

# Detection of Gene Deletions by PCR Analysis in a Malaysian Patient with Duchenne Muscular Dystrophy

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## Summary

Duchenne muscular dystrophy (DMD), the commonest X-linked disorder, is a progressive, eventually fatal disease. With the advent of molecular genetics, the Duchenne gene and its protein product, *dystrophin*, have been characterised. Molecular diagnosis of DMD, identification of carriers and antenatal diagnosis are now possible. We describe here the use, in a Malaysian boy with DMD, of a recent innovation, multiplex polymerase chain reaction (PCR), to obtain molecular diagnosis by detection of dystrophin gene deletions.

**Key words:** Multiplex PCR, gene deletion, Duchenne muscular dystrophy, *dystrophin*.

## Introduction

Duchenne muscular dystrophy (DMD) is a progressive form of muscular dystrophy affecting boys and leading to death from respiratory failure at the end of the second decade. It is the commonest X-linked disorder in man, occurring in approximately 1 in 3,500 male births. Previously, diagnosis of DMD had been based upon clinical features, pedigree studies, muscle enzymes, electromyography and muscle biopsy. However, diagnostic uncertainty might arise in cases of the milder sex-linked Becker form of muscular dystrophy (BMD), the rare female with DMD, autosomal recessive muscular dystrophy and spinal muscular atrophy. Of practical relevance, for the purpose of genetic counselling, there is a need for confirmation by molecular diagnosis, and to determine in a particular kindred, whether the disease has arisen from a spontaneous new (non-hereditary) somatic mutation. With this information it has been possible to offer to parents the option of antenatal diagnosis<sup>1</sup>. Female carrier relatives may also be identified<sup>2</sup>.

## Molecular Genetics of DMD

Since 1983, when the gene responsible for DMD was mapped to a specific region of the X chromosome, dramatic progress in molecular genetics has resulted in the identification of the gene responsible for DMD, the largest yet discovered (2,300 kilobases (kb) in size)<sup>3</sup>. Mutation of this gene results in DMD and its allelic variant BMD. With the use of 'reverse genetics', the protein product coded by the dystrophin gene has been determined. *Dystrophin* comprises 3,685 amino acids with a molecular weight of 427 kilodaltons. Current evidence suggests that it is a sarcolemmal membrane-associated component of the cytoskeleton of skeletal muscle fibres<sup>4,5</sup>.

*Dystrophin* is absent or undetectable in the muscles of patients with DMD on immunohistochemical staining with antidystrophin antibodies (reviewed in Arahata *et al*, 1991<sup>6</sup>). This is attributed to gene mutations which disrupt the translational reading frame (out-of-frame deletion or duplication). In BMD, however, gene mutations, which are probably in-frame, result in production of abnormal dystrophin which shows faint patchy staining of sarcolemmal membranes.

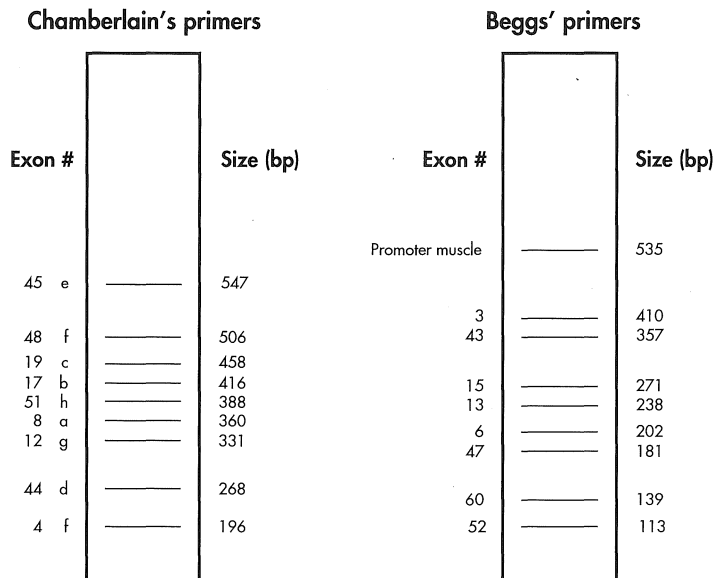
### Molecular Diagnosis of DMD

Two-thirds of DMD arise from intragenic deletions detectable by the use of an array of dystrophic gene probes which are either cDNA or genomic clones. In the remaining one-third of families, where deletions are not detectable, the dystrophin gene may be tracked by segregation analysis of restriction fragment length polymorphisms (RFLPs) which have been identified to be genetic markers of the dystrophin gene in a particular family.

