to 41 unrelated families with HbF levels ranging from 3.5% to 10.5% and with normal or decreased HbA\(_2\) were identified during survey studies performed in Spain. Forty of these patients were studied, before the standardization of the PCR method, by restriction endonuclease digestion of genomic DNA and blot hybridization (Southern blot method) and tested later by PCR. The remaining 59 patients were only tested by the PCR method. For comparison purposes, a patient with homozygous δβ-thalassemia was also included in the study.

Basic hematologic parameters were obtained by the Technicon H\(^{+}\)2 System (Bayer Diagnostics). HbA\(_2\) levels were calculated by microchromatography and HbF percentage by alkali denaturation.

Restriction endonuclease mapping of DNA. DNA was prepared from peripheral blooduffy coat by phenol-chloroform extraction and digested with appropriate restriction enzymes (BamHI, Bgl II, Pvu I, EcoRI, HindIII, and Pst I). According to the manufacturer’s recommendations, DNA fragments were separated on agarose gel (0.8% to 1.2%) and transferred to nitrocellulose filters. The filters were hybridized to the genomic probes corresponding to the A\(_{\text{δ}}\), a 2.7-kb fragment of EcoRI containing the A\(_{\text{δ}}\) gene, pseudo-β gene (a 1.8-kb fragment of Bgl II/Xho I), and HBS (a 4.4-kb fragment of Pst I/HindIII containing the β gene). The DNA probe for Spanish (δβ)\(^{-}\)-thalassemia was kindly supplied by the INSERM (Hopital Henry Mondor, Paris, France).

PCR method. PCR reaction of the 3’ breakpoint region was performed using Taq I DNA polymerase and three specific oligonucleotide primers obtained from normal 5’ DNA sequence within RIH region (N\(_1\)), normal 3’ DNA complementary sequence (N\(_2\)), and DNA complementary sequence to the Spanish (δβ)\(^{-}\)-thalassemic 3’ breakpoint region (Sp98) (Fig 2). The DNA se-
sequences of the three oligonucleotides N1, N2, and Spsp used in the present study are: N1, 5'-ATG GGT ATT TCA CIT GTT AT-3'; N2, 5'-ACT TTG TCT GTT AAT TCC AA-3'; Spsp, 5'-ACT GTG GGA GCC CCT TTC TG-3'.

Amplification of normal DNA with N1 and N2 primers gives a single band of 685 bp, whereas in DNA with homozygous Spanish (δβ)⁺-thalassemia deletion, no bands are obtained by using N1 and N2 primers and a single fragment of 299 bp is obtained with N1 and Spsp oligonucleotides.10

RESULTS

Hematologic findings. The results (mean ± SD) relative to the 99 subjects heterozygous for (δβ)⁺-thalassemia are shown in Table 1. These values were compared with those of the β-thalassemia trait and the α-thalassemia trait (homozygous and heterozygous) also obtained in our laboratory. The single case with homozygous Spanish (δβ)⁺-thalassemia showed the following hematologic data: RBC, 4.3 ± 0.7 x 10¹²/L; Hb, 109 ± 5 g/L; mean corpuscular volume (MCV), 80 ± 4 fl; RDW, 28.8%; reticulocytes, 6%; HbA₂, 0.65%; Hbf, 86.7%. The Kleihauer Test showed a homogenous pattern.

Southern blot analysis. Digestion of patients' DNA with HindIII and EcoRI restriction enzymes and hybridization to the Aᵦ genomic probe showed, in all cases, a normal pattern. The same pattern was obtained by digesting with BamHI enzyme and hybridizing with H8S. Hybridization with β probe provided normal fragments when PstI and BglI restriction enzymes were used, whereas abnormal bands appeared with PvuI and HindIII digestion, indicating that the 5' breakpoint of the deletion is situated between the 3' BglI II restriction site of the normal β gene and the 5' PvuI restriction site of the deleted δ gene.

