Becker muscular dystrophy: correlation of deletion type with clinical severity

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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

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.

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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 *Pst*I, *Hind*III, and *Msp*I. 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 × 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

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

Figure 2 Deletions detected near the centre of the dystrophin cDNA on exon containing *Hind*III 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.

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

Patient code	Date of birth	Age at diagnosis (y)	Wheelchair	CK
15	1973	9	No	8000
B41	1966	5	18	2248
H26	1961	11	No	1398
B10	1946	20	32	801
H25	1947	34	No	2499
20	1950	18	No	994
9	1971	12	No	5050
14	1965	11	No	1880
Group A 2	1954	21	No	1730
5	1947	17	No	610
6	1956	21	No	1700
7	1967	11	No	4620
10	1969	13	No	4220
13	1966	20	No	2750
18	1956	11	No	8820
22	1949	20	No	1180
23	1935	25	No	739
B2	1945	26	No	729
B11	1965	13	No	1223
B38	1926	32	No	912
B67	1957	25	No	691
H4	1970	13	No	4164
H6	1946	5	No*	1487
H8	1961	19	No*	2119
H11	1936	15	No*	415
H12	1960	11	No	2331
H19	1950	19	No*	816
H21	1963	12	No	1514
Group A Mean (SD)	34.1 (11.8)	17.5 (6.4)	None	2139 (1964)
Group B 4	1949	26	No	908
11	1970	11	No	1430
B24	1964	18	No	2391
H14	1959	17	No	4038
Group C 3	1966	11	17	2630
B4	1948	10	30	1967
B6	1940	20	31	397
B43	1947	13	40	2278
Group D				
H17	1945	9	29	718
H20	1970	11	No	4079
31	1931	36	53	499
B16	1967	13	No	4000
H22	1965	15	No	3416
TH	1931	45	No	609

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/l.

*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 re-initiation 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.

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

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 (SD)	29.4 (8.7)	13.3 (7.0)	—	2997 (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).

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

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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