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.

SINCE THE INTRODUCTION of prenatal diagnosis programs for thalassemia in several Mediterranean countries, the number of homozygous $\beta$ thalassemia newborns has decreased dramatically. However, in many parts of the world where 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. 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. The recently introduced nonradioactive technique for labeling oligonucleotide probes makes the prenatal diagnosis procedure even easier. 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. 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. The amplified DNA was analyzed on polyacrylamide gel electrophoresis using two sets of primers to amplify the $\beta$-globin gene region. The mutation present in $\beta$ thalassemia worldwide, the actual number of mutations in any one region is much smaller. In southern China for $\beta$ thalassemia, the number of 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 $\beta^4$ gene. 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 β Thalassemia Mutations

<table>
<thead>
<tr>
<th>Mutation</th>
<th>5'-Sequence-3'</th>
<th>Hybridization and Wash Temperatures (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codon 41-42</td>
<td>(GAGGTITGAGTCCTT)</td>
<td>42</td>
</tr>
<tr>
<td>IVS2 position 654</td>
<td>(TGCTATTACCTTAA)</td>
<td>37</td>
</tr>
<tr>
<td>Codon 17</td>
<td>(TTTACCTTATT)</td>
<td>44</td>
</tr>
<tr>
<td>TATA box - 28</td>
<td>(CTGACTTCTATGCC)</td>
<td>42</td>
</tr>
<tr>
<td>IVS1 position 5</td>
<td>(CAGGTTG)</td>
<td>40</td>
</tr>
<tr>
<td>Codon 71-72</td>
<td>(TGGTGGTAAGGCCC)</td>
<td>44</td>
</tr>
<tr>
<td>(\beta^+) codon 26</td>
<td>(TGGTG)</td>
<td>37</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 β 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 β 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.
fied 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