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Topography of the Duchenne Muscular Dystrophy (DMD) Gene: FIGE and cDNA Analysis of 194 Cases Reveals 115 Deletions and 13 Duplications

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Summary

We have studied 34 Becker and 160 Duchenne muscular dystrophy (DMD) patients with the dystrophin cDNA, using conventional blots and FIGE analysis. One hundred twenty-eight mutations (65%) were found, 115 deletions and 13 duplications, of which 106 deletions and 11 duplications could be precisely mapped in relation to both the mRNA and the major and minor mutation hot spots. Junction fragments, ideal markers for carrier detection, were found in 23 (17%) of the 128 cases. We identified eight new cDNA RFLPs within the DMD gene. With the use of cDNA probes we have completed the long-range map of the DMD gene, by the identification of a 680-kb *Sfi*I fragment containing the gene's 3′ end. The size of the DMD gene is now determined to be about 2.3 million basepairs. The combination of cDNA hybridizations with long-range analysis of deletion and duplication patients yields a global picture of the exon spacing within the dystrophin gene. The gene shows a large variability of intron size, ranging from only a few kilobases to 160–180 kb for the P20 intron.

Introduction

One of the first genes isolated by means of 'reverse genetics' was the Duchenne muscular dystrophy (DMD) gene (Monaco et al. 1986; Burghes et al. 1987; Koenig et al. 1987). DMD, allelic with the milder Becker muscular dystrophy (BMD) (Emery 1988), is an X-linked myopathy resulting in death of the patients by early adulthood. With an incidence of 1/3,500 live male births, it is one of the commonest severe hereditary diseases known (Emery 1988). The DMD gene covers more than 2 million bp (Mbp) (Kenwrick et al. 1987; Van Ommen et al. 1987; Burmeister et al. 1988) and encodes a 14-kb mRNA (Monaco et al. 1986; Koenig et al. 1987), which is translated into a membrane-associated protein of 3,685 amino acids (430 kD), named "dystrophin" (Hoffman et al. 1987; Arahata et al. 1988; Bonilla et al. 1988; Koenig et al. 1988; Watkins et al. 1988; Zubrzycka-Gaarn et al. 1988).

BMD/DMD carrier detection and prenatal diagnosis were initially performed with randomly isolated genomic probes flanking the gene (Wieacker et al. 1983; Bakker et al. 1985). Later, intragenic and deletion-junction probes were isolated, and ultimately the cDNA was cloned (Monaco et al. 1985, 1986; Ray et al. 1985; Burghes et al. 1987; Koenig et al. 1987; Van Ommen et al. 1987). Genomic, cDNA, and field-inversion gel electrophoresis (FIGE) studies (Den Dunnen et al. 1987; Forrest et al. 1987; Koenig et al. 1987; Darras et al. 1988) have shown independently that a majority of all DMD mutations are partial gene deletions.

Current long-range maps of the DMD region were derived from hybridizations with (mostly) randomly isolated genomic probes. (Den Dunnen et al. 1987; Kenwrick et al. 1987; Van Ommen et al. 1987; Burmeister et al. 1988). However, not all fragments inferred from partial digestion have been detected individually, and

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the 3′ end of the gene has not yet been defined. This has precluded a definite estimate of the total gene size, and it left the possibility of undetected interspersed fragments. The dystrophin cDNA constitutes a probe with numerous target sites over the entire region. We have used it in a FIGE analysis to resolve the remaining uncertainties.

Furthermore, the use of cDNA probes for the diagnosis of deletions or duplications in carrier females, especially when no patients are alive, requires precise quantitative Southern analysis. In those cases, FIGE analysis has the advantage of yielding an altered fragment next to a normal internal control (Den Dunnen et al. 1987; Chen et al. 1988). However, as we have pointed out elsewhere (Den Dunnen et al. 1987), small rearrangements may be missed, and altered FIGE fragments might not always involve expressed sequences.

To address these questions, and to attempt to detect directly the underlying mutation in more cases, we have studied, with the dystrophin cDNA, all our BMD/DMD patients. In this study, we report the results of three different approaches used to define the genetic defects: (1) deletion/duplication detection with cDNA probes, (2) long-range mapping by FIGE analysis, and (3) haplotype analysis with genomic probes. The combined study results in an overall topographical picture of the DMD locus, including a refined localization of genomic probes, restriction-enzyme sites, and exons spread over the 2.3 Mb covered by the dystrophin gene.

Material and Methods

DNA Probes

DNA probes (and loci) referred to in the present paper are 754 (DXS84), pERT84–10 (DXS142), XJ1.1 (DXS206), pERT87–1 (DXS164), JBir (DXS270), P20 (DXS269), GMGX11 (DXS239), J66 (DXS268), L1 (DXS68), C7 (DXS28), and B24 (DXS67) (Davies et al. 1987). Dystrophin cDNA probes (provided by L. Kunkel [Koenig et al. 1987]) are indicated according to their mRNA position. We used, successively this order, cDNA8, cDNA0–2a, cDNA5b–7, cDNA2b–3a, cDNA3b–5a, cDNA9–10 (7,875–9,786 bp), cDNA11 (9,786–11,137 bp), and cDNA13–14 (12,096–13,973 bp); cDNA12 was not used. cDNA8 was divided into cDNA8a and cDNA8b at the *Bgl*II site (7,420 bp). Initially, we used cDNA0–2 (cDNA-XJ10, provided by R. Worton [Burghes et al. 1987]), but this gives a more complex hybridization pattern and does not detect exon 1 as well as does cDNA0–2a.

Screening of Patients

Isolation of high-molecular-weight DNA, both in solution and in agarose blocks, from whole-blood leukocytes or from Epstein-Barr virus–transformed cell lines; their restriction-enzyme digestion; gel electrophoresis (conventional or FIGE); blotting; and hybridization have been described elsewhere (Bakker et al. 1985, 1989; Den Dunnen et al. 1987). Probes were labeled with a multiprime kit (Amersham). FIGE electrophoretic equipment used was the Gene-Tic (Biocent, NL) (Den Dunnen et al. 1987).

To confirm deletions/duplications and to exclude RFLPs, we performed at least two different digestions (*Hin*dIII and *Eco*RI) with the DNA sample of each patient. We also often used *Eco*RV and *Pst*I digestions or existing blots, made in the course of our routine family haplotype analysis (Bakker et al. 1989).

Nomenclature of Exonic HindIII Fragments

The 14-kb cDNA detects 65 genomic *Hin*dIII fragments (Hd fragments) (Koenig et al. 1987, 1989). They have been numbered from 5′ to 3′, and we describe them, e.g., as Hd33 for the 0.5-kb Hd fragment 33 (table 1). We find Hd10, described as a 4.2-kb fragment, to be 3.9 kb in size. Hd64 and Hd66 are considered to be the same fragment of 6.0 kb (McCabe et al. 1989). Most Hd fragments correspond to only one exon of the DMD gene, but at least Hd8, Hd9, and Hd12 contain two exons (Koenig et al. 1987; Monaco et al. 1988). The cDNA has five internal *Hin*dIII sites, resulting in only three cases in single exons hybridizing detectably to two genomic Hd fragments. This applies to Hd36+37, Hd42+43, and Hd64+65, which straddle cDNA *Hin*dIII sites at positions 7,193, 8,012, and 12,972 bp, respectively (Chamberlain et al. 1988; Koenig et al. 1988; Baumbach et al. 1989). cDNA sequence and patient deletion data indicate that Hd34 (1.5 kb) and Hd35 (10 kb) each contain a different exon. The exon on Hd34 contains cDNA *Hin*dIII site 6,953 bp and extends for 12 bp into the adjacent Hd35 which contains a second exon further downstream (Baumbach et al. 1989). Similarly, *Hin*dIII site 9,281 is located at the extreme 3′ end of the J66 exon (see Results).

