Efficiency and cost effectiveness: PAGE-SSCP versus MDE and Phast gels for the identification of unknown β thalassaemia mutations

A Gupta, S Agarwal

Background: Prenatal diagnosis for β thalassaemia has proved to be very effective in preventing the birth of an affected child and hence in controlling the disease. The success of prenatal diagnosis depends on the delineation of the underlying mutations in the population at risk. Each population carries a limited number of frequent defects (89–91%) and a variable number of rare alleles (4–5%), whereas 2–3% of alleles remain uncharacterised. To offer prenatal diagnosis when the parental mutation is unknown, the application of a non-specific detection method (such as single stranded conformational polymorphism [SSCP]) to localise the mutation, followed by direct sequencing of the amplified gene sequence, is required. With this objective in mind, this study was designed to devise the best protocol and system of SSCP for the rapid screening of unknown mutations in the β globin gene.

Methods: To detect mutations in this disease, three different systems—Phast gels, MDE gels, and polyacrylamide gels—were used under varying conditions.

Results: Polyacrylamide gels were found to be the most efficient, both in terms of resolution and cost. Conclusion: Polyacrylamide gels are the most rapid, efficient, reliable, and cost effective means for DNA mutation analysis of the β globin gene.
Electrophoresis was carried out for 14 hours at constant power (8 W), at room temperature (in an air conditioned room at 20–25°C).

Polyacrylamide gels

A 10% polyacrylamide gel was prepared and pre-run for one hour at a constant power of 60 W. A 1 μl aliquot of the αdCTP labelled PCR products was added to 9 μl of loading dye containing 96.5% formamide. Samples were denatured at 95°C for five minutes and chilled on ice. These denatured samples (3 μl aliquots) were loaded on to the gel and run at room temperature in 1× TBE for 16 hours, at 8 W. An additional 1.5% of formamide was used to improve the resolution.

The polyacrylamide and Hydrolink-MDE gels were transferred to Whatman 1MM and 3MM paper, respectively. They were then dried in a gel drier (Rapid Dry, Atto, Japan), exposed to x-ray film (Kodak) at −70°C for 24 hours, and developed.

RESULTS AND DISCUSSION

Mutational analysis of the β globin gene using the Phast and MDE gels produced mutant bands were as intense as the normal ones and the separation was good (figs 2, 3). The Phast system was very rapid—the entire procedure was completed in less than two hours.

"Using a higher concentration of formamide (96.5%) than normal (95%) gave better results, with no smiling bands”

Different conditions were tested with the polyacrylamide gels, such as pre-running the gels, using denaturants, adding glycerol, and varying the ionic strength of the electrophoretic buffer and the percentage of the polyacrylamide. The

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Primer</th>
<th>Sequence</th>
<th>Fragment (bp)</th>
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<tbody>
<tr>
<td>I</td>
<td>Primer 1</td>
<td>CCAAGGACACGGTACGGCCTTCAC 3′</td>
<td>322</td>
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<tr>
<td></td>
<td>Primer 2</td>
<td>TAAATGCACTGGCCTCACCACATCC 3′</td>
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<td>Primer 5</td>
<td>CCTGCTTTCAGTAACATCTCAGG 3′</td>
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<td></td>
<td>Primer 9</td>
<td>GGAACAAAGGAACCTTTAATAG 3′</td>
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Table 1 The sequence and size of the primers used for single stranded conformational polymorphism analysis of the β globin gene

Figure 1 A schematic representation of the human β globin gene with the location of the primers, and the region and the size (bp) of the fragments they amplify. IVS, intervening sequence (intron).

Figure 2 Autoradiograph of the Phast gel. Lanes 1 and 6, samples heterozygous for the CD 41/42 β thalassaemia mutation; lanes 2 and 3, samples homozygous for the CD 41/42 β thalassaemia mutation; lane 4, normal.

Figure 3 Autoradiograph of the MDE gel. Lanes 1 and 2, normal; lanes 3 and 4, samples heterozygous for the CD 41/42 β thalassaemia mutation.

Figure 4 Autoradiogram of the polyacrylamide single stranded conformational polymorphism gel run at constant 8 W for 16 hours at room temperature. Lanes 1 and 4, samples heterozygous for the CD 41/42 β thalassaemia mutation; lanes 2 and 3, samples heterozygous for the CD 47/48 mutation.
resolution was good when the gel was pre-run at 60 W for one hour. Using a higher concentration of formamide (96.5%) than normal (95%) gave better results, with no “smiling” bands. The band resolution was not affected by the addition or absence of 5% glycerol. Three ionic strengths of the TBE buffer were used: 0.5×, 1.0×, and 1.5×. Normal strength (1×) TBE yielded the sharpest SSCP bands using 8–12% gels. The sharpest and most consistent bands were obtained with 10% gels (fig 4).

Thus, polyacrylamide gels were found to be the most rapid, efficient, reliable, and cost effective method for DNA mutation analysis in β thalassaemia.

ACKNOWLEDGEMENTS
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REFERENCES

Take home messages
• Polyacrylamide gels are the most rapid, efficient, reliable, and cost effective means for DNA mutation analysis of the β globin gene

Protein kinase regulates IL16 transcription in arthritis
Molecular researchers have suggested that pathways dependent on protein kinase C may regulate transcription of interleukin 16 (IL16), a proinflammatory cytokine abundant in arthritic joints.

They compared the effect of various chemical agents on steady state (IL16) mRNA transcripts in growing synovial fibroblasts from six patients with rheumatoid arthritis (RA) and three with osteoarthritis (OA). The reverse transcriptase PCR method they used enabled them to obtain results that were semiquantitative.

Early passage synovial fibroblasts from patients with RA or OA transcribed IL16 mRNA when incubated with growing medium without additives. Protein kinase inhibitor staurosporine enhanced IL16 steady state mRNA in both types of synovial fibroblasts and specific protein kinase C activator phorbol-12-myristate-13-acetate reduced transcription. Other agents—the calcium ionophore ionomycin, protein kinase A stimulator cyclic AMP, and G protein activator MAS-7—gave minor, variable responses. Phosphatase inhibitor okadaic acid and protein kinase inhibitor H-7 dihydrochloride reduced mRNA transcripts, maybe because of their killing the fibroblasts. This response pattern suggests that IL16 is regulated by protein kinase C dependent mechanisms, say the researchers.

Please visit the Molecular Pathology website [www.molpath.com] for link to this full article.

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