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Correlation of clinical and deletion data in Duchenne and Becker muscular dystrophy

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SUMMARY Cloned cDNA sequences representing exons from the Duchenne/Becker muscular dystrophy (DMD/BMD) gene were used for deletion screening in a population of 287 males affected with DMD or BMD. The clinical phenotypes of affected boys were classified into three clinical severity groups based on the age at which ambulation was lost. Boys in group 1 had DMD, losing ambulation before their 13th birthday; those in group 2 had disease of intermediate severity, losing ambulation between the ages of 13 and 16 years; and boys in group 3 had BMD, being ambulant beyond 16 years. A fourth group consisted of patients too young to be classified. Clinical group allocation was made without previous knowledge of the DNA results.

A gene deletion was found in 124 cases where the clinical severity group of the affected boy was known. The extent of the deletions was delineated using cDNA probes. There were 74 different deletions. Fifty-five of these were unique to individual patients, but the other 19 were found in at least two unrelated patients.

The different clinical groups showed generally similar distributions of deletions, and the number of exon bands deleted (that is, deletion size) was independent of phenotype. Some specific deletion types, however, correlated with the clinical severity of the disease. Deletion of exons containing *Hind*III fragments 33 and 34 and 33 to 35 were associated with BMD and were not found in patients with DMD. Deletions 3 to 7 occurred in four patients with the intermediate phenotype and one patient with BMD. Other shared deletions were associated with DMD, although in four cases patients with disease of intermediate severity apparently shared the same deletion with boys with DMD.

The range of phenotypes observed, and the overlap at the genetic level between severe and intermediate and mild and intermediate forms of dystrophy, emphasises the essential continuity of the clinical spectrum of DMD/BMD.

There were no characteristic deletions found in boys with mental retardation or short stature which differed from deletions in affected boys without these features.

Duchenne muscular dystrophy (DMD) is an X linked recessive neuromuscular disorder occurring in about one in 3000 males. Affected boys become wheelchair bound by 13 years of age and few live beyond the second decade. Becker muscular dystrophy (BMD) has a similar distribution of muscle weakness, but the course of the disease is slower and milder: ambulation is retained beyond 16 years and affected males may have a normal life span. About one third of boys with DMD have some degree of mental retardation,[1 2] whereas intelligence in BMD is usually normal.[3] There are also affected boys whose clinical course is intermediate between BMD and classical DMD, who become unable to walk between 13 and 16 years of age.

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The DMD and BMD loci are allelic and have been localised within Xp21.[4 5] The genomic sequences pERT87[6] and pXJ1.1[7] were found to detect submicroscopic deletions, by non-hybridisation of patient DNA to these sequences, in a proportion of patients with both DMD and BMD.[8 9]

cDNAs representing exons of the DMD/BMD gene (which codes for a 14 kb mRNA[10–14]) have been used to screen for exon deletions in affected males, and have shown deletions in over half the cases of DMD and BMD.[12 13 15]

The size of the deletion is not simply correlated with the severity of disease.[16 17] It has been postulated that some exon deletions cause DMD while others cause the milder BMD, because deletions causing DMD shift the translational open reading frame, resulting in a severely truncated protein gene product, whereas in BMD the translational reading frame may be maintained in phase, resulting in an abnormal, but partially functional, protein product.[17–19] This would be compatible with the observation that cases of BMD often have an abnormal size of dystrophin, whereas DMD patients almost invariably have no demonstrable dystrophin.[20]

Exon deletions, both at the 5′ and in the central region of the gene, have been described which appear specifically to be associated with a milder phenotype.[19 21] A 5′ deletion (of exons 3 to 7) has been found in six BMD, five intermediate, and two DMD patients.[21] It is predicted that this deletion disrupts the translational reading frame. These mild phenotypes may be the result of differential splicing, an alternative in frame translation start site, or a second promoter site, which could compensate for the effects of this frameshift mutation.

Since dystrophin may be expressed in neurones,[22 23] it may be important for normal neuronal development or function, and variable mental retardation may be an inherent effect of the DMD mutation.[1 24] We set out to correlate phenotypic characteristics of affected males with the position and extent of exon deletions detected using cDNA probes.

Patients

A total of 287 patients with DMD or BMD was ascertained from the Paediatric Muscle Clinic at the Hammersmith Hospital, and from the South-East Thames Regional Genetics Centre, Guy's Hospital, London. Clinical details were obtained personally or from the hospital records, and in a few cases by contacting the referring physician. In all cases the diagnosis was confirmed by the finding of markedly raised serum creatine kinase (CK) levels and characteristic muscle biopsy findings.[24] Information about developmental milestones, school performance, and height centiles was obtained from the clinical records, and was available for most cases ascertained through the Hammersmith and Guy's Hospital clinics. Physiotherapists' records were used to assess clinical severity in cases who had not yet lost ambulation at the time of study. In most cases no formal assessment of intelligence was possible; IQ tests had been performed on a few cases attending the Muscle Clinic at Hammersmith Hospital. In other cases an estimate of intelligence was made from the school performance record. Where the affected boy was attending a school for the educationally subnormal, this was taken to indicate educational subnormality (ESN).

Cases were divided into groups according to the age at which ambulation was lost, or the age reached without loss of ambulation. Most Duchenne cases had undergone Achilles tenotomies and the fitting of lightweight orthoses (calipers) when they were unable to walk any distance but could still stand alone and take a few steps. For the purposes of this survey this was used to indicate the age at which ambulation was lost.[25] In three cases who were still ambulant between 11½ and 12 years at the time of study (cases 46036, 46167, and 45049), the clinical impression was that they would lose ambulation between 13 and 16 years; these boys were therefore included in the group of intermediate severity. Cases 42403 and 45987, ambulant at 15 years of age, had only mild disability and were included in the BMD group (3). Cases were classified into the following groups.