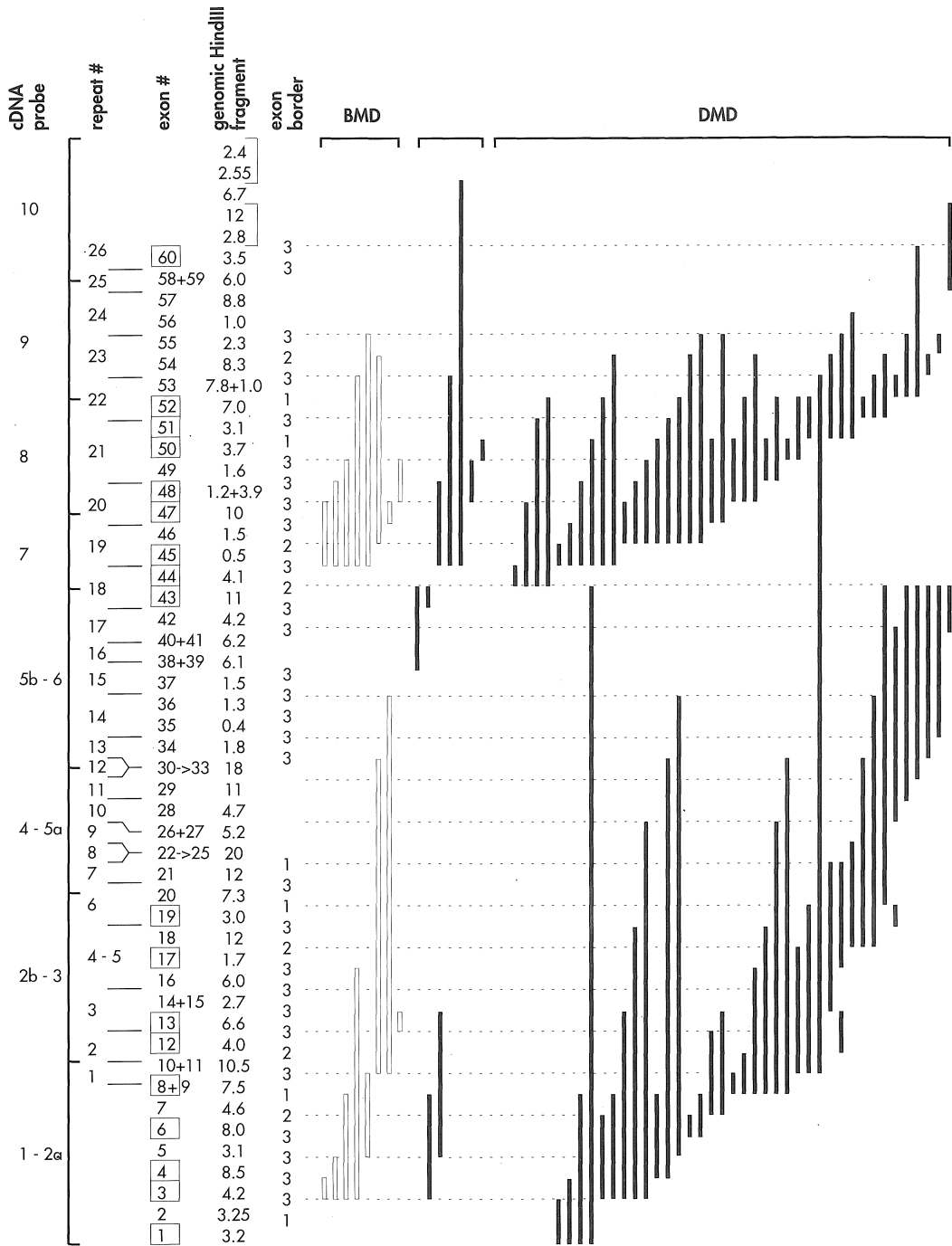
Both deletion and linkage analysis have earlier been performed using Southern blotting. This method is time-consuming and technically demanding. Recently, with the widespread use of the multiplex polymerase chain reaction (PCR) method, deletion analysis and linkage studies have become faster and easier. In multiplex PCR, a gene region of interest is amplified and examined for mutations or RFLPs<sup>7</sup>. Alternatively, a specific region of DNA may be amplified to rapidly identify deletions in genomic DNA from patients. This method detects over 98% of gene deletions<sup>8</sup>. PCR method of diagnosis of carriers is now available<sup>2</sup>.

### Materials and Methods

High molecular-weight genomic DNA was isolated from leukocytes in heparinised blood samples using the salting out method<sup>9</sup>. PCR analysis was performed using the multiplex oligonucleotide primers of Chamberlain<sup>7</sup> and Beggs<sup>8</sup>. In a 0.5 ml microfuge tube was added: 4 µl of dNTPs (Perkin-Elmer Cetus) containing 1.25 mM each, 18 µl (25 ng) of each primer (Chamberlain's and Beggs' primers: see Figs 1 and 2), 5 µl of 10 x *Taq* buffer,



**Fig 1:** Dystrophin gene analysis using multiplex PCR. Schematic illustration of the DMD gene indicating the relative positions of the exons within DNA segments amplified by PCR with the use of Chamberlain's and Beggs' primers.