Sequencing

Fragments of interest were subcloned in pKUN1 (Konings et al. 1986) and were sequenced with the dideoxy chain termination method on double-stranded DNA by using SEQUENASE protocols (UBS Corp.).

Results

cDNA Analysis of Patients

Of our 322 BMD/DMD families in 194 cases, DNA of the proband was available for this study (34 BMD and 160 DMD). Although not all probes were tested on each sample, screening with the dystrophin cDNA enabled us to detect the mutation in 128 cases (65%): 115 deletions, including 17 in BMD, and 13 duplications (one in BMD). So far, we have not observed deletion and duplication together in one patient.

Deletions

The majority of the mutations detected were genomic deletions. An example is shown in figure 1*A*. Patient DL129.3 is deleted for Hd36–41 (detectable with cDNA8). *Hin*dIII digests screened with cDNA5b–7 and cDNA9–14 show no abnormalities. Although Hd35 is normal (lane 1), the corresponding *Eco*RI fragment (table 1) is altered (lane 4), so the deletion begins just distal to Hd35.

Figure 2 shows all 106 deletions which were precisely mapped in relation to the dystrophin cDNA. Individual deletions vary greatly in length, and, while they may affect any part of the cDNA, they clearly have a nonrandom distribution, consistent with previous publications and parallel studies (Den Dunnen et al. 1987; Forrest et al. 1987; Koenig et al. 1987; Darras et al. 1988; Baumbach et al. 1989; Koenig et al. 1989). A minor deletion hot spot is found in the 5′ XJ region, around cDNA position 1 kb. A major hot spot lies just beyond the middle of the gene, in the P20 region (Wapenaar et al. 1987; Blonden et al. 1989), around cDNA position 7 kb. The deletions at the 5′ end tend to span larger regions and are more heterogeneous in size than the central deletions. In the region of 3.0–5.8 kb, i.e., between p87 and JBir, deletions are rare. Deletions at the 3′ end of the cDNA, distal to the J66 region beyond 9.5 kb, are rarer yet.

Duplications

In addition to the deletions, a number of the patients have a duplication of genomic DNA. Figure 1*B* shows the cDNA5b–7 hybridization to DNA of patient DL117.4 (lanes 2 and 4). Hd33 and Hd35 (asterisks) show a stronger intensity, and so do the corresponding *Eco*RI fragments (lane 4; table 1). The increased intensity of Hd34 is masked by the strong hybridization of the comigrating Hd27 fragment. cDNA8 detects duplicated bands as well (not shown). Thus, DL117.4 carries a partial intragenic duplication, including exons on Hd33–35 and extending toward the 3′ end. Unfortunately, we have no cell line available from this patient to size this duplication by FIGE analysis.

Exact duplication borders are hard to establish, since dosage comparisons of hybridization intensities are difficult. Two duplications have not been defined precisely. Two of the 11 duplications, which could be precisely mapped in relation to the cDNA (fig. 2), were first detected by FIGE analysis. We find that duplications usually span only a few exons, and so far they are located near the two mutation hot spots. Notably, duplications were found more frequently near the minor hot spot than near the major one (fig. 2). Our figure of 10% (13/128) duplications in BMD/DMD mutations exceeds previous figures. While only few studies report duplications (Den Dunnen et al. 1987; Hu et al. 1988, 1989), and while some report none at all (Forrest et al. 1987; Koenig et al. 1987; Darras et al. 1988), we think that these discrepancies are probably due to the ease with which duplications are overlooked.

Junction Fragments

In 23 (17%) of 128 deletion and duplication cases, we could identify junction fragments (fig. 1*A* and 1*C*), locating their breakpoints close to the adjacent exon. The presence of junction fragments greatly facilitates the identification of carrier females (fig. 1*C*): BMD patient BL19.1 (lane 1) has a deletion starting in cDNA5b–7 (Hd33) and ending in Hd35 (all restriction enzymes tested show an altered fragment). His sister (lane 2), a healthy uncle (lane 4), and two nieces (lanes 5 and 6) lack the altered fragment. However, his aunt (lane 3) has the altered fragment, so she definitely is a carrier.

In only four cases (3%), all restriction enzymes tested produced junction fragments, locating the breakpoints either extremely close to or within an exon. Probably only one of these four actually affects exonic sequences, since in this case the junction fragments also showed a decreased hybridization intensity. In a gene of 2.3 Mbp (see below) containing 14-kb (0.6%) coding sequences, rearrangement breakpoints are expected to occur within exons in about 1.2% of the cases. We conclude, therefore, that at least 97% of the BMD/DMD deletions, while having heterogeneous breakpoints at the DNA level, should involve deletions of integral sets of exons at the mRNA level.

New RFLPs

cDNA analysis of our family material (Belgian and

Table I

Order of Exon-containing Fragments and Position on the Long-Range *Sfi*I Map

A.

Probe[a]	Hd Fragment	*Hin*dIII Size (kb)	*Hin*dIII Number[b]	*Sfi*I Fragment
1a	1	3.2	7a	BC
1a	2	3.25	7b	BC
1a	3	4.2	6	BC
1a	4	8.5	2	BC
1b	5	3.1	8	BC
1b	6	8.0	3	BC
1b	7	4.6	5	BC
2a	8[c]	7.5	4	BC
2a	9[c]	10.5	1	CD
	9[c,d]	10.5	1b	CD
2b	10	3.9	5	CD
2b	11	6.6	3	CD
2b/3a	12[c]	2.7	7	CD
				CD
3a	13	6.0	4	CD
3a	14	1.7	8	CD
3b	15[c]	12.0	1a	CD
3b	16	3.0	6	CD
3b	17	7.3	2	CD
	17	7.3	3	CD
4a	18	11	2b	CD
4a	19	20	1a	CD
4a	20	5.2	4	CD
4a	21[e]	12	2a	CE
4b	22[e]	4.7	7	CE
5a	23a[f]	18	1b	DE
5b	23b[f]	18	1c	DE
	23b[f]	18	1	DE
6a	24[g]	.45	12	DE
6a	25[g]	1.3	10	DE
6a	26[g]	1.8	8	DE
6b	27[d]	1.5	9b	DE
6b	28	6.0	5	DF
7a	29	6.2	4	DF
7a	30	4.2	6	DF
7b	31	11.0	2	EF

B.