Group 1: DMD where ambulation was lost before the 13th birthday. This group was subdivided into: (a) more severe DMD: ambulation lost before the age of 10 years. There were 37 boys in this group (29·8% of the total); (b) less severe DMD: ambulation lost between 10 years and 12 years 11 months of age. There were 30 boys in this group (24·2%).

Group 2: intermediate severity where ambulation was lost (or, in three cases, expected to be lost) between 13 years and 15 years 11 months of age. There were 12 boys in this group (9·7%).

Group 3: BMD where ambulation was maintained beyond 16 years of age (13 boys) (10·5%). Case 44230 lost ambulation at 16 years five months. All other boys in group 3 were still ambulant at 16 years of age or over (including cases 42403 and 45987, referred to above) and had mild disability on clinical assessment.

TABLE 1 *Proportions of clinical parameters found in each clinical severity group.*

Delay in walking	
Group 1 (a)	14/34 (41%)
Group 1 (b)	12/27 (44%)
Group 2	4/10 (40%)
Group 3	2/8 (25%)
Speech delay	
Group 1 (a)	20/28 (71%)
Group 1 (b)	17/21 (81%)
Group 2	4/4 (100%)
Group 3	2/3 (67%)
Stature=or<3rd centile for age	
Group 1 (a)	3/27 (11%)
Group 1 (b)	7/25 (28%)
Group 2	2/12 (17%)
Group 3	0/6
Mental retardation (severe or moderate)	
Group 1 (a)	3 severe+2 mild/28 (18%) [severe 11%]
Group 1 (b)	6 severe+6 mild/22 (55%) [severe 27%]
Group 2	4/10 (none severe) (40%)
Group 3	1/11 (none severe) (9%)

TABLE 2 *Clinical details of boys in each group.*

GROUP 1: DMD

Group 1 (a): lost ambulation before 10 years of age.

No	*First walked (mth)*	*First sentences*	*Height (centile)*	*Age ceased to walk*	*IQ*
33202	16	3 y	10	8 y 6 mth	SL R
46364	14	2 y	10	9 y	ESN (M)
33172	16		50	8 y 6 mth	N
46878	18	Late	10	8 y 6 mth	N
41622	24			7 y	N
43052	12–14		25	9 y	N
45061	13	18 mth	3	9 y 6 mth	
43053	24	3 y 6 mth	75	6 y 6 mth	
46162	15	2 y	25	8 y 6 mth	N
40106	18	2 y 6 mth	25	7 y 2 mth	R
33189	26	3 y	25	9 y 6 mth	R
33196	17	15 mth	<3	9 y 4 mth	N
48439				7 y	
44416	19	2 y 6 mth	25	8 y 6 mth	N
33180	30	>2 y	25	8 y	
33169	21	2 y		8 y	N
42915	16		10	8 y	N
44979	20	15 mth		9 y	N
46958	13		75	9 y	N
42203	18	2 y	10	7 y	N
38777	12	Early		6 y 9 mth	
44765	11	18 mth	50	9 y	
46038	17	2 y	50	8 y	N
33374	24		25	9 y	N
44966				9 y	N
45263	12	2 y 6 mth		8 y 1 mth	
41165	14	2 y 6 mth	25	9 y	N
13011	13	2 y		8 y	N
42915	24	2 y	<3	8 y	
48698	N			9 y	N
44988	21	3 y	50	8 y 3 mth	
45976	19	18 mth	50	8 y 9 mth	N
35706	15	N	25	9 y 3 mth	N
44601	12–18	Late		8 y 11 mth	N
33190	15	3 y	25	8 y 6 mth	IQ 84
45056		21 mth	50	9 y 8 mth	N
33197	14	2 y	25	9 y	N

Delay in talking >2 y: 20/28. Delay in walking >17 mth: 14/34.
Stature at or below 3rd centile: 3/27. Mentally retarded: 5/28 (3 severe).

Group 1 (b): lost ambulation between 10 years and 12 years 11 months of age.

No	*First walked (mth)*	*First sentences*	*Height (centile)*	*Age ceased to walk*	*IQ*
41790	42	2 y		12 y	N
33211	48	2 y 6 mth	50	12 y	ESN (M)
33175	24	4 y 6 mth	50	10 y	R
32936	15	1 y 6 mth	<3	11 y	
17748	18	2 y	50	10 y 9 mth	IQ 87
33187	14	3 y 6 mth	50	11 y	ESN (M)
45527	18		<3	10 y	SL R
33217	18		50	11 y 6 mth	
45070	15			10 y 9 mth	
37965	18	2 y	97	11 y 6 mth	N
48462			<3	10 y 6 mth	
46020	17	18 mth		10 y	
41443	17	18 mth	<3	12 y	N
42706	24	4 y 6 mth	3	10 y	
33216	12		<3	10 y	SL R
33205	15	3 y	10	11 y 6 mth	N
42512	24	3 y	10	12 y	SL R
53176			25	10 y	
20832	15	3 y 6 mth	10	11 y 6 mth	IQ 85
44189	24	5 y	10	12 y	IQ 71
45637				12 y	
20708	17	Delayed	10	10 y	N
43111	14		3–10	12 y	ESN
42513	12		<3	10 y	N
46018	27	Delayed	10	10 y 6 mth	N
42018	15	3 y	25	10 y	ESN
16861	18	2 y 6 mth		12 y	ESN (M)
44987	15	2 y	50	10 y 3 mth	IQ 112
45515	15	2 y 6 mth	25	11 y 6 mth	N
34517	15	Early	90	12 y 9 mth	N

Delay in talking >2 y: 17/21. Delay in walking >17 mth: 12/27.
Stature at or below 3rd centile: 7/25. Mentally retarded: 12/22 (6 severe).

