Rapid Prenatal Diagnosis of \( \beta \) Thalassemia Using DNA Amplification and Nonradioactive Probes


We used in vitro DNA amplification by the polymerase chain reaction and nonradioactive probes for prenatal diagnosis of \( \beta \) thalassemia in Chinese from the Guangdong province. Exact molecular diagnoses were made in all 20 fetuses studied over a 6-month period. We conclude that this method of prenatal diagnosis for \( \beta \) thalassemia is a viable approach in many parts of the world where this disease is common.

\* 1989 by Grune & Stratton, Inc.

Since the introduction of prenatal diagnosis programs for thalassemia in several Mediterranean countries, the number of homozygous \( \beta \) thalassemia newborns has decreased dramatically.\(^1\) However, in many parts of the world this disorder is common, implementation of such programs has been slow. A major problem has been the complexity of the diagnostic procedures of fetal blood and DNA analysis.\(^2\) The newly developed method of in vitro DNA amplification by the polymerase chain reaction (PCR) coupled with oligonucleotide probe detection provides a rapid and simple approach to prenatal diagnosis.\(^3\)\(^,\)\(^4\) The recently introduced nonradioactive technique for labeling oligonucleotide probes makes the prenatal diagnosis procedure even easier.\(^5\) In this study, we applied these new techniques to prenatal diagnosis of \( \beta \) thalassemia in pregnancies at risk from the Guangdong province of China. The carrier rate for \( \beta \) thalassemia in this province is \(~3\%\). We successfully diagnosed 20 cases in 6 months.

Materials and Methods

According to the approved protocol of the institute, informed consent was obtained from the 20 families who requested prenatal diagnosis. DNA was extracted from the parents’ peripheral blood cells and, when available, from the homozygously affected children.\(^6\) DNA was also obtained from chorionic villi at 7 to 10 weeks of gestation or from amniotic fluid cells at approximately the fifteenth week. In vitro amplification of DNA was performed as described using two sets of primers to amplify the \( \beta \)-globin gene region.\(^7\) The amplified DNA was analyzed on polyacrylamide gel electrophoresis (PAGE), and equal amounts (usually one tenth) of the amplified DNA was dotted onto nylon filters as described.\(^7\) The filters were then hybridized with horseradish peroxidase (HRP)-labeled probes.\(^3\) Seven pairs of probes were prepared, each 15 nucleotides long, corresponding to the six common \( \beta \) thalassemia mutations found in the Chinese, and the HbE mutation (Table 1). The filters were washed at the temperatures indicated, and the HRP reaction was developed as described previously.\(^5\) Each sample was tested with the six probes corresponding to the mutant \( \beta \) thalassemia sequences, and with the \( \beta^\beta \) probe when the diagnosis of HbE was evident from hemoglobin electrophoresis. When both parents carried the same mutation, the probes corresponding to both mutant and normal sequences were used to hybridize the amplified DNA.\(^7\)

Results

The results of the 20 cases of prenatal diagnosis of \( \beta \) thalassemia are shown in Fig 1. The mutation present in DNA from the paternal and maternal chromosomes is indicated. In four cases, the fetus inherited the nontalassemic \( \beta \)-globin alleles from both the father and the mother and hence was homozygously normal. Ten fetuses inherited one thalassemia gene from one parent and the normal allele from the other and were heterozygous for \( \beta \) thalassemia. Six fetuses inherited the \( \beta \) thalassemia alleles from both parents and had homozygous \( \beta \) thalassemia.

Hybridization results representing each of the three clinical diagnoses are shown in Fig 2. In case 2, the father carries the 41/42 frameshift mutation, and the mother has a \(-28\) TATA box mutation. The fetal DNA did not hybridize with either of the two mutant probes and is therefore homozygously normal. The fetus in case 7 inherited the codon 17 nonsense mutation from the father but did not inherit the 41/42 frameshift mutation from the mother and is therefore heterozygous for \( \beta \) thalassemia. The fetus shown in case 15 inherited the \(-28\) TATA mutation from the father and the IVS-2 position 654 mutation from the mother and is homozygous for \( \beta \) thalassemia.

