Rapid Prenatal Diagnosis of β 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 β 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 β thalassemia is a viable approach in many parts of the world where this disease is common.

RESULTS

The results of the 20 cases of prenatal diagnosis of β thalassemia are shown in Fig 1. The mutation present in DNA from the paternal chromosome is indicated. In four cases, the fetus inherited the nonthalassemic β-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 β thalassemia. Six fetuses inherited the β thalassemia alleles from both parents and had homozygous β 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 β 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 β thalassemia.

DISCUSSION

We describe an approach to prenatal diagnosis of β 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 β 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 β thalassemia revealed only six β thalassemia mutations and the βθ gene. 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.
PRENATAL DIAGNOSIS OF β THALASSEMIA IN CHINA

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>
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<tbody>
<tr>
<td>Codon 41-42</td>
<td>G A G G T T G A G 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 C C A C T T A A C T</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 T</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 C T</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 T</td>
<td>40</td>
</tr>
<tr>
<td>Codon 71-72</td>
<td>T G C C T T T A T G T G A T G T</td>
<td>37</td>
</tr>
<tr>
<td>β², codon 26</td>
<td>T G G T G G T A A G G C C C C T</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 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.

as compared with the phenotypic diagnosis obtained by fetal blood sampling or linkage analysis with DNA polymorphism.

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-
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