**Fig 2: Chromosome map of the dystrophin gene showing detection of deleted exons using PCR. Source: Am J Hum Genet 1989;45 : 498-506.**

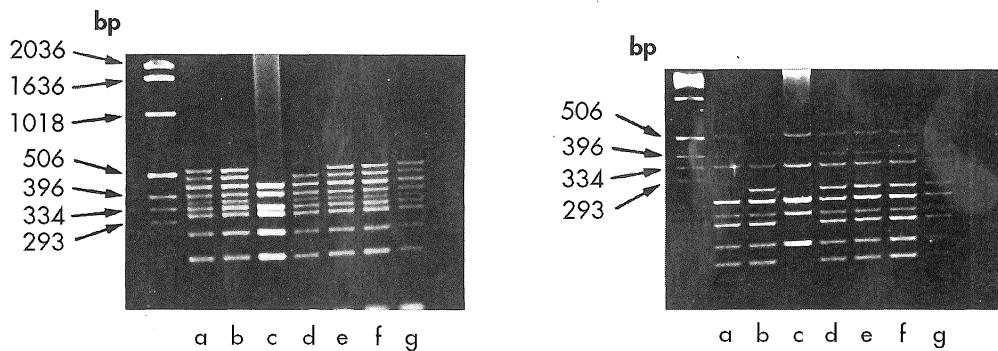
and DNA template of approximately 250-500 ng (concentration of 5-10 ng/ $\mu$ l). Distilled water was added to a final volume of 50  $\mu$ l. To the reaction mixture, 1  $\mu$ l of *Taq polymerase* (5U *Amplitaq*, Perkin-Elmer Cetus) was added. This was topped with 25  $\mu$ l of paraffin oil and the mixture centrifuged for 5 seconds. The sample was placed in an automatic thermocycler using the programmes of either Chamberlain or Beggs. Fifteen  $\mu$ l of the reaction products were electrophoresed on a 1.4% agarose mini-gel containing 0.2  $\mu$ g/ml ethidium bromide at 35 mA for 3 hours (approximately 3.7 V/cm) in 1x TBE (10 mM Tris base, 90 mM boric acid, 1 mM EDTA). The results were photographed with a Polaroid camera.

### Case History

The patient, an 8 year old Chinese boy, product of a non-consanguineous marriage, had normal development until he developed symmetrical proximal weakness of the lower limbs at 6 years of age. Speech was normal and there was no bulbar weakness. His father, elder sister aged 14, and elder brother aged 13 were clinically normal. His mother was asymptomatic, with serum CK level of 100 IU/L. There was no family history of muscular disorder. Physical examination of the proband revealed mild lumbar lordosis and waddling gait. There was pseudohypertrophy of the calves, and Gower's sign was positive. Tendon reflexes were depressed. The heart was normal. Creatine kinase (CK) level was 1436 IU/L and lactic dehydrogenase 1497 IU/L. The electrocardiogram and chest radiograph were normal.

### Results

The proband was found to have an intragenic deletion of exon 45 (547 bp in size), using Chamberlain's primers (Fig 3a). No deletion was detected with Beggs' Primers (Fig 3b). This deletion site corresponds with dystrophin cDNA probe 7, which hybridises to a site near the centre of the dystrophin gene (Fig 2). DNA analysis of his elder brother was normal.



a : Deletion of exon 45 (547 kb) within the dystrophin gene of proband (lane d) confirms diagnosis of DMD.

b : Proband shows no deletion on PCR using Beggs' primers.

**Fig 3:** Amplification products of multiplex PCR using primers of Chamberlain<sup>7</sup> (a) and Beggs<sup>8</sup> (b). No deletion was detected in mother (e), sister (f), or brother (g).

## DETECTION OF GENE DELETIONS BY PCR ANALYSIS

### Discussion

Multiplex PCR analysis demonstrated a single deletion in exon 45 of the dystrophin gene in the proband. This single deletion was likely to have been an 'out-of-frame' deletion which resulted in severe clinical disease. In his brother, the absence of dystrophin gene deletion excluded the disease. A direct, non-radioactive method of quantitative PCR amplification has been shown to diagnose deletion and duplication carriers<sup>2</sup>. In such identified carrier mothers, PCR method of antenatal diagnosis may be offered<sup>1</sup>. Carrier status of female relatives of the proband may be similarly established.

PCR amplification is technically less demanding than methods using Southern blotting, and requires shorter time (minimum of 48 hours). Rapid analysis is particularly important in antenatal diagnosis where chorionic villus biopsy may only be safely performed at 14 weeks gestation and management decisions need to be made speedily. PCR multiplex primers are now commercially available.

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