GROUP 2: INTERMEDIATE

Group 2: lost ambulation between 13 and 16 years of age.

No	*First walked (mth)*	*First sentences*	*Height (centile)*	*Age ceased to walk*	*IQ*
46036	14		50		SL R
46167	14		25		
46405	22	3 y	25	14 y	SL R
08630	24		90	15 y	N
42786	14		25	14 y 6 mth	SL R
33193	13	2 y	10	13 y	N
33212	22		25	13 y	N
33174		3 y 6 mth	3	13 y	N
41688	22		10	13 y	N
46034	16	4 y	75	13 y	SL R
45049	12		3		N
33381			10	13 y 4 mth	

Delay in talking >2 y: 4/4. Delay in walking >17 mth: 4/10.
Stature at or below 3rd centile: 2/12. Mentally retarded: 4/10 (0 severe).

GROUP 3: BMD

Group 3: still walking after 16 years of age.

No	First walked (mth)	First sentences	Height (centile)	Age at investigation	IQ
44230	21	3 y	10	16 y 6 mth*	N
45002	14		50	18 y	N
44434				18 y	
33213	18		50	18 y 10 mth	N
38604	16			18 y	N
42403	14		97	15 y†	N
46115				17 y 4 mth	
45987	Normal			15 y†	SL R
39744				40 y	N
45996	14	2 y mth	10	23 y	N
43848				33y	N
43055	14	Normal	10	20 y	N
32060				25 y	

Delay in talking >2 y: 2/3. Delay in walking >17 mth: 2/8.
Stature at or below 3rd centile: 0/6. Mentally retarded: 1/11 (0 severe).
*Just off feet. †Still walking well at 15 y; expected to maintain ambulation beyond 16 y.

Group 4: less than 10 years at time of study; still ambulant.

No	First walked (mth)	First sentences	Height (centile)	Estimated group	IQ	Age
43991				1	N	6 y
44047	17	Normal			N	8 y
46535	12	2 y	50		N	3 y 6 mth
18402	12	2 y 6 mth	25	2	N	7 y 6 mth
41821	21	2 y 6 mth	25		N	8 y 6 mth
44268	14			1	N	6 y
45050	15	20 mth	3		N	3 y
40203	18	2 y 6 mth	3			8 y
39487	13	18 mth	25			8 y
43484	12			1		5 y 6 mth
45436	17	14 mth	10	1	SL R	9 y
42657	20					6 y
42583	12	20 mth	25			6 y 6 mth
42021	12	Late	10	1	N	7 y
48942	18		50			4 y
43485				1	N	7 y
44247	12	2 y	50		N	6 y
42095	23	3 y	25	1	N	8 y
40335	18	15 mth	25	1		6 y
41832	13	18 mth				6 y
36504	18	Normal		1	N	6 y
40329	18	18 mth			R	7 y
12376	Affected grandfather lost ambulation in 40s			3		5 y
37229			3	1	N	9 y
40784	19	2 y 6 mth				5 y
45480	14	3 y	10	3	R	7 y 6 mth
45036	15	>18 mth	10		SL R	3 y 6 mth
37797	20	3 y 6 mth	3–10			6 y
49951	14	2 y	25		N	5 y
42582	18	Slow	50			5 y 6 mth
46751	13	18 mth	<3	3	N	9 y
46116	24	2 y		1		8 y 6 mth

Delay in talking >2 y: 12/24. Delay in walking >17 mth: 12/29.
Stature at or below 3rd centile: 4/20. Mentally retarded: 4/19 (2 severe).
ESN=educationally subnormal. SL R=slight mental retardation.
N=normal. R=mental retardation.
Height given as centile of height for age.

TABLE 3 *Numbers assigned to HindIII exon bands.*

Exon band		No
5′		
9–7	3·2	1
	3·2	2
	4·2	3
XJcDNA1→	8·5	4
	3·1	5
	8·0	6
	4·6	7
	7·5	8
	10·5	9
30.2	4·2	10
	6·6	11
	2·7	12
	6·0	13
	1·7	14
	12·0	15
	3·0	16
30.1	7·3	17
	11·0	18
	20·0	19
	5·2	20
	12·0	21
	4·7	22
47.4	18·0	23
	0·45	24
	1·3	25
	1·8	26
	1·5	27
	6·0	28
	6·2	29
CF23a→	11·0	30
	4·2	31
	4·1	32
	0·5	33
	1·5*	
44.1	10·0	34
	1·25	35
	3·8	36
	1·6	37
	3·7	38
	3·1	39
	7·0	40
63.1 (9)	7·8	41
	8·3	42
	1·0	43
	2·3	44
	1·0	45
	8·8	46
63.1 (10)	6·0	47
	3·5	48
	2·6	49
	2·8	50
	6·6	51
	12·0	52
	2·4	53
63.1 (11–14)	1·45	54
	1·5	55
	1·9	56
	2·1	57
	5·2?	58
	6·8	59
	10·0	60
	1·8	61
	5·9	62
	7·8	63
	6·0	64
3′		

*Hind*III band fragments detected by cDNA probes used in this study. Exon fragments are numbered 1 to 64, ordered on data from Koenig *et al.*[13] Malhotra *et al.*[21] Burghes *et al.*[30] and Wapenaar *et al.*[31] Sizes of *Hind*III fragments are in kilobases. The order of the bracketed exon fragments is not known. Band 58 was not evident on our autoradiograms.
*This exon fragment is not numbered as it forms part of the next 10 kb exon fragment, but is split by a *Hind*III site in the cDNA. The exact position of other split exons is not known.