Probe	Hd Fragment	*Hin*dIII Size (kb)	*Hin*dIII Number[b]	*Eco*RI Size (kb)	*Eco*RI Number[b]	*Pst*I Size (kb)	*Pst*I Number[b]	*Eco*RV Size (kb)	*Eco*RV Number[b]	*Bgl*II Size (kb)	*Bgl*II Number[b]	*Sfi*I Fragment
7b	32[h]	4.1	7	25	1		4	2	8	3.4		EF
7b	33[h]	0.5	11	4.2	5	3.5		14	3	2.8		EF
8a	34	1.5	9a	12	2a	5	8	20	1	3.3		EF
8a	35	10	3							3.5		EF
	35	10	1	25	2	5.0	3	20	1	3.5	3a	EF
8a	36[i]	1.25	6	13.5	3	4.7	4	13	3	11	1	EF
8a	37[i]	3.8	3a									
8a	38	1.6	5	10.5	5	5.6	2	14	2	7.4	2	FG
8b	39	3.7	3b	28	1	1.0	6	5.9	5	2.6	4	FG
8b	40	3.1	4	11	4	3.4	5	0.85	6	1.0	5	FG
8b	41	7.0	2	6.2	6	11.8	1	11	4	3.5	3b	FG

(*continued*)

Table 1 (continued)

C.				
		*Hin*dIII		
PROBE[a]	Hd FRAGMENT	Size (kb)	Number	*Sfi*I FRAGMENT
8b	42[j,k]	7.8	3b	FG
8b	43[j,k]	1.0	11a	FG
9a.....	44[d,k]	8.3	3a	FG
9a.....	45	2.3	10	FG
9b	46[g]	1.0	11b	FH
9b	47[g]	8.8	2	FH
9b	48	6.0	5	FH
10a.....	49	3.5	6	FH
10a.....	50[l]	2.4	9	GI
10a.....	51[g]	12	1	GI
10b	52[d,m]	6.6	4	GI
10b	53[n]	2.8	7	HJ
10b	54[d,l]	2.55	8	HJ
	54[d,l]	2.55	4	HJ
10b	55[g]	1.45	8	HJ
11a.....	56[g]	1.5	7	IJ
11a.....	57[g]	6.8	2	IJ
11a.....	58[o]	2.1	5	IJ
11a.....	59[g]	5.2	3	IJ
11b	60[p]	1.9	6	IJ
11b	61	10.0	1	IJ
	61	10.0	1	IJ
12a.....	62	1.8	5	IJ
12b	63	5.9	4	IJ
13/14	64[p]	7.8	2	IJ

NOTE.—Table sections A and C provide mapping data for Hd, and section B, covering a cDNA region which has been studied more intensively, provides data for five enzymes as indicated. Horizontal subdivisions of the tables represent the individual cDNA subclones used in the analysis (see Material and Methods).

[a] Subdivisions a (1–500 bp) and b (501–1,000 bp) are made according to the cDNA sequence (Koenig et al. 1989).

[b] Band-pattern order (numbered from largest to smallest).

[c] Hd fragments containing two exons (Koenig et al. 1987; Monaco et al. 1988).

[d] *Hin*dIII double digestions with rare-cutter enzymes identified sites for *Sal*I in the region spanned by cDNA0–2a, cDNA5b–7, cDNA9 (Hd44), and cDNA10 (Hd54); for *Nae*I in cDNA2b–3a (Hd9 or Hd15); for *Nar*I in cDNA8, cDNA9, and cDNA10; for *Sfi*I in cDNA10 (Hd52); and for *Sac*II in cDNA5b–7 (Hd27, cDNA position 5,440 bp). cDNA3b–5a and cDNA11–14 were not tested.

[e] DA3-g (table 2).

[f] Hd23 is a doublet (see text).

[g] Fragments in unknown order.

[h] Wapenaar et al. (1987).

[i] Source: Chamberlain et al. (1988).

[j] Two Hd fragments together containing one exon.

[k] DL44.1 (table 2).

[l] Overlap between subclones split at cDNA *Bam*HI site 9,786 interchanges the 2.55-kb and 2.4-kb bands.

[m] DL211.6 (table 2).

[n] DA4.6 (table 2).

[o] DL128.3 (table 2).

[p] Source: Darras and Francke (1988*a*) and McCabe et al. (1989; patient CM).

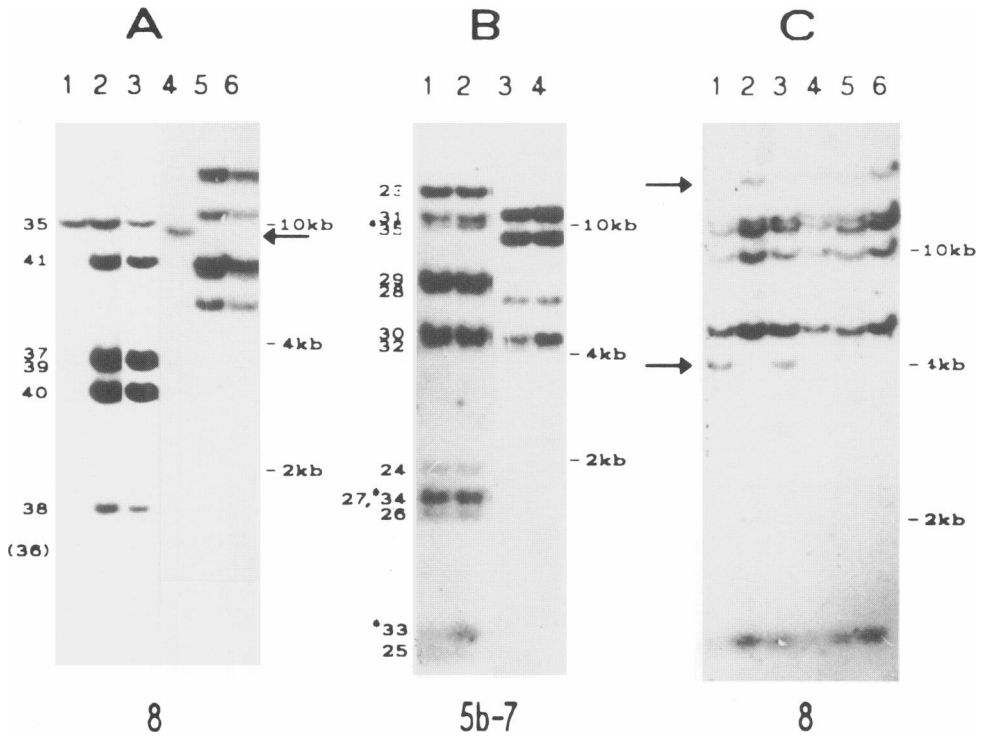

Figure 1 Hybridizations of dystrophin cDNA subprobes (indicated at bottom) to detect BMD/DMD mutations. cDNA detectable Hd fragments are numbered (table 1), and fragment sizes are indicated. *A*, *Hin*dIII (lanes 1–3) and *Eco*RI (lanes 4–6) digestions detecting a deletion of exonic sequences in DNA of patient DL129.3 (lanes 1 and 4). DL162.1 (lanes 2 and 5), and DL167.3 (lanes 3 and 6) show no deletion. The arrow indicates the altered *Eco*RI fragment. *B*, *Hin*dIII (lanes 1 and 2) or *Eco*RI (lanes 3 and 4) digestions detecting a duplication of exonic sequences (marked with asterisk) in DNA of patient DL117.4 (lanes 2 and 4). DL114.5 (lanes 1 and 3) serves as a control. *C*, *Eco*RV digestion of BMD family BL19 (see text). The junction fragment (arrow) is derived from fragment 1. DNA in lane 5 is not digested completely. The complete deletion involves also cDNA5b–7 sequences.

Dutch Caucasian populations) revealed eight new RFLPs (table 2). Because of small sample sizes, allele frequencies have not been determined in most cases. The *Eco*RV RFLPs of cDNA5b–7 and cDNA9–10 are frequent and have proved to be useful to us in standard haplotype analysis. The cDNA3b–5a *Eco*RI RFLP is also frequent, but the small allelic size differences make it difficult to use. The minor allele of the cDNA5b–7 *Eco*RI RFLP (upper band) was observed in only three of 126 males tested. The cDNA9–10 *Taq*I RFLP is probably the same as that described by Darras and Francke (1988*b*) for cDNA10.

Order of Exonic Hd Fragments

Cosmid walks around the P20 (DXS269) and J66 (DXS268) regions (Van Ommen et al. 1987; Wapenaar et al. 1987; Blonden et al. 1989) were screened for the presence of exons. In both walks only one exon was found, corresponding, respectively, to Hd33 (0.5 kb) and Hd49 (3.5 kb). Both were subcloned and sequenced. Approximately 10 kb 3′ to the distal P20 segment a 176-bp exon was found which maps at 6,647–6,822 bp on the cDNA sequence. It starts in register with the reading frame and ends out of register, after triplet position 2 (Baumbach et al. 1989; Blonden et al. 1989).

The J66 region contained a 147-bp exon 22 kb proximal to J66-H1, which is the last exon deleted in patient DL66.6. It covers the cDNA from 9,146 to 9,292 bp, contains a *Hin*dIII site at position 9,281, and begins and ends in register with the reading frame.

As Koenig et al. (1987) first showed for *Hin*dIII, combinations of hybridization data of different deletions provide the genomic order of exonic fragments (tables 1 and 3, fig. 2). Our patient material allows further ordering of Hd fragments 21/22, 42+43/44, 50+51/52/53, 55–57/58+59 (tables 1 and 3). For example, deletion patient DL44.1 orders Hd42+43/Hd44 (tables

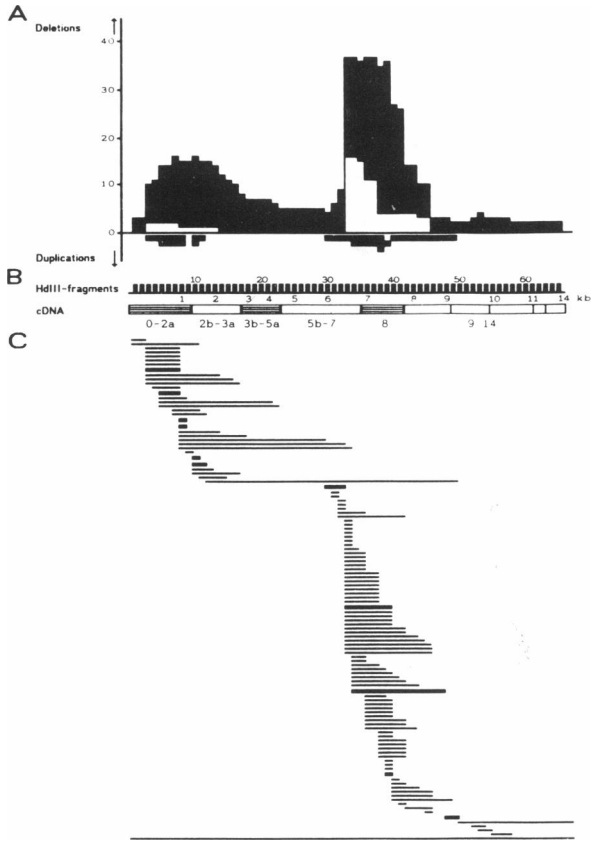

Figure 2 Schematic graphic representation of the location of 106 deletions and 11 duplications in relation to the dystrophin cDNA. Of nine deletions and two more duplications the borders have not yet been determined precisely. *A*, The number of times that each individually detectable cDNA Hd fragment (Koenig et al. 1987) is deleted (above X-axis) or duplicated (below X-axis). White and black bars represent, respectively, BMD and DMD mutations. *B*, Schematic drawing of the dystrophin cDNA subclones (*bottom*), as used in the hybridization analysis, and the 65 genomic Hd fragments it detects (*top*). Each fragment is represented by a black box whose position in relation to the mRNA sequence (in kb) is shown. *C*, Localization of each individual deletion (line) or duplication (block).

Table 2

RFLPs

Probe and Enzyme	Fragment Size (kb)	
	Major Allele	Minor Allele
0–2a:		
*Xmn*I	2.0	2.1
3b–5a:		
*Eco*RI	9.4	9.7
5b–7:		
*Eco*RV	7.3	10
*Eco*RI	10.5	13
*Xmn*I	4.7	4.4
9–10:		
*Eco*RV	12	14
*Pvu*II	9.3	11.0
*Taq*I	2.1	1.7

Note.—Given are the combinations of cDNA probe and restriction enzyme detecting an RFLP.

1 and 2). The 7.8- and 1.0-kb fragments (Hd42+43) are the last ones missing, while the 8.3-kb fragment (Hd44) is present; so Hd42+43 are 7.8 and 1.0 kb, and Hd44 is 8.3 kb.

A cDNA3b–5a hybridization to DNA of patient BB (LCL119) (Francke et al. 1985) shows two abnormal Hd fragments but only one abnormal fragment for *Eco*RI, *Bam*HI, and *Bgl*II. A cDNA5b–7 hybridization shows the absence of the 18-kb Hd23 and the presence of the smallest of the altered Hd fragments, but it shows no abnormalities for the other enzymes. Therefore, we conclude that Hd23 is a doublet of two genomically adjacent 18-kb fragments, designated Hd23a and Hd23b. BB then lacks Hd1–22 and shows a junction fragment for Hd23a. Hd23b results most likely from a rare *Hin*dIII RFLP.

Less is known about the order of fragments for other restriction enzymes. Some data have been published for *Eco*RI (Malhotra et al. 1988), *Pst*I (Forrest et al. 1987), and *Bgl*II (Darras et al. 1988). Table 1 summarizes our data (they match the other published data) for other restriction enzymes in the region of Hd32–41. Deletions in this region can now be related to deletions of Hd fragments.

The Hd fragment order presented in table 3 differs at two points from those of other studies (Koenig et al. 1989; McCabe et al. 1989). A 3.3-kb fragment, detectable with cDNA12a (McCabe et al. 1989), is not found in the present and other studies (Koenig et al. 1987; Darras and Francke 1988*a*). The order of Hd21/Hd22 is derived from one deletion case, DA3.9 (table 3), and is the reverse of that determined by Koenig et al. (1989). The explanation of this inconsistency requires further study. A potential cause for such discrepancies might be a junction which disguises itself as a bona fide exon fragment located at the opposite end of a deletion.

Muscular Dystrophy Phenotype and the Reading Frame

It has recently been shown that many BMD patients have an altered dystrophin, while in DMD patients no protein product can be detected (Hoffman et al. 1988).

Table 3

Extent of Specific DMD/BMD Mutations

Patient	Deletion		Deletion Size (kb)	Junction[b]
	Hd Fragments[a]	*Sfi*I Sites		
BB	1–22	A–D	>2,000	Hd23a
DL120.7[c]	...	...	150	
DL103.7	2–22	C–E	1,220	
DA3.9	5–21	ND	ND	
DL13.5	5–22	C–D	ND	Hd23a
DL66.6	12–49	D–G	1,160	J66
DL23.4	32–35	...	200	
DL149.6	33	...	160	
BL19.1.....	33–34	...	180	Hd35
DL207.1	33–35	...	35	P20i[d]
DL125.2	33–39	F	180	P20i[d]
DL117.4[c]	33–39	ND	ND	P20i[d]
DA11.8	33–40	F	230	P20i[d]/Hd40
DL48.1	33–41	F	210	P20i[d]
DL44.1	33–43	ND	ND	
DL104.4	34–41	F	210	
DL70.7	36–41	F	230	
DL129.3	36–41	F	180	
DL101.13 ...	38–41	...	90	
DL145.5	38–41	...	120	
DL46.1	40–45	...	100	
DL182.4	40–48	ND	ND	GMGX11
DL140.6	50–end	H–J	>2,000	
DL211.6	52–53	ND	ND	
DA4.6	53–54	ND	ND	
DL128.3	55–57	...	60	

NOTE.—ND = not determined.
[a] Depleted/duplicated.
[b] Probe with which junction fragments were found.
[c] Duplication.
[d] Identified in intronic sequences near P20 (Blonden et al. 1989).

