Becker muscular dystrophy: correlation of deletion type with clinical severity

A M Norman, N S T Thomas, H M Kingston, P S Harper

Abstract
Molecular deletion screening with cDNA probes from the dystrophin gene was undertaken in patients with Becker muscular dystrophy from 58 separate families. Deletions were found in 41 (71%) of these families. Thirty-four (83%) of the deletions started in the same intron near the centre of the gene, and although there was no precise correlation between clinical severity and deletion pattern, the commonest deletion pattern, which was present in 49% of all deletion families, is associated with a mild phenotype.

Becker muscular dystrophy (BMD) has been a major interest of this department since 1981, and we were among the first to show that BMD and Duchenne muscular dystrophy (DMD) were likely to be allelic.1 The cloning of the DMD/BMD gene,2 and the discovery of its protein product, dystrophin,3 has confirmed that mutations in the same gene are indeed responsible for the clinical spectrum of DMD/BMD, but the details of how the varying severity of phenotypes can be explained by differences in the underlying mutation are not yet fully worked out, though some progress has been made.4 Study of BMD, with its greater range of clinical severity and relative homogeneity of molecular deletions, is likely to be more fruitful than study of DMD, where a more narrowly defined phenotype is produced by a wide range of molecular deletions. Several groups have already described series of DMD deletions,5-10 but few have included large series of BMD patients.11 We report here the 41 deletions discovered in 58 separate BMD families.

Methods
Patients and families with BMD were collected from three sources. Firstly patients were referred for confirmation of diagnosis and genetic counselling because of the known interest in muscle disease of our department. Secondly, multigeneration families were collected for the original linkage analysis.1 Thirdly, isolated male patients were collected as part of an attempt to distinguish BMD from autosomal recessive limb-girdle muscular dystrophy by means of

Institute of Medical Genetics, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN.
A M Norman, N S T Thomas, P S Harper

Regional Genetics Centre, St Mary’s Hospital, Manchester.
H M Kingston

Correspondence to Dr Norman.

Received for publication 21 September 1989.
Accepted for publication 4 October 1989.

Figure 1  Deletions detected near the centromeric end of the dystrophin cDNA on exon containing HindIII genomic fragment map as published by Darras et al.4 Numbers at the top of deletion lines represent number of separate families studied with that deletion. Codes at bottom refer to patients indicated in table 1. *Note patient H25 has a duplication of exon 10, not a deletion.
dystrophin cDNA probes. All patients were examined by one of us and had a proximal limb-girdle pattern of muscle weakness, calf hypertrophy, and either a family history compatible with X linked inheritance or muscle pathology characteristic of a primary muscular dystrophy or both.

DNA was extracted from venous blood and aliquots were digested to completion with PstI, HindIII, and MspI. They were then subjected to electrophoresis on 0.9% agarose gels and blotted onto nylon membranes (Hybond N⁺, Amersham) by the method of Southern. The membranes were hybridised overnight with cDNA probes that had been labelled with ³²P by the random hexanucleotide primed method. Membranes were washed at 65°C in 1 × SSC (SSC=0.15 mol/l sodium chloride, 0.015 mol/l sodium citrate), 0.1% sodium dodecyl sulphate, then exposed to Fuji x ray film with intensifying screens at −70°C for one to seven days.

The cDNA probes used represent a complete clone from the dystrophin gene. Molecular deletions are indicated by alteration of the normal band pattern on the autoradiographs. The deletions were mapped onto the HindIII genomic fragment map as published by Darras et al.

Results
Useful data were obtained on patients from 58 separate BMD families. Molecular deletions were detected in 41 (71%) of these, but only one patient (H25) appeared to have a duplication. Deletion patterns are summarised diagrammatically in figs 1 and 2. Clinical data for each deletion patient or group are summarised in table 1 and for those without a deletion in table 2. In 20 (49%) of the families with a deletion, there was a common pattern of deleted exons (0-5, 1-5, and 10 kb). In total, 34 (83%) of the deletions started in the same intron (between 4-1 and 0-5 kb exons). The extent of the deletion within this intron was variable, as shown in table 3 by the results of deletion screening with the intronic probe P20.

Discussion
We report here an extensive series of BMD deletions. Our finding that 71% of BMD families have a molecular deletion detectable with cDNA probes agrees with the work of others. Our results show that 83% of these deletions start in the same intron and confirm the findings of Forrest et al. The start site of the deletion within this ‘hotspot’ is variable (table 3). Other workers have disagreed with these conclusions but have only reported small numbers.

It is difficult to correlate clinical severity with deletion type within BMD and this is probably in part the result of individual and personal factors that are likely to affect age at diagnosis and age of acceptance of a wheelchair for mobility in any slowly progressive, chronic disease such as BMD. Furthermore, patients are being seen at different points in the natural history of their disease and this makes assessment of clinical severity difficult, especially in the young isolated case. Nevertheless, the common BMD deletion appears to predict a mild phenotype, as the index patients studied were all still ambulant at a mean age of 34, and in familial cases no patient in older generations had been confined to a wheelchair before the age of 41. This particular deletion pattern is rarely seen in DMD. In contrast to this, it can be seen by inspection of figs 1 and 2 and table 1 that other deletion patterns have been associated with more divergent phenotypes, as has also been reported by others.