Group 4 comprised boys still ambulant but below the age of 10 years at the time of the study. There were 32 boys in this group (25·8% of the total). Where assessment of severity could be made, 11 were thought to be in group 1, one in group 2, and three in group 3. This group was, however, analysed separately.

Four clinical parameters were assessed wherever possible.

(1) The age at which the child first walked (based on history from the parents).
(2) The age at which two or three words were first used together to form sentences (based on history from the parents).
(3) The height centile taken in the clinic before ambulation was lost.
(4) The level of intelligence.

When onset of walking was delayed to 18 months of age or beyond, this was categorised as abnormally delayed. Speech delay was defined as the inability to make simple sentences after the age of two years.[26]

Clinical details of probands are shown in tables 1 and 2.

This study includes only those affected males who were found to have deletions of one or more exons. Clinical data were categorised into severity groups without knowledge of the patient deletion data. Similarly, deletions were characterised without knowledge of the clinical details.

DNA analysis

Genomic DNA was made as previously described[27] or, more recently, using a 340A Nucleic Acid Extractor (Applied Biosystems Ltd). DNA restriction endonuclease digestion, agarose gel electrophoresis, and Southern blotting were carried out using standard procedures.[28] Probe DNA was radioactively labelled using a hexanucleotide primed reaction.[29] Filters were prehybridised for a minimum of two hours in 3×SSC (20×SSC=3 mol/l sodium chloride, 0·3 mol/l trisodium citrate), 0·1% sodium dodecyl sulphate, 100 μg/ml denatured salmon sperm DNA, 2×Denhardt's solution (100×=2% Ficoll 400, 2% polyvinyl-pyrrolidone, and 2% bovine serum albumin (pentax fraction V)) and 8% dextran sulphate. Hybridisation was carried out overnight at 65°C in the same solution, with addition of denatured radioactive probe. Filters were washed down to 0·2×SSC, 0·1% SDS at 65°C for 30 minutes each. Autoradiography was carried out at −70°C using intensifying screens.

The complete cloned cDNA from the DMD/BMD gene[13] was made available as six inserts in Bluescript or Bluescribe plasmid vectors, called 9.7, 30.2, 30.1,

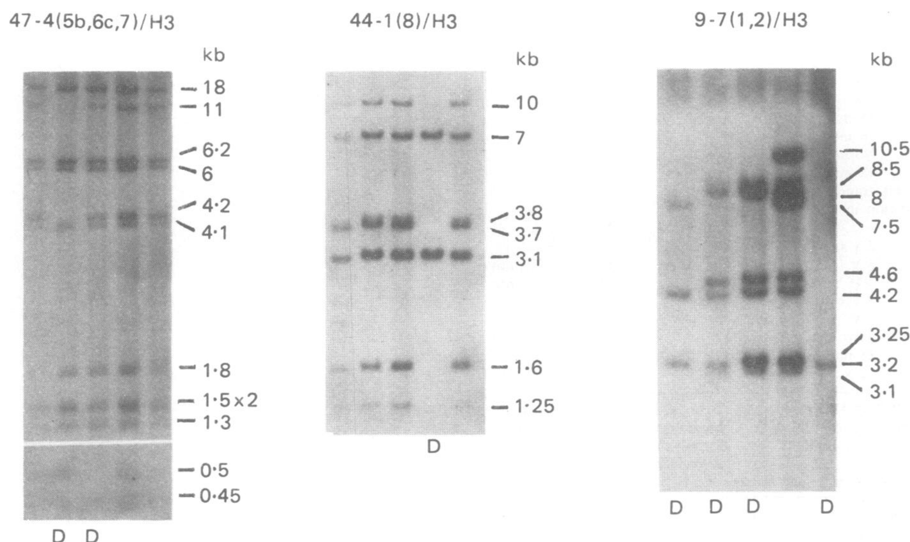


FIG 1 *Examples of HindIII restriction endonuclease digestions of genomic DNA from DMD/BMD patients, probed with the cDNA clones 9–7, 47–4, and 44–1.[14] The normal complement of fragments detected by each probe is shown on the right of each autoradiograph. Sizes are given in kilobases (kb). Lanes showing deletion of one or more fragments are identified by a D.*

47.4, 44.1, and 63.1 (in 5′ to 3′ order). They detected 65 *Hind*III fragments on genomic DNA, with five *Hind*III sites in the cDNA, thus representing a minimum of 60 exons. The 14 kb cDNA had been divided into 14 short probes to produce band patterns simple enough to be interpreted.[13] In this study, purified inserts were used as probes for 9.7, 30.2, 30.1, 47.4, and 44.1, corresponding approximately to probes 1+2, 2+3, 4+5a, 5b+6c+7, and 8, respectively. 63.1 was digested with *Bam*HI to give three smaller inserts that were used as separate probes, namely 63.1/9, 63.1/10, and 63.1/11–14, corresponding to probes 9, 10, and 11+12+13+14, respectively.[13] cDNA clones XJcdna1[11] and CF23a[12] were also used in this study. XJcdna1 detected the same exon fragments as 9.7 and part of 30.2, while CF23a detected the same exon fragments as the 3′ end of 47.4 (6c+7). Exon band ordering was as reported for *Hind*III fragments.[11 13 30 31] For simplicity, the exon containing fragments were numbered 1 to 64 from the 5′ end of the gene (table 3).

Genomic deletions[32] were further mapped by hybridisation against cDNA probes mapping to the deleted loci. Further deletions were detected by routine screening with cDNA probes 44.1, 47.4 or CF23a, and XJcdna1. Remaining undeleted patients were screened with 30.2, 30.1, 63.1/9, 63.1/10, and 63.1/11–14 cDNAs. Deletions detected were delineated in full with the requisite cDNAs.[33]

Results

A total of 163 of the 287 patients initially screened was found to have molecular deletions in the dystrophin gene, and in 142 of these the deletion was delineated with cDNA probes.[33] In 124 of these patients there was sufficient clinical information to allow clinical severity group allocation. In those families where there were multiple DMD/BMD cases, the disease severity and the deletion were concordant in the different affected males. Only one affected boy per kindred was included in this survey. As far as we are aware, the deletions in this study therefore represent 124 independent mutations.

Deletions were usually defined by the presence or absence of hybridisation signals (fig 1). The solid lines in figs 2 and 3 represent the extents of the deletions. In some cases it was not possible to define the boundary of the deletion clearly (owing to the neighbouring exon fragment giving an equivocal or obscured signal). This is denoted by a question mark in the diagram. In other cases, a junction fragment was indicated by the appearance of an aberrant sized band; this is shown by a 'J' in the diagrams (figs 2 and 3). Most deletions were simple linear deletions, but one (46116 in group 4) appeared to be a complex mutation, because of the presence of two abnormally sized bands.[33] Fig 2 shows the sites and extents of the deletions found in groups 1 to 4, indicating those associated with short stature and mental retardation. Fig 3 shows the total range of deletions.

It can be seen that 74 different deletions were identified; 55 were found in only one affected boy, whereas 19 deletions were found in more than one unrelated subject. Table 4 summarises the clinical groups to which the boys found to have common deletions were assigned. The phenotypes were all severe (DMD, group 1) in deletions 32, 33–38, 34, 36–38, 37, and 38. Only mild BMD phenotypes (group 3) were found in deletions 33–34 and 33–35. The intermediate phenotype was predominant in deletion 3–7, but one case had BMD. In each of deletions 8, 33, 33–36, and 39, one case with the intermediate phenotype (group 2) was found in addition to cases of the DMD phenotype.

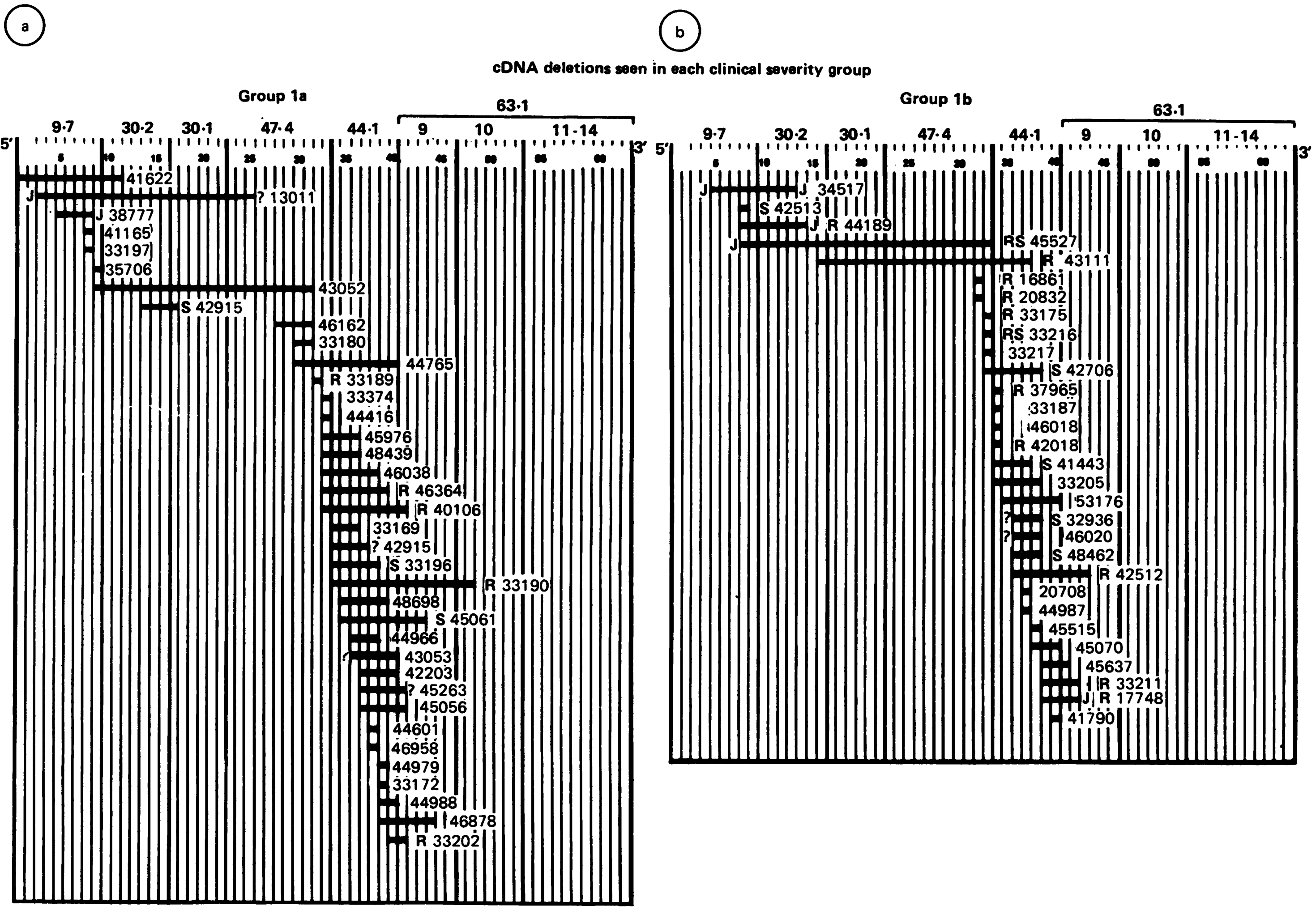
In nine of the group 4 cases sharing common deletions, where severity could be judged, there was concordance between the assessed severity of group 4 cases and those in groups 1 to 3 found to have the same deletions (see table 4).

There was no obvious difference between the distribution and size of deletions in boys with mental retardation and those of normal intelligence (fig 2, table 4). Cases with mental retardation were not found to have deletions confined to any one region of the gene, and large deletions occurred both in boys with normal and in those with low intelligence. There was similarly no obvious difference between the distribution and size of deletions found in cases with short stature and those with normal stature.

Discussion

Within the DMD/BMD spectrum, DMD has been defined by the loss of ambulation by the age of 13 years, whereas ambulation retained beyond the age of 16 years was diagnostic of BMD.[3] It can be seen from our clinical data that there is no clear dividing line between DMD and BMD, and that 10% of patients have disease of intermediate severity, where ambulation is lost between 13 and 16 years of age. This concurs with observations from other groups.[34 35]

Analysis of the deletions (table 4, figs 2 and 3) shows that they are heterogeneous in size and widely distributed within the DMD/BMD gene. There are two regions of the gene prone to deletions, as noted by others.[12 13 32 36] Where two or more boys have apparently the same deletion, they are of similar


a
b
cDNA deletions seen in each clinical severity group
Group 1a
Group 1b
9·7
30·2
30·1
47·4
44·1
63·1
9
10
11-14
5'
3'
41622
J
? 13011
J 38777
41165
33197
35706
43052
S 42915
46162
33180
44765
R 33189
33374
44416
45976
48439
46038
R 46364
R 40106
33169
? 42915
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46018
R 42018
S 41443
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R 17748
41790

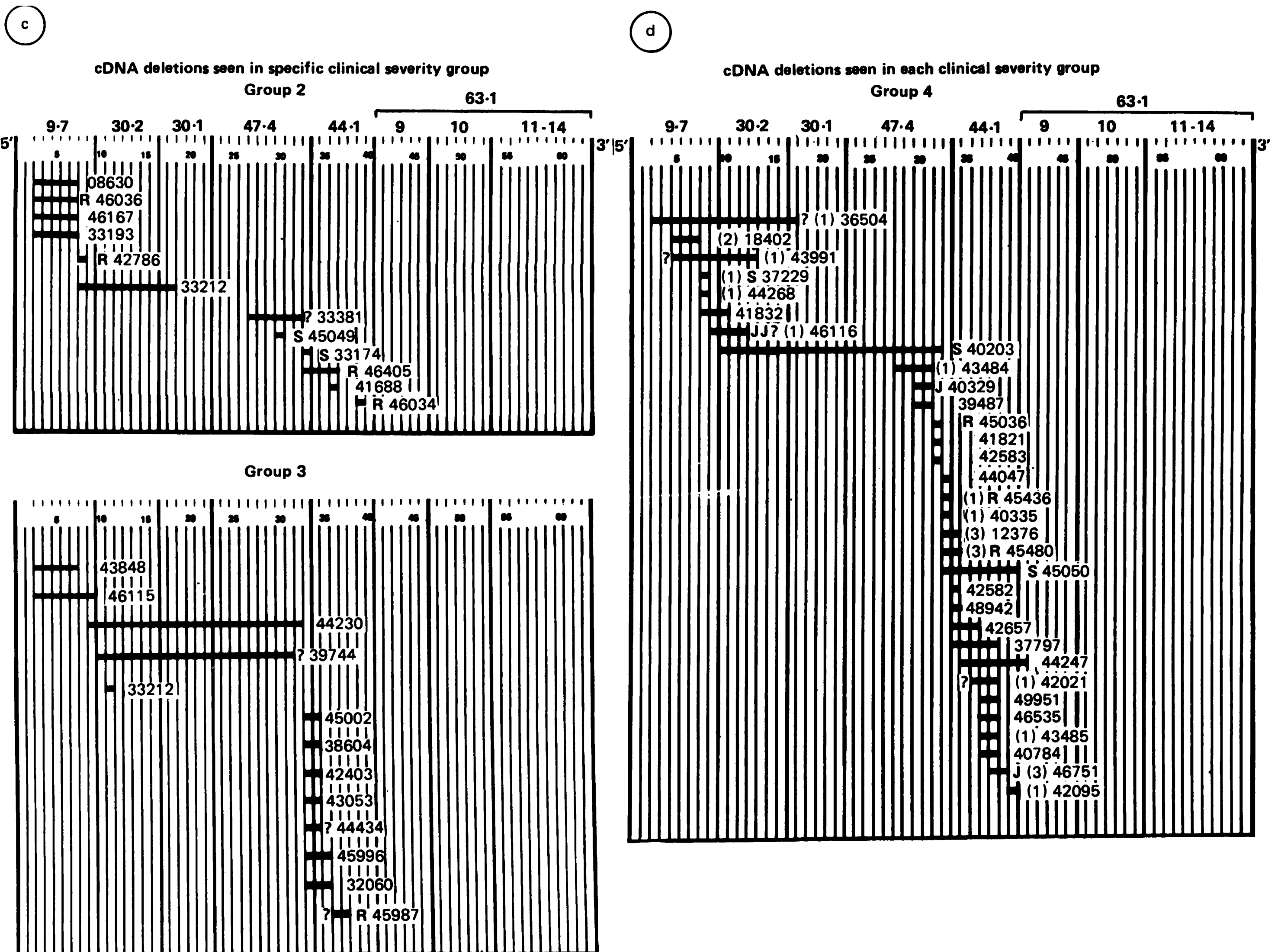

FIG 2 *cDNA deletions seen in each clinical severity group. Each horizontal bar represents the extent of the deletion found in each patient (identified by a number). The exon bands are indicated at the top of the vertical lines, numbered 1 to 64, corresponding to exon fragments as shown in table 3. J=junction fragment. ?=boundary of deletion not defined with certainty. R=mental retardation in the affected subject with the deletion. S=short stature in the affected subject with the deletion. In group 4, suggested severity group is indicated in brackets where applicable.*