PCR analysis. One fragment of 685 bp was obtained in all cases when the oligonucleotides N1 and N2 were used, and a second fragment of 299 bp was also apparent when the DNA amplification was performed in the presence of the N1 and Spsp oligonucleotides. In normal controls and in the case of homozygous Spanish (δβ)⁺-thalassemia (one case), only single fragments of 685 and 299 bp, respectively, were obtained (Fig 3).

DISCUSSION

Spanish (δβ)⁺-thalassemia is a mild thalassemic condition characterized by increased levels of Hbf production during adult life. It is known that the 5' breakpoint of the Spanish (δβ)⁺-thalassemia deletion lies within the AluI repeat, between the 4 and δ-globin genes immediately 3' to the RIH probe,11 and extends at 11 and 17 kb downstream to the 3' endpoints of HPFH-1 and HPFH-2, respectively (Fig 1). Up to now, only the Japanese (δβ)⁺-thalassemia deletion has been found to be at least 16 kb longer than the Spanish (δβ)⁺-thalassemia and its 3' breakpoint extends further downstream to the 3' end of the Spanish (δβ)⁺-thalassemia.12,13 Knowing the 3' restriction sites of this breakpoint has allowed us to identify the presence of the specific deletion by using the PCR.

In the present study, a PCR screening method for the Spanish (δβ)⁺-thalassemia deletion was undertaken in a

---

**Fig 1.** Map of the human β-globin gene cluster showing the location and extent of various deletions associated with HPFH and δ-thalassemia.

**Fig 2.** Sequence of normal DNA in the region corresponding to the 3' end of the deletion causing Spanish (δβ)⁺-thalassemia.
Table 1. Comparison of General Hematologic Data Between Patients With δβ-Thalassemia, Other Heterozygote Thalassemias, and Normal Controls

<table>
<thead>
<tr>
<th>Trait</th>
<th>δβ-Thal</th>
<th>β-Thal</th>
<th>α-Thal</th>
<th>α'-Thal</th>
<th>Normal Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (×10^12/L)</td>
<td>5.5 ± 0.7</td>
<td>5.8 ± 0.7</td>
<td>5.6 ± 0.5</td>
<td>5.1 ± 0.5</td>
<td>5.4 ± 1.5</td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>117 ± 12</td>
<td>123 ± 13</td>
<td>127 ± 12</td>
<td>137 ± 15</td>
<td>150 ± 20</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>67.4 ± 5.4</td>
<td>68 ± 6.6</td>
<td>72.4 ± 6.6</td>
<td>82.9 ± 5.6</td>
<td>90 ± 10</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>314 ± 32</td>
<td>313 ± 12</td>
<td>314 ± 14</td>
<td>324 ± 11</td>
<td>330 ± 20</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>18.6 ± 1.42</td>
<td>15 ± 1.2</td>
<td>14.7 ± 0.62</td>
<td>13.7 ± 0.89</td>
<td>13.7 ± 0.4</td>
</tr>
<tr>
<td>HDW (g/L)</td>
<td>31.4 ± 2.86</td>
<td>28.4 ± 2.9</td>
<td>26.8 ± 1.9</td>
<td>24.3 ± 2.25</td>
<td>24.8 ± 2.2</td>
</tr>
<tr>
<td>WBC (×10^9/L)</td>
<td>6.9 ± 1.9</td>
<td>7.0 ± 1.5</td>
<td>7.9 ± 3.2</td>
<td>7.7 ± 2.1</td>
<td>7.5 ± 3.5</td>
</tr>
<tr>
<td>Platelets (×10^11/L)</td>
<td>253 ± 65.6</td>
<td>253 ± 57</td>
<td>282 ± 56</td>
<td>246.5 ± 64.8</td>
<td>275 ± 125</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>1.73 ± 0.71</td>
<td>1.20 ± 0.60</td>
<td>0.98 ± 0.38</td>
<td>1.06 ± 0.44</td>
<td>1.25 ± 0.75</td>
</tr>
<tr>
<td>HbA2 (%)</td>
<td>2.75 ± 0.41</td>
<td>5.3 ± 1.8</td>
<td>2.6 ± 0.40</td>
<td>2.52 ± 0.33</td>
<td>2.25 ± 0.75</td>
</tr>
<tr>
<td>HbF (%)</td>
<td>6.7 ± 3.29</td>
<td>0.3 ± 0.10</td>
<td>0.58 ± 0.45</td>
<td>0.53 ± 0.36</td>
<td>≤2</td>
</tr>
<tr>
<td>No. of cases</td>
<td>99*</td>
<td>54</td>
<td>27</td>
<td>79</td>
<td>50</td>
</tr>
</tbody>
</table>