Discussion

We describe an approach to prenatal diagnosis of \( \beta \) thalassemia which should be applicable in many countries where this disease is common. First, the molecular basis of the mutations in an area where thalassemia is common must be delineated. Although > 50 different point mutations cause \( \beta \) thalassemia worldwide, the actual number of mutations in any one region is much smaller. In southern China for example, a previous study of 40 patients with homozygous \( \beta \) thalassemia revealed only six \( \beta \) thalassemia mutations and the \( \beta^\alpha \) gene.\(^7\) Once the most common mutations have been determined, the appropriate probes can be synthesized and used for DNA hybridization. Parents at risk (and, when available, previously affected children) are screened with these probes, and the oligonucleotides corresponding to the mutations they carry are then used to test the fetus. Oligonucleotide hybridization provides an exact molecular diagnosis.
Table 1. Oligonucleotide Probes Corresponding to Common Chinese \( \beta \) Thalassemia Mutations

<table>
<thead>
<tr>
<th>Mutation</th>
<th>5'-Sequence-3'</th>
<th>Hybridization and Wash Temperatures (°C)</th>
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<tbody>
<tr>
<td>Codon 41-42</td>
<td>G A G G T T G A T C C T T T T</td>
<td>42</td>
</tr>
<tr>
<td>IVS2 position 654</td>
<td>T G C T A T T A C C T T A A C</td>
<td>37</td>
</tr>
<tr>
<td>Codon 17</td>
<td>T T C A C C T A G C C C C A C</td>
<td>44</td>
</tr>
<tr>
<td>TATA box -28</td>
<td>C T G A C T T C T A T G C C C</td>
<td>42</td>
</tr>
<tr>
<td>IVS1 position 5</td>
<td>C A G G T T G C T A T C A A G</td>
<td>40</td>
</tr>
<tr>
<td>Codon 71-72</td>
<td>T G C C T T T A G T G A T G</td>
<td>37</td>
</tr>
<tr>
<td>( \beta^+ ), codon 26</td>
<td>T G G T G G T A A G G C C C</td>
<td>44</td>
</tr>
</tbody>
</table>

* Horseradish peroxidase was conjugated to the 5' end. Point mutations are underlined and deletions and additions are indicated by arrows. † Sequences corresponding to the sense and antisense strands, respectively.

Fig 1. Diagnosis in 20 fetuses at risk for \( \beta \) thalassemia: Mutations in paternal (squares) and maternal (circles) chromosomes. Open symbols indicate that the fetus inherited the normal alleles; closed symbols represent the thalassemic alleles. In cases 10 through 14, both parents carry the same \( \beta \) thalassemia mutation; hence, the parental origin of the mutation could not be determined.

Fig 2. Three representative clinical diagnoses from cases 2, 7, and 15 shown in Fig 1. Each strip was hybridized with the nonradioactive probe corresponding to the indicated mutant sequence.

Prenatal diagnosis by PCR offers several distinct advantages over previous methods. In vitro DNA amplification can be performed rapidly using either an automated machine or manually with water baths. The increased number of target sequences following amplification permits use of nonradioactive probes. All reagents, DNA primers, DNA probes, and heat-stable enzymes for DNA amplification can be stored for prolonged periods. The method has the additional advantage of speed. Amplification, hybridization, and detection require only one day, and a diagnosis can be achieved within two days. When used in conjunction with chorionic villus biopsy, a fetal diagnosis can be made before the tenth week of gestation.

We found a new TATA box mutation at position -30 (case 9). Initially, we were unable to determine the mutation on the paternal chromosome using the panel of probes, but after in vitro amplification and direct sequencing of ampli-
fled DNA, we were able to identify it (S.-P. Cai, J.-Z. Zhang, and Y.W. Kan, manuscript in preparation). Even in this complicated case, prenatal diagnosis was accomplished within 2 weeks.

Based on our experience with these 20 cases in a 6-month period, we conclude that prenatal diagnosis for β thalassemia is now a viable approach in many countries where this disease is common. Implementation will decrease the number of homozygous births in parts of the world where optimum treatment for β thalassemia is not readily available.

REFERENCES

Rapid prenatal diagnosis of beta thalassemia using DNA amplification and nonradioactive probes

SP Cai, CA Chang, JZ Zhang, RK Saiki, HA Erlich and YW Kan