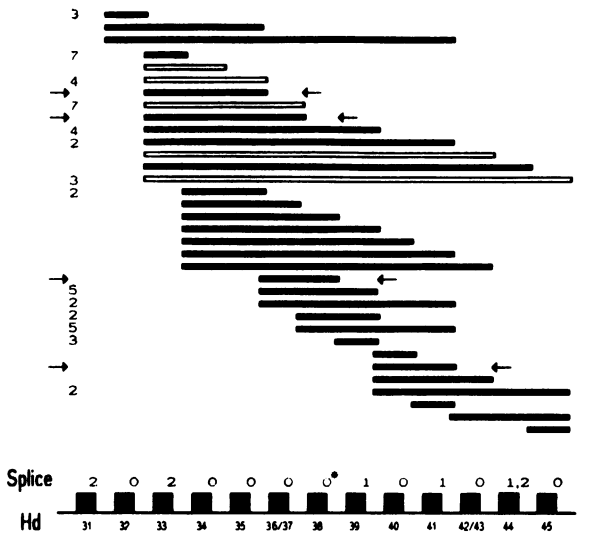

Figure 3 Exon phasing model derived from DMD or BMD phenotypes caused by deletions in the P20 mutation hot spot. Each Hd fragment is shown as a black square. The splice position deduced (see text) is shown on top: e.g., 1 indicates a 5′ splice site after nucleotide 1 of a coding triplet. The extent of the deletions is shown above the Hd fragments, as is the disease phenotype; open boxes for BMD and closed boxes for DMD. The total number of patients deleted for a specific set of exons is given at the left; for only one patient no number is given. Exceptions to a consistent model are marked with arrows. The asterisk marks a splice site deduced from the data of two BMD patients (Darras et al. 1988), one missing Hd33–38 and the other missing Hd36–38.

It is presently believed that most BMD patients have in-frame deletions or duplications generating dystrophin of abnormal size, while DMD deletions mostly disrupt the reading frame, generating truncated dystrophins of decreased stability (Malhotra et al. 1988; Monaco et al. 1988).

Using the coding sequence of exons 1–21 (Koenig et al. 1988; Malhotra et al. 1988) and of the newly generated P20 and J66 exon sequences, we have analyzed the potential correlation between deletions and the BMD/DMD phenotype in our patient material. Of two BMD and 31 DMD cases deleted within these regions, 30 can be explained by frameshifts followed by premature translation termination. We found three exceptions to the "reading frame hypothesis", namely, patients BL39.3, DL66.6, and DA14.8. BMD patient BL39.3 is deleted for Hd3–7 and falls in the category of exceptions described by Malhotra et al. (1988). DMD patient DL66.6 lacks Hd12–49, corresponding to a 1.2-Mb genomic deletion (Van Ommen et al. 1987), while his dystrophin reading frame is not disrupted. However, the deletion removes 7.0 kb of the mRNA, i.e., more than 60% of the 11-kb reading frame, which is probably a large enough segment to affect the function of the protein significantly. The deletion in DMD patient DA14.8 removes Hd3–15 and leaves the reading frame intact. This cannot be explained with our current insights, especially since BMD patient BL15.5 has both a similar deletion and the expected BMD phenotype.

Within the central mutation hot spot we noticed that deletions of specific sets of exons resulted in either a BMD or a DMD phenotype. Given, therefore, that the reading-frame hypothesis appears largely correct, we applied it to the P20 deletion hot spot region of Hd31–45. When only the 176-bp P20 exon (Hd33) is deleted, the reading frame is distorted, resulting in a DMD phenotype, confirmed by seven cases (fig. 3). Deletion of one (one case) or two (four cases) additional

exons results in a BMD phenotype (fig. 3), suggesting that the 3′ splice sites of Hd34 and Hd35, like that of Hd32, are located between two coding triplets. Likewise, the 3′ splice site of Hd37, Hd43, and Hd45 should also be located between two coding triplets (fig. 3); that is, the 3′ splice site of Hd41 can be derived by combining the DMD-causing deletions of Hd33–41 (i.e., splicing after positions 1 or 2) and Hd34–41, leaving splicing after position 1 as the only possibility remaining. The results deduced from this analysis independently cross-confirm each other in many ways, while recent results of sequenced exons in this region also confirm the proposed splice positions (Chamberlain et al. 1988; Baumbach et al. 1989).

The exceptions to the reading-frame rule can be explained in many ways. Deletions may either invisibly affect the edge of an exon or have secondary effects on transcription, splicing, or translation of the gene; also, the influence of extragenic effects cannot be excluded. We believe that a careful analysis, at the mRNA level, is required for meaningful correlation of muscular dystrophy phenotype and reading-frame effect.

Completion of the Megabase Map

The use of the cDNA on genomic *Sfi*I digests, separated on FIGE gels, confirms the published megabase map (Den Dunnen et al. 1987; Kenwrick et al. 1987; Van Ommen et al. 1987; Burmeister et al. 1988; Meitinger et al. 1988). All previously described *Sfi*I fragments are detected. The extreme 5′ cDNA detects the *Sfi*I fragment designated BC (*Sfi*I/BC) but not *Sfi*I/AB (fig. 4). The 5′ end of the DMD gene, exon 1, was found near pERT84 (Koenig et al. 1987), which maps within 150 kb distal to *Sfi*I/B (Burmeister and Lehrach 1986). An extra band of 680 kb is detected with cDNA9–14 (fig. 4). This fragment, designated *Sfi*I/IJ, therefore contains the 3′ end of the dystrophin gene (Meitinger et al. 1988). It has not been detected previously with genomic probes. Internal fragments *Sfi*I/DE and *Sfi*I/HI have not been detected independently with genomic probes but are clearly visualized with cDNA probes (fig. 4*B*), which implies that they contain several exons.

Our recent data suggest that the *Sfi*I/CD (fig. 4), formerly estimated to be 280 kb (Den Dunnen et al. 1987; Van Ommen et al. 1987), only spans 230 kb, a finding consistent with other estimates (Kenwrick et al. 1987; Burmeister et al. 1988). The long-range electrophoresis produces some striking inconsistencies between fragment sizes obtained by subtraction of larger, partial fragments and those of the fragments themselves. *Sfi*I/DE, inferred to be 130 kb by subtraction (Van Ommen et al. 1987); Burmeister et al. 1988), migrates at 200 kb by itself. In contrast, *Sfi*I/HI, by subtraction estimated to be 50 kb (Den Dunnen et al. 1987; Kenwrick et al. 1987; Van Ommen et al. 1987; Burmeister et al. 1988; Meitinger et al. 1988), migrates below 35 kb as a separate FIGE fragment (fig. 4). To reduce confusion, until more definite sizes are obtained from mapping and cloning, we have kept the previously published sizes, as these provide internally consistent size measurements.