Abbreviations: WBC, white blood cell count.

*The results of the single case with homozygous Spanish (δβ)-thalassemia are not included here.

A large group of subjects with slightly increased HbF. Three different oligonucleotides were used: two (N1 and N2) for the normal DNA and two (N1 and SPδβ) for the thalassemia DNA. In normal subjects, the amplification of DNA in the presence of N1 and N2 provides a single fragment of 685 bp, whereas in homozygous Spanish (δβ)-thalassemia, a unique fragment of 299 bp is observed only if N1 and SPδβ oligonucleotides are used. Therefore, by using the three oligonucleotides, the genotype of heterozygous Spanish (δβ)-thalassemia is characterized by two different fragments of 685 bp and 299 bp, respectively. It is noteworthy that in the present study all the subjects with heterozygous δβ-thalassemia phenotype analyzed for the presence of the Spanish (δβ)-thalassemia deletion showed the presence of the 299-bp fragment, characteristic of this deletion. Because in all the cases the normal 685-bp fragment was also apparent when the N1 and N2 primers were used, it can be concluded that all the subjects were carriers for the Spanish (δβ)-thalassemia deletion.

Although δβ-thalassemia has been observed in different ethnic groups, it has been described mainly in subjects of Mediterranean extraction. In Spain, δβ-thalassemia is not a rare condition, and about 7% of thalassemia trait carriers are heterozygous for δβ-thalassemia. Spanish (δβ)-thalassemia was first described by Ottolenghi and Giglioni in 1982, but its incidence among the Spanish population is presently unknown. Except for the report of hematologic studies performed in homozygous Spanish (δβ)-thalassemia patients by Baiget et al. in 1983, there are no other references to this specific type of deletion. The present study performed on a large group of δβ-thalassemia carriers shows that Spanish (δβ)-thalassemia is the most frequent trait producing high HbF in Spain and contributes to a better definition of this type of thalassemia trait at both the hematologic and molecular levels. Furthermore, the fact that all of the patients studied here were from Spain suggests that the Spanish (δβ)-thalassemia may originate from that part of the world.

ACKNOWLEDGMENT

We are indebted to Drs Michel Vidaud and Nada Ganem for providing us with the oligonucleotides used for the PCR method and to Prof Goosens (Hôpital Henry Mondor, Paris, France) for the critical review of the manuscript.

![Fig 3. Three percent agarose electrophoresis of PCR performed in DNA samples of patients with Spanish (δβ)-thalassemia and in normal controls. M: molecular weight marker; lanes 1 and 7: normal controls; lanes 2, 3, 4, 6, 8, 9, and 10: heterozygotes for the Spanish (δβ)-thalassemia deletion; lane 5: homozygous patient for Spanish (δβ)-thalassemia.](image-url)
REFERENCES

Rapid detection of Spanish (delta beta)zero-thalassemia deletion by polymerase chain reaction

JL Vives-Corrons, MA Pujades, A Miguel-Garcia, A Miguel-Sosa and S Cambiazzo

Updated information and services can be found at:
http://www.bloodjournal.org/content/80/6/1582.full.html

Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml