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,³⁻¹⁰ but few have included large series of BMD patients.¹¹ We

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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



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.⁸ 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.¹² 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. ¹³ Membranes were washed at 65°C in $1 \times SSC$ (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.² Molecular deletions are indicated by alteration of the normal band pattern on



Figure 2 Deletions detected near the centre of the dystrophin cDNA on exon containing HindIII fragment map as published by Darras et al.⁸ (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.

the autoradiographs. The deletions were mapped onto the *Hind*III genomic fragment map as published by Darras *et al.*⁸

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.⁵ ¹¹ Our results show that 83% of these deletions start in the same intron and confirm the findings of Forrest *et al.*¹¹ The start site of the deletion within this 'hotspot' is variable (table 3).¹⁴ Other workers have disagreed with these conclusions but have only reported small numbers.¹⁵

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.⁶ ¹⁶

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*⁴ that deletions which disrupt the codon

| 15 1973 9 No 8000 B1 1966 1 1 No 1398 B10 1946 20 32 801 H25 1947 34 No 2499 20 1950 18 No 9499 9 1971 12 No 5050 14 1965 11 No 1880 Group A 2 1954 21 No 1730 6 1966 10 No 4100 1700 7 1967 11 No 1880 4220 7 1967 13 No 4100 4220 13 1966 10 No 4220 No 223 13 1965 13 No 1221 No 2223 14 1965 13 No 1222 1449 20 No 1180 2245 No 7239 <th>Patient code</th> <th>Date of birth</th> <th>Age at diagnosis (y)</th> <th>Wheelchair</th> <th>СК</th> | Patient code | Date of birth | Age at diagnosis (y) | Wheelchair | СК |
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| 9 1971 12 No 5050 14 1965 11 No 1880 Group A 2 1954 21 No 1730 6 1947 17 No 1730 6 1956 21 No 1700 7 1967 13 No 4220 10 1969 20 No 67759 12 1935 25 No 7799 13 1965 13 No 1233 14 1955 13 No 1233 153 1945 25 No 1233 164 1970 13 No 1233 170 13 No 1447 1447 184 1956 15 No 1447 1914 1956 15 No' 415 11 1956 15 No' 2139 11 1950 <td>20</td> <td>1950</td> <td>18</td> <td>No</td> <td>994</td> | 20 | 1950 | 18 | No | 994 |
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| I0 1966 20 No 2220 13 1966 11 No 8820 22 1949 20 No 739 33 1935 25 No 739 B2 1945 26 No 729 B11 1965 13 No 1223 B38 1926 32 No 911 B67 1957 25 No 691 H4 1970 13 No* 4164 H6 1946 5 No* 4187 H8 1961 19 No* 2331 H11 1936 12 No 1514 H7 1960 19 No* 2331 H12 1960 19 No* 1514 Group A Mean 34-1 17.5 None 2139 Group B 4 1949 26 No 303 B24 1940 | 10 | 1907 | 11 | No | 4220 |
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| Group C 3 1966 11 17 2630 B4 1948 10 30 1967 B6 1940 20 31 397 B43 1947 13 40 2278 Group D | HI4 | 1959 | 17 | NO | 4050 |
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| B43 1947 13 40 2278 Group D H17 H20 99 1970 29 11 718 No 4079 31 1931 36 53 499 B16 H22 1965 13 15 No 4000 4079 TH 1931 45 No 609 | Bo | 1940 | 20 | 51 | 2278 |
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| B16196713No4000H22196515No3416TH193145No609 | 31 | 1931 | 36 | 53 | 499 |
| B16 1967 13 No 4000 H22 1965 15 No 3416 TH 1931 45 No 609 | | | | N- | 4000 |
| H22 1965 15 No 3416 TH 1931 45 No 609 | B16 | 1967 | 13 | No | 4000 |
| TH 1931 45 No 609 | H22 | 1965 | 15 | No | 3416 |
| | ТН | 1931 | 45 | No | 609 |

Table 1 Summary of clinical details of patients in study with a deletion (or duplication).

Patient codes without a prefixed letter identify patients included in the previous study of isolated cases.¹² Patient codes prefixed by letter H identify patients included in the original linkage study.¹ Other patients are taken from the Wales BMD register. Deletions for each patient or

group are shown in figs 1 and 2. CK=serum creatine kinase activity in IU/1. *Maternal uncle in wheelchair at ages 55, 43, 49, and 41 respectively.

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 reinitiation from a fresh start site allows production of functional dystrophin.¹⁷ 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.¹⁸ This patient certainly has very mild disease.

| Patient code | Date of birth | Age at diagnosis (y) | Wheelchair | CK (IU/l) |
|--------------|---------------|----------------------|------------|-----------|
| 1 | 1957 | 30 | No | 3210 |
| 8 | 1966 | 10 | No | 2650 |
| 12 | 1969 | 11 | 17 | 1880 |
| 16 | 1942 | 11 | Yes | 396 |
| 17 | 1954 | 11 | No | 8820 |
| B1 | 1950 | 20 | 37 | 380 |
| B8 | 1968 | 8 | No | 10097 |
| B23 | 1968 | 14 | No | 4198 |
| B28 | 1968 | 9 | No | 2105 |
| B29 | 1970 | 3 | 14 | 2000 |
| B48 | 1958 | 12 | No | |
| B61 | _ | | | |
| H9 | 1947 | 13 | 33 | 76 |
| H18 | 1959 | 11 | No | 367 |
| H23 | 1955 | 8 | 15 | 4870 |
| H30 | 1960 | 13 | 23 | _ |
| Mean | 29.4 | 13.3 | _ | 2997 |
| (SD) | (8.7) | (7.0) | | (2997) |

Table 2 Summary of clinical details of patients in study without a deletion.

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).

| All bands deleted | 21 |
|-----------------------------|----|
| Some bands deleted | 2 |
| No bands deleted | 5 |
| Altered band size | 6 |
| (implies junction fragment) | |

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

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