To map the 3′ end of the DMD gene more precisely,

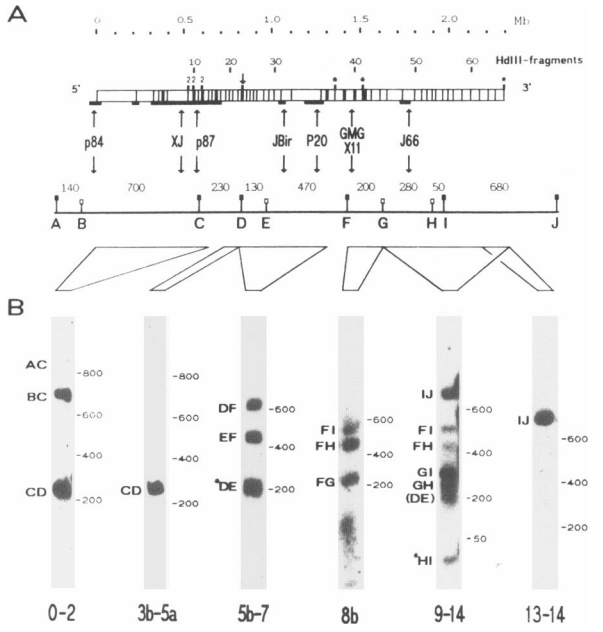

Figure 4 Megabase map of the DMD gene. *A*, *Sfi*I physical map of the DMD region (bottom line) showing partially or fully digestible *Sfi*I sites as open or closed boxes, respectively. Individual sites are marked with letters (Den Dunnen et al. 1987), and fragment lengths are indicated in kilobases. The top line shows the localization of exon-containing genomic Hd fragments (vertical bar) in relation to the *Sfi*I map. Heavy lines at the bottom of exonic fragments show the extension of cloned regions. Asterisks indicate two Hd fragments containing one exon; 2's indicate Hd fragments containing two exons, and the arrow identifies Hd23, drawn as a doublet. Localization of genomic probes (central line) is indicated in relation to the long-range and exonic maps. Deletion breakpoints located between Hd33–35 and Hd38–40 very frequently show junction fragments on conventional Southern blots, so these exons are probably located close to each other. Because of the paucity of deletions, the spacing of the exons in *Sfi*I/IJ is undetermined and arbitrarily drawn to fill the available space. *B*, cDNA hybridizations to FIGE gels in relation to the megabase map. *Sfi*I-digested DNAs were hybridized to specific cDNA subclones (*bottom*). Letter symbols (*left*) mark each fragment detected (see panel *A*); asterisks mark abnormally migrating fragments (see text); and brackets mark a signal of a previous hybridization not removed fully after stripping. Fragments sizes are indicated in kilobases, at the right.

we used several other restriction enzymes (*Sac*II, *Sal*I, *Nae*I, and *Nar*I). Single and double digests were analyzed on FIGE blots hybridized with parts of cDNA9–14 (data not shown). We found that the DMD gene spans no more than the proximal 400 kb of *Sfi*I/IJ, so its total size can be determined to be about 2.3 Mb.

In our FIGE study of 46 patients, a random subset of all our patients for which we have cultured cells available, we detected 28 aberrations (61%) (Den Dunnen et al. 1989). The validity of the FIGE analysis was proved by comparison with the cDNA data of the same patient set, which showed that the chromosomal rearrangements detected in almost all cases involved expressed exonic sequences. Two small one-exon duplications were missed by FIGE analysis, a possibility mentioned at the outset (Den Dunnen et al. 1987). Inclusion of these two cases raises the total of detectable anomalies in the set of patients tested by FIGE analysis to 30/46 or 65%, i.e., identical to that in the total patient material. It is interesting, however, that two patients with FIGE rearrangements have no detectable cDNA alterations (see Discussion).

Long-Range Exon Map

Our genomic and cDNA deletion map (fig. 4, tables 1 and 2) essentially agrees with previously published data and parallel studies (Koenig et al. 1987; Wapenaar et al. 1987; Malhotra et al. 1988; Koenig et al. 1989). J66-H1 maps between Hd49 and Hd50, as anticipated (Koenig et al. 1987). GMGX11 maps between Hd39 and Hd40 (fig. 4), on the basis of its absence in DL139.4 (missing Hd36–39), its presence in DL171.8 (missing Hd38–39) and DL146.7 (missing Hd40–45), and the detection of a junction fragment in DL182.4 (missing Hd40–48; see table 3).

Other useful genomic markers in the DMD region are the *Sfi*I sites themselves (fig. 4, tables 1 and 3) (Den Dunnen et al. 1987). Sites /A and /B both lie 5′ of the DMD gene, while site /J is located 3′. Site /C maps between Hd8 and Hd9 (Monaco et al. 1986; Malhotra et al. 1988). The BB deletion ends between sites /D and /E, lacks Hd1–22, and has an altered Hd23a (table 3). A *Hin*dIII/*Sac*II double digestion shows that Hd27 contains the *Sac*II site (table 1) which has been located between /D and /E (Burmeister et al. 1988). Thus, Hd23–27 lies on the /DE fragment. Site /F maps between Hd37 and Hd38, since it is absent from DL70.7 and DL129.3 (both missing Hd36–41), and present in DL37.5, DL145.5, and DL101.13, (missing Hd38–41) and since Hd36+37 share one exon.

Deletions in the 3′ region of the gene are rare (fig. 2), so sites /G, /H, and /I can only be mapped approximately, on the basis of reasoning similar to that presented above (tables 1 and 3). Site /G maps between Hd45 and Hd49, and sites /H and /I map between Hd49 and Hd55. The latter localization is confirmed by the fact that Hd52 contains a partially digestible *Sfi*I site (table 1).

The combined cDNA-and-FIGE analysis (table 3) enables a further refinement of the exon map. An example of this is shown in figure 5, where the length of the P20 intron was deduced from some partially overlapping deletions. DL149.6 provides a minimum size of 160 kb, and DL23.4 provides a maximum size of 180 kb. Similarly, the deletion in DL207.1, whose 5′ breakpoint was located in the cloned P20 region (Blonden et al. 1989), shows that the introns distal to Hd33 and Hd34 each cover only 10–20 kb. The combined cDNA- and FIGE-deletion data, together with the data published for the XJ (Malhotra et al. 1988) and pERT87 regions (Monaco et al. 1986, 1988), resulted in the long-range exon map of the dystrophin gene (fig. 4*A*).

The dystrophin coding sequence contains a structural repeat motif which is present in 26 or 24 copies, of which the first seven are spread over 14 exons (Koenig et al. 1988, 1989; Malhotra et al. 1988). An estimation of the total number of exons, based on extrapolation and excluding differential splicing (Feener et al. 1989; Nudel et al. 1989), would add up to about 75 exons: eight exons for the N-terminal sequence in front of the repeat domain, 52 exons for 26 two-exon repeat units, two exons for the two segments interspersed between repeats 20/21 and 24/25, and 13 exons for the remainder of the sequence, spread over 14 cDNA-detectable Hd fragments (the last two share one exon). In this setting, Hd33 would contain exon 45, and the end of the repeat unit would be exon 62, corresponding to Hd50, which is further documented in a parallel study (Koenig et al. 1989). This model predicts that many Hd fragments between Hd19 and Hd32 (Fig. 4*B*) contain more than one exon, indicating that the exon

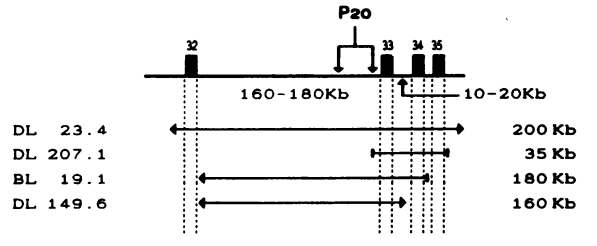

Figure 5 Estimation of intron lengths after combining cDNA and FIGE deletion data. Exonic Hd fragments, numbered according to table 1, are represented by black boxes. Deletion sizes are indicated at the right, and patient numbers are at the left.

spacing between p87 and JBir is much denser than that in the 5′ and 3′ parts of the gene. However, the essential conclusion (fig. 4*A*)—i.e., that the highly uneven exon spacing found for the 5′ half of the gene continues in the 3′ half, with introns ranging from a few kilobases to the record size, thus far, of 160–180 kb for the P20-containing intron—remains unaffected.

Discussion

Location of Mutations

After the discovery of the DMD gene, the application of long-range techniques, short-range cloning, and nucleotide sequencing together are rapidly yielding a much better insight into the morbid anatomy of the DMD gene than each of the techniques separately would have allowed.

It is now well documented (Den Dunnen et al. 1987; Forrest et al. 1987; Koenig et al. 1987; Wapenaar et al. 1987; Darras et al. 1988; present study) that there is a striking nonrandom distribution of deletions/duplications in the DMD gene. In our family material the 200-kb region located around P20 contains 55 rearrangement sites, while the 200-kb region directly proximal, around JBir, contains only nine such sites. The underlying mechanism seems not to be related to deletion or duplication of integral copies of the repeated structural units identified in the dystrophin gene (Koenig et al. 1988), since the large majority of breakpoints does not involve exons at all (see Results).

We have set out to study the possible involvement of specific intronic sequences or other properties of the region, such as chromosomal structure (Blonden et al. 1989) or interdigitation of the DMD gene with other genetic units. It is remarkable that both mutation hot spots are located in rather large introns (fig. 4*A*), even for the dystrophin gene (Malhotra et al. 1988; Blonden et al. 1989; present study). One might expect that evolution would shorten intronic regions prone to pathological deletions. The fact that this has not happened suggests the presence of essential sequences in these introns. These could be other genes, whose expression may even be independent from DMD gene expression. The localization of one human gene within another was recently reported for the factor VIII gene (Levinson et al. 1988).

Detection Efficiency of Mutations

In families without living patients it is difficult to detect deletions or duplications by using conventional cDNA studies, since quantitative assessment of differences in band intensities is required. Duplications, causing triple instead of double band intensities are especially hard to find. In our hands, mutations in carrier females can best be detected using FIGE analysis (Den Dunnen et al. 1987, 1989; Chen et al. 1988). The technique gives clear results, by producing easily resolvable normal and altered fragments, both for deletions and for duplications.

We believe that the presently detected fraction of DMD mutations, i.e., about 65% (Forrest et al. 1987; Koenig et al. 1987; Chamberlain et al. 1988; Darras et al. 1988; Baumbach et al. 1989; present study), is a low estimate. Not all probes were tested extensively, and not only duplications are hard to detect but so also are very small deletions, duplications, and insertions. A few unclear cases, i.e. abnormal fragments detected with one restriction enzyme only, require further study, on either conventional or FIGE blots. These cases may result either from rare RFLPs or from more-complex rearrangements. It is notable that translocations, insertions, and inversions starting in intronic sequences will generally not be detected by conventional analysis. FIGE analysis allows their detection, since one normal fragment should be broken into one or more different ones. A case in point is DL120.7, having a larger *Sfi*I/BC fragment, suggesting a 150-kb duplication (or insertion) in the 5′ region of the gene. However, we have not detected any anomaly by conventional cDNA hybridizations. We envisage three possible explanations: first, the event may affect exon sequences not present in the cDNA used, i.e., resulting from alternative splicing (Feener et al. 1989; Nudel et al. 1989); second, the event may involve sequences directly upstream of the gene in the promotor region; and, third, intronic sequences may be altered in a way affecting splicing. Whatever the nature of these anomalies, however, their detection highlights the affected chromosome and can be used as such in diagnostic haplotype analysis.

In conclusion, we have extended the insight into the long-range map and regional topography of this remarkable gene, which we show to measure 2.3 Mb and which contains only 0.6% coding sequence, divided into some 75 exons. This study provides, for the first time, a correlation between the exonic Hd fragments and the *Sfi*I long-range map, both in normal and in mutated DNA. Several questions remain to be answered, such as the nature of the deletion hot spots and the background of the exceptions to the reading-frame rule. These features will be the aim of much future study.

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References

Arahata K, Ishiura S, Ishiguro T, Tsukahara T, Suhara Y, Eguchi C, Ishihara T, et al (1988) Immunostaining of skeletal and cardiac muscle surface membrane with antibody against Duchenne muscular dystrophy peptide. Nature 333: 861–863

Bakker E, Bonten EJ, Veenema H, Den Dunnen JT, Grootscholten PM, Van Ommen GJB, Pearson PL (1989) Prenatal diagnosis of Duchenne muscular dystrophy: a three-year experience in a rapidly evolving field. J Inherited Metab Dis 12:174–190

Bakker E, Hofker MH, Goor N, Mandel JL, Wrogemann K, Davies KE, Kunkel L, et al (1985) Prenatal diagnosis and carrier detection of Duchenne muscular dystrophy with closely linked RFLPs. Lancet 1:655–658

Baumbach LL, Chamberlain JS, Ward PA, Farwell NJ, Caskey CT (1989) Molecular and clinical correlations of deletions leading to Duchenne and Becker muscular dystrophies. Neurology 39:465–474

Blonden LAJ, Den Dunnen JT, Van Paassen HMB, Wapenaar MC, Grootscholten PM, Ginjaar HB, Bakker E, et al (1989) High resolution deletion breakpoint mapping in the DMD-gene by whole cosmid hybridization. Nucleic Acids Res 17:5611–5621

Bonilla E, Schmidt B, Samitt CE, Miranda AF, Hays AP, de Oliveira ABS, Chang HW, et al (1988) Normal and dystrophin-deficient muscule fibers in carriers of the gene for Duchenne muscular dystrophy. Am J Pathol 133: 440–445

Burghes AHM, Logan C, Hu V, Belfall B, Worton RG, Ray PN (1987) A cDNA clone from the Duchenne/Becker muscular dystrophy gene. Nature 328:434–437

Burmeister H, Lehrach H (1986) Long-range restriction map around the Duchenne muscular dystrophy gene. Nature 324:482–485

Burmeister H, Monaco AP, Gillard EF, Van Ommen GJB, Affara NA, Ferguson-Smith MA, Kunkel LM, et al (1988) A 10-megabase map of human Xp21, including the Duchenne muscular dystrophy gene. Genomics 2:189–202

Chamberlain JS, Gibbs RA, Ranier JE, Nguyen PN, Caskey CT (1988) Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. Nucleic Acids Res 23:11141–11156

Chen J-D, Denton MJ, Morgan G, Pearn JH, Mackinlay AG (1988) The use of field-inversion gel electrophoresis for deletion detection in Duchenne muscular dystrophy. Am J Hum Genet 42:777–780