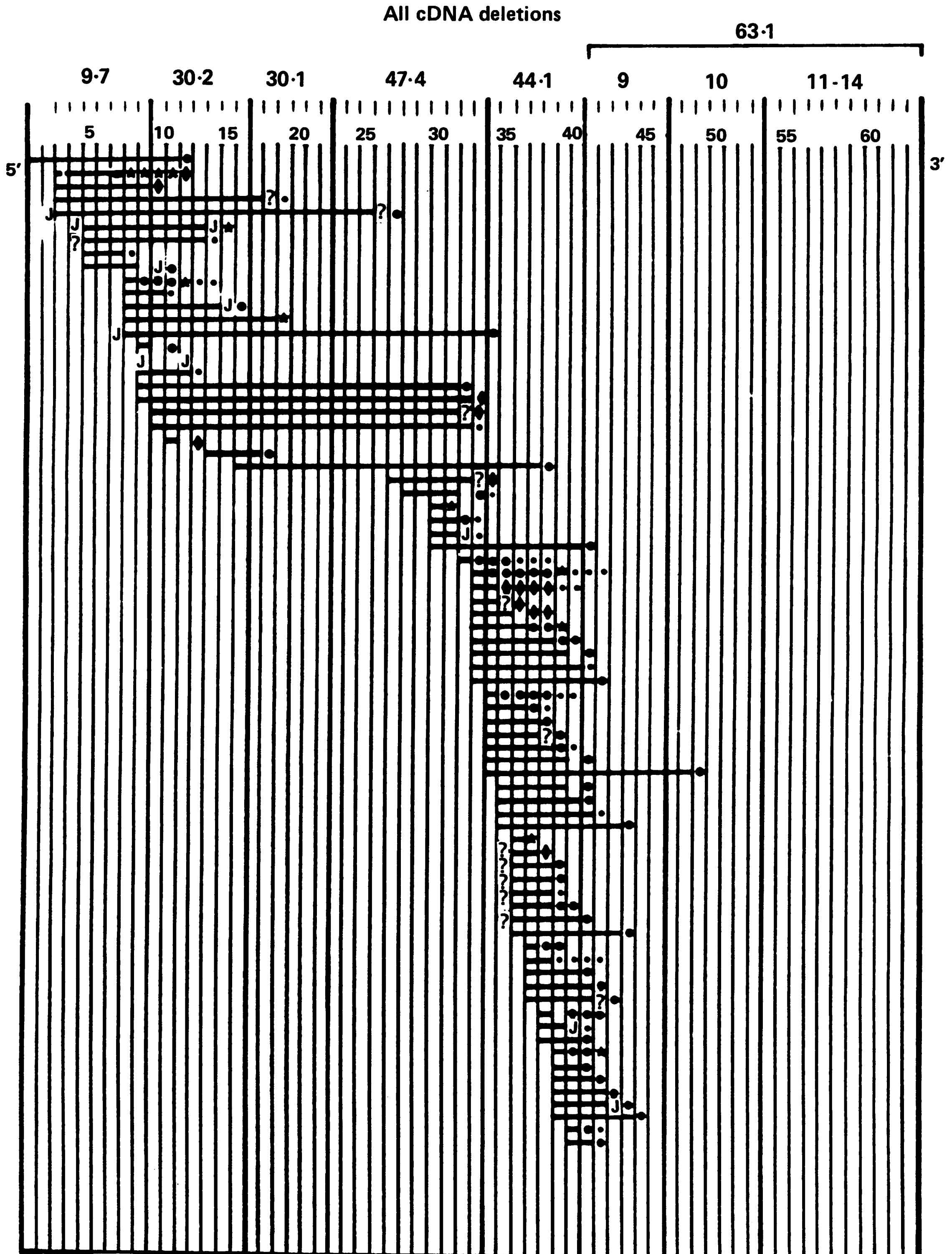

FIG 3 *All cDNA deletions. Each separate deletion defined in this survey is represented by a horizontal bar. Some deletions were found in one subject only, but others were found in more than one. The symbols indicate the clinical group of the subject with that deletion, and where more than one subject had the deletion, each one is represented by one symbol, shown next to the relevant horizontal bar; the number of symbols thus indicates the number of boys shown to have the deletion.* ●=*patient from group 1.* ★=*patient from group 2.* ◆=*patient from group 3.* •=*patient from group 4.*

TABLE 4 *Clinical severity and frequency of cases occurring with shared deletions.*

Deletion type	*Clinical severity (by group)*					
	Group 1 *a*	*b*	*Group 2*	*Group 3*	*Group 4* *[estimated severity in brackets]*	
3–7			4 (R, N, N)	1 (N)		
8	2 (N, N)	1 (S)	1 (R)		2 (SN, N)	[2 gp 1]
28–31	1 (S)				1	[gp 1]
30–31	1				1	
32	1 (R)	2 (R, R)			3 (R, N)	
33	2 (N, N)	3 (R, R, S)	1 (S)		3 (R, N)	[3 gp 1]
33–34				4 (N, N, N)	2 (R)	[1 gp 3]
33–35				2 (N)		
33–36	2 (N)		1 (R)			
33–38	1 (N)	1 (S)				
34		4 (R, N, R, R)			2	
34–36	1 (N)				1	
34–38		1 (N)			1	
36–38	1	1 (S)				
37		2 (N)				
37–38					4 (N, N, N)	[1 gp 1]
38	2 (N, N)	1 (N)				
39	2 (N, N)		1 (R)			
40		1 (N)			1 (N)	[1 gp 1]

R=mental retardation
N=normal intelligence
S =short stature
} where any of these are known.
Numbers in each column indicate numbers of affected subjects in that group bearing the deletion indicated.

phenotype (table 4). In some cases, however, the same deletion was found in boys with severe (group 1) and intermediate (group 2) phenotypes.