Correlation of phenotype and genotype between DMD and BMD is a different matter. Others have shown that DMD deletions are varied in position and extent; our data clearly show that BMD deletions are much more homogeneous. It has been proposed by Monaco et al that deletions which disrupt the codon

---

**Figure 2** Deletions detected near the centre of the dystrophin cDNA on exon containing HindIII fragment map as published by Darras et al. (Order of fragments in brackets has not been established. Horizontal arrow indicates position of P20 intron.) Numbers at top of deletion lines represent number of separate families with that deletion. Codes at bottom refer to patients indicated in table 1. Arrows indicate that end of deletion has not been found yet.
reading frame lead to DMD and those which maintain
an open reading frame lead to BMD. However, three
of our patients (15, B41, H26), all deleted for exons 3
to 7 (fig 1), have previously been reported to have a
frameshift deletion. It has been proposed that re-
initiation from a fresh start site allows production of
functional dystrophin. The deletion in patient 9 is
also of interest because Kunkel recently hypothesised
that deletions upstream of the 4·1/0·5 kb intron
(Wapenaar's hotspot) would lead to a very slight
defect with either very mild symptoms or none at all,
and this might account for the rarity of such
deletions. This patient certainly has very mild
disease.
Table 2  Summary of clinical details of patients in study without a deletion.

<table>
<thead>
<tr>
<th>Patient code</th>
<th>Date of birth</th>
<th>Age at diagnosis (y)</th>
<th>Wheelchair</th>
<th>CK (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1957</td>
<td>30</td>
<td>No</td>
<td>3210</td>
</tr>
<tr>
<td>8</td>
<td>1966</td>
<td>10</td>
<td>No</td>
<td>2650</td>
</tr>
<tr>
<td>12</td>
<td>1969</td>
<td>11</td>
<td>Yes</td>
<td>17880</td>
</tr>
<tr>
<td>16</td>
<td>1942</td>
<td>11</td>
<td>No</td>
<td>396</td>
</tr>
<tr>
<td>17</td>
<td>1954</td>
<td>11</td>
<td>No</td>
<td>8820</td>
</tr>
<tr>
<td>B1</td>
<td>1950</td>
<td>20</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>B8</td>
<td>1968</td>
<td>8</td>
<td>No</td>
<td>10097</td>
</tr>
<tr>
<td>B23</td>
<td>1968</td>
<td>14</td>
<td>No</td>
<td>4198</td>
</tr>
<tr>
<td>B28</td>
<td>1968</td>
<td>9</td>
<td>No</td>
<td>2105</td>
</tr>
<tr>
<td>B29</td>
<td>1970</td>
<td>3</td>
<td>14</td>
<td>2000</td>
</tr>
<tr>
<td>B48</td>
<td>1958</td>
<td>12</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>B61</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>H9</td>
<td>1947</td>
<td>13</td>
<td>33</td>
<td>76</td>
</tr>
<tr>
<td>H18</td>
<td>1959</td>
<td>11</td>
<td>No</td>
<td>367</td>
</tr>
<tr>
<td>H23</td>
<td>1955</td>
<td>8</td>
<td>15</td>
<td>4870</td>
</tr>
<tr>
<td>H30</td>
<td>1960</td>
<td>13</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

Mean (SD) 29.4 (8.7) 13 (3) — (2997)

Patient codes as for table 1.
*Maternal uncle in wheelchair at age 45.

Table 3  Summary of findings with the intronic probe P20 in deletion patients with start point in this intron (n = 34).

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>All bands deleted</td>
<td>21</td>
</tr>
<tr>
<td>Some bands deleted</td>
<td>2</td>
</tr>
<tr>
<td>No bands deleted</td>
<td>5</td>
</tr>
<tr>
<td>Altered band size</td>
<td>6</td>
</tr>
</tbody>
</table>

Examination of muscle dystrophin in this cohort of patients is likely to illuminate further the relationship between gene deletion pattern and clinical severity.

We thank L M Kunkel (Boston, USA), R G Worton (Toronto, Canada), Kay Davies (Oxford), and G van Ommen (Leiden, The Netherlands) for kindly donating DNA probes. This work was supported by the Muscular Dystrophy Association of America and the Muscular Dystrophy Group of Great Britain.

10 Upadhaya M, Smith KA, Thomas NST, Norman AM, Harper PS. Intragenic deletions in 164 boys with Duchenne muscular dystrophy studied with the dystrophin cDNA. Clin Genet (in press).
Becker muscular dystrophy: correlation of deletion type with clinical severity.
A M Norman, N S Thomas, H M Kingston and P S Harper

doi: 10.1136/jmg.27.4.236

Updated information and services can be found at:
http://jmg.bmj.com/content/27/4/236

Email alerting service

These include:
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/