Darras BT, Blattner P, Harper JF, Spiro AJ, Alter S, Francke U (1988) Intragenic deletions in 21 Duchenne muscular dystrophy (DMD)/Becker muscular dystrophy (BMD) families studied with the dystrophin cDNA: location of breakpoints on *Hin*dIII and *Bgl*II exon-containing fragment maps, meiotic and mitotic origin of the mutations. Am J Hum Genet 43:620–629

Darras BT, Francke U (1988*a*) Myopathy in complex glycerol kinase deficiency patients is due to 3′ deletions of the dystrophin gene. Am J Hum Genet 43:126–130

——— (1988*b*) Normal human genomic restriction-fragment patterns and polymorphisms revealed by hybridization with the entire dystrophin cDNA. Am J Hum Genet 43:612–619

Davies KE, Mandel JL, Weissenbach J, Fellous M (1987) Report of the Committee on the Genetic Constitution of the X and Y Chromosome. Cytogenet-Cell Genet 46:277–315

Den Dunnen JT, Bakker E, Klein-Breteler EG, Pearson PL, Van Ommen GJB (1987) Direct detection of more than 50% Duchenne muscular dystrophy mutations by field inversion gels. Nature 329:640–642

Den Dunnen JT, Bakker E, Van Ommen GJB, Pearson PL (1989) The DMD gene analysed by field inversion gel electrophoresis. Br Med Bull 45:644–658

Emery AEH (1988) Duchenne muscular dystrophy. Oxford Monographs on Medical Genetics, no 15. Rev ed. Oxford University Press, Oxford

Feener CA, Koenig M, Kunkel LM (1989) Alternative splicing of human dystrophin mRNA generates isoforms at the carboxy terminus. Nature 338:509–511

Forrest SM, Cross GS, Speer A, Gardner-Medwin D, Burn J, Davies K (1987) Preferential deletion of exons in Duchenne and Becker muscular dystrophies. Nature 329:638–640

Francke U, Ochs HD, de Martinville B, Giacalone J, Lindgren V, Distèche C, Pagon RA, et al (1985) Minor Xp21 chromosome deletion in a male associated with expression of Duchenne muscular dystrophy, chronic granulomatous disease, retinitis pigmentosa, and McLeod syndrome. Am J Hum Genet 37:250–267

Hoffman EP, Fischbeck KH, Brown RH, Johnson M, Medori R, Loike JD, Harris JB, et al (1988) Characterization of dystrophin in muscle-biopsy specimens from patients with Duchenne's or Becker's muscular dystrophy. New Engl J Med 318:1363–1368

Hoffman EP, Knudson CM, Campbell KP, Kunkel LM (1987) Subcellular fractionation of dystrophin to the triads of skeletal muscle. Nature 330:754–757

Hu X, Burghes AHM, Bulman DE, Ray PN, Worton RG (1989) Evidence for mutation by unequal sister chromatid exchange in the Duchenne muscular dystrophy gene. Hum Genet 44:855–863

Hu X, Burghes AHM, Ray PN, Thompson MW, Murphy EG, Worton RG (1988) Partial gene duplications in Du-

chenne and Becker muscular dystrophies. J Med Genet 25:369–376

Kenwrick S, Patterson M, Speer A, Fischbeck K, Davies KE (1987) Molecular analysis of the Duchenne muscular dystrophy region using pulsed field gel electrophoresis. Cell 48:351–357

Koenig M, Hoffman EP, Bertelson CJ, Monaco AP, Feener C, Kunkel LM (1987) Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals. Cell 50:509–517

Koenig M, Monaco AP, Kunkel LM (1988) The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. Cell 53:219–228

Koenig M, Beggs AH, Moyer M, Scherpf S, Heindrich K, Bettecken T, Meng G, et al (1989) The molecular basis for Duchenne versus Becker muscular dystrophy: correlation of severity with type of deletion. Am J Hum Genet 45:498–506

Konings RNH, Luiten RGM, Peeters BPH (1986) Mike, a chimeric filamentous phage designed for separate production of either DNA strand of pKUN vector plasmids by F^+ cells. Gene 46:269–276

Levinson B, Lakich D, Silvera P, Kenwrick S, Gitschier J (1988) A gene contained within a factor VIII intron is identified by a CpG island. Am J Hum Genet 43:A192

McCabe ERB, Towbin J, Chamberlain J, Baumbach L, Selzer WK, Van Ommen GJB, Kunkel LM, et al (1989) cDNA probes for the Duchenne muscular dystrophy locus demonstrate a previously undetectable deletion in a patient with dystrophic myopathy, glycerol kinase deficiency and congenital adrenal hypoplasia. J Clin Invest 83:95–99

Malhotra SB, Hart KA, Klamut HJ, Thomas NST, Bodrug SE, Burghes AHM, Bobrow M, et al (1988) Frame-shift deletions in patients with Duchenne and Becker muscular dystrophy. Science 242:755–759

Meitinger T, Boyd Y, Anand R, Craig W (1988) Mapping of Xp21 translocation breakpoints in and around the DMD gene by pulsed field gel electrophoresis. Genomics 3:315–322

Monaco AP, Bertelson CJ, Liechti-Gallati S, Moser H, Kunkel LM (1988) An explanation for the phenotypic differences between patients bearing partial deletions of the DMD locus. Genomics 2:90–95

Monaco AP, Bertelson CJ, Middlesworth W, Coletti C-A, Aldridge J, Fischbeck KH, Bartlett R, et al (1985) Detection of deletions spanning the Duchenne muscular dystrophy locus using a tightly linked DNA segment. Nature 316: 842–845

Monaco AP, Neve RL, Colletti-Feener C, Bertelson CJ, Kurnit DM, Kunkel LM (1986) Isolation of candidate cDNAs for portions of the Duchenne muscular dystrophy gene. Nature 323:646–650

Nudel U, Zuk D, Einat P, Zeelon E, Levy Z, Neuman S, Yaffe D (1989) Duchenne muscular dystrophy gene product is not identical in muscle and brain. Nature 337:76–78

Ray PN, Belfall B, Duff C, Logan C, Kean V, Thompson MW, Sylvester JE, et al (1985). Cloning of the breakpoint of an X:21 translocation associated with Duchenne muscular dystrophy. Nature 318:672–675

Van Ommen GJB, Bertelson CE, Ginjaar HB, Den Dunnen JT, Bakker E, Chelly J, Matton M, et al (1987) Long-range genomic map of the Duchenne muscular dystrophy (DMD) gene: isolation and use of J66 (DXS268), a distal intragenic marker. Genomics 1:329–336

Wapenaar MC, Kievits T, Hart KA, Blonden L, Den Dunnen JT, Bakker E, Bobrow M, et al (1987) A deletion hotspot in the Duchenne muscular dystrophy gene. Genomics 2:101–108

Watkins SC, Hoffman EP, Slayter HS, Kunkel LM (1988) Immunoelectron microscopic localization of dystrophin in myofibres. Nature 333:863–866

Wieacker P, Davies KE, Pearson PL, Ropers H-H (1983) Carrier detection in Duchenne muscular dystrophy by use of cloned DNA sequences. Lancet 2:1325–1327

Zubrzycka-Gaarn EE, Bulman DE, Karpati G, Burghes AHM, Belfall B, Klamut HJ, Talbot J, et al (1988) The Duchenne muscular dystrophy gene product is localized in sarcolemma of human skeletal muscle. Nature 333:466–469