The hypothesis that DMD results from a frameshift deletion, and that BMD results from an in frame deletion, is supported by some of our data. Several examples were found in which deletions differing by one exon containing fragment were associated with disease of mild phenotype in one case and severe in another. This suggested that the deletion of a specific exon containing fragment had altered the translational reading frame. For example, deletion of exon fragment 33 occurred in boys with DMD, but deletion 33–34, longer by the inclusion of exon band 34, occurred in boys with BMD. Conversely, a deletion of exon fragments 33–35 was found in two boys with BMD, but the addition of deletion of fragment 36 to this was associated with DMD, suggesting that a deletion of fragment 36 alone renders the translational reading frame out of phase. Disease of intermediate type in a boy who lost ambulation at 13 years of age was found in a patient with a deletion of fragment 36 only. Similarly, deletion of exon bands 9–31 gave a DMD phenotype (fig 3), while deletion of exon bands 9–32 gave a BMD phenotype. This would suggest that exon band 32 contains a frameshift exon (or exons) to bring the BMD case back in frame. This is substantiated by the demonstration that deletion of exon band 32 alone produced a DMD phenotype in three cases.

Deletion of exon bands 33 with 34 gave rise to BMD, and inclusion of the next exon band (35) in the deletion still caused a BMD phenotype. Smaller deletions within these BMD deletions gave rise to DMD; for example, exon band 33 only or exon band 34 only, suggesting that these smaller deletions each bring the reading frame out of phase, but when deleted together they result in an in frame message.

The finding of the same deletion (3–7) in five boys, four of whom have the intermediate phenotype (group 2) and one of whom has BMD, strongly suggests that this particular deletion is associated with disease of intermediate or mild severity.[21 33] Seven of our patients (Nos 46036, 33193, 08630, 42786, 18402, 41165, and 37229) with deletions within the first 10 exons of the DMD/BMD gene were included in the paper by Malhotra *et al.*[21] Four further cases from other centres show 3–7 exon deletions in association with a BMD or intermediate phenotype, despite the fact that exon/codon border analysis suggests that this deletion would cause a translational frameshift.[21]

Four domains of dystrophin have been postulated, based on the predicted amino acid sequence.[16] The 5′ end (exons 3 to 9) is homologous with the actin binding domain of α actinin[37]; a long triple helical repeat segment is then found before a cystein rich domain and the c′ terminal domain. Large deletions of the helical segment may be possible without much reduction in function of the protein product. This is borne out by the observation that a long 10–31

deletion was found in BMD patient 39744 and deletion 9–32 in BMD patient 44230. Deletion 3–7 occurs within the actin binding homologous region.

Deletions in patients with the milder phenotypes are more homogeneous than the DMD deletions in this series. Of the 25 deletions found in phenotypic groups 2 and 3, five delete exons 3–7, five delete exons 33–34, and two delete exons 33–35. Thus only 13 in this group are unique deletions, and of the 13 BMD deletions only five are unique. Read *et al*[19] also reported a high proportion of 33–34 (seven cases) and 33–35 (three cases) deletions in 14 BMD patients.

Some deletions are found in patients with both the intermediate and the severe phenotype. Patients from groups 1 and 2 share the same deletion for exons 8, 33, 33–36, and 39, suggesting that this degree of variation in phenotype can result from non-allelic variation, related either to genetic background or to environmental factors. In dystrophic dogs with no demonstrable dystrophin, the severity of their disease varies between litters even when they are presumed (by descent from the same affected male) to carry the same mutation.[38]

As previously suggested,[1 39–41] we found that the degree of mental retardation in muscular dystrophy was related to the severity of the disorder. It occurred in 34% of DMD patients, in 40% of those in group 2, and in 9% of boys with BMD. Severe retardation was not found in boys in groups 2 and 3, but occurred in 18% of those in group 1 (table 1). Delayed speech occurred in 74% of boys in group 1, and 100% of boys in group 2, but also in 67% of boys in group 3 (table 1). It is doubtful whether this data on delayed speech, depending on a retrospective history from the parents, is of any discriminative value.

The distribution of deletions in boys with mental retardation was similar to that seen in all patients and showed no unique abnormalities. Three deletion types are represented by more than one patient with mental retardation (table 4; these are deletions 32, 33, and 34), but patients without mental retardation are also found to have these deletions.

Despite the suggestion that short stature with growth hormone insufficiency may be associated with amelioration of the progression of DMD,[42] it appears from our survey that short stature is found more commonly in boys with severe DMD; 19% of boys in group 1 and 17% of those in group 2, but none of the boys in group 3, were at or below the 3rd centile for height. Short stature may therefore be one of the manifestations of the disease itself.[43]

Affected boys with short stature were found to have deletions throughout the deletion spectrum, and boys with and without short stature share deletions 8, 28–31, 33, 33–38, and 36–38 (table 4). It appears, therefore, that short stature and mental retardation are not the result of specific deletion mutations of the DMD gene.

It is hoped that studies correlating the molecular abnormalities of dystrophin with the deletion data in these patients will lead to further understanding of the pathogenetic mechanisms involved in Duchenne and Becker muscular dystrophy.

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