*Am. J. Hum. Genet. 45:498–506, 1989*

# The Molecular Basis for Duchenne versus Becker Muscular Dystrophy: Correlation of Severity with Type of Deletion

M. Koenig,* A. H. Beggs,* M. Moyer,* S. Scherpf,† K. Heindrich,† T. Bettecken,† G. Meng,† C. R. Müller,† M. Lindlöf,‡ H. Kaariainen,‡ A. de la Chapelle,‡ A. Kiuru,§ M.-L. Savontaus,§ H. Gilgenkrantz,‖ D. Récan,‖ J. Chelly,‖ J.-C. Kaplan,‖ A. E. Covone,# N. Archidiacono,# G. Romeo,# S. Liechti-Gallati,** V. Schneider,** S. Braga,** H. Moser,** B. T. Darras,†† P. Murphy,†† U. Francke,†† J. D. Chen,‡‡ G. Morgan,‡‡ M. Denton,‡‡ C. R. Greenberg,§§ K. Wrogemann,§§ L. A. J. Blonden,‖‖ H. M. B. van Paassen,‖‖ G. J. B. van Ommen,‖‖ and L. M. Kunkel*

*Division of Genetics, Howard Hughes Medical Institute at Children's Hospital, Boston; †Institut für Humangenetik der Universität Würzburg, Würzburg, Federal Republic of Germany; ‡Department of Medical Genetics, University of Helsinki, Helsinki; §Department of Medical Genetics, University of Turku, Turku, Finland; ‖Institut de Pathologie Moléculaire, INSERM, Hopital Cochin, Paris; #Laboratory of Molecular Genetics, Istituto G. Gaslini, Genova Quarto, Italy; **Medical Genetics, University Department of Pediatrics, Inselspital, Berne; ††Department of Human Genetics, Yale University School of Medicine, New Haven, CT; ‡‡Department of Pathology, Prince of Wales Hospital, Sydney; §§Departments of Biochemistry and Human Genetics, University of Manitoba, Winnipeg; and ‖‖Department of Human Genetics, Sylvius Laboratories, Leiden.

## Summary

**About 60% of both Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) is due to deletions of the dystrophin gene. For cases with a deletion mutation, the "reading frame" hypothesis predicts that BMD patients produce a semifunctional, internally deleted dystrophin protein, whereas DMD patients produce a severely truncated protein that would be unstable. To test the validity of this theory, we analyzed 258 independent deletions at the DMD/BMD locus. The correlation between phenotype and type of deletion mutation is in agreement with the "reading frame" theory in 92% of cases and is of diagnostic and prognostic significance. The distribution and frequency of deletions spanning the entire locus suggests that many "in-frame" deletions of the dystrophin gene are not detected because the individuals bearing them are either asymptomatic or exhibit non-DMD/non-BMD clinical features.**

## Introduction

Duchenne muscular dystrophy (DMD) is a severe X-linked myopathy with an incidence of 1/3,500 male births (Engel and Banker 1986). DMD is allelic with Becker muscular dystrophy (BMD), a clinically similar but less severe form of myopathy affecting 1/30,000 males. The gene that, when defective, results in DMD or BMD consists of a minimum of 65 exons spread over ~2,000 kb (Koenig et al. 1987; van Ommen et al. 1987; Burmeister et al. 1988). Intragenic deletions appear to be the most common gene defect leading to DMD or BMD (Forrest et al. 1987; Koenig et al. 1987). On the basis of analysis of a limited set of deletions, Monaco et al. (1988) proposed that DNA deletions resulting in the clinically less severe BMD bring together exons that maintain the translational reading frame of the messenger RNA. Such deletions should allow the production of an internally deleted dystrophin protein that may be at least partially functional. Conversely, deletions resulting in the more severe DMD bring together exons that disrupt the translational reading frame, which should result in the production of a se-

Received April 4, 1989; revision received May 19, 1989.

Address for correspondence and reprints: Michel Koenig, Département de Génétique Humaine, Institut de Chimie Biologique CNRS-INSERM, 11 rue Humann, 65085 Strasbourg Cedex, France.



0002-9297/89/4504-0003$02.00

verely truncated molecule. Western blot analysis of the gene product, dystrophin, supports this hypothesis, as DMD patients have no detectable protein while most BMD patients have dystrophin of reduced size (Hoffman et al. 1988). Recently, however, Malhotra et al. (1988) described deletions within the first 10 exons of the dystrophin gene that apparently contradict the reading-frame model. Here we present our analysis of deletions spread over most of the dystrophin gene. We have determined the consequence of these deletions on the dystrophin mRNA reading frame and have correlated this information with the clinical severity of muscle weakness. In addition, the large number of different deletions gathered in this collaborative study allows analysis of the relative importance of dystrophin domains with respect to the disease.

## Material and Methods

### *Clinical Classification of Patients*

We studied only patients older than 8 years because classification of younger patients is less reliable, especially if no other individuals in the family are affected. Patients were classified as DMD or BMD, depending on the severity of muscle weakness (Engel and Banker 1986). The age when patients became permanently wheelchair bound was the main clinical parameter used for classification, but for young patients the progression of muscle weakness at present age was used. Patients wheelchair bound by age 13 years, as well as ambulatory patients between 8 and 13 years of age and with severe muscle weakness typical of DMD, were classified as DMD. BMD patients were those who remained ambulatory past the age of 15 years. Patients younger than 15 years of age and with a myopathy less severe than that of typical DMD, as well as patients who became wheelchair bound between ages 13 and 15 years, were classified as being in an intermediate group, to indicate clinical uncertainty.

### *Cloning of Genomic Fragments Containing Exons of the Dystrophin Gene*

Lambda phages or cosmids containing exons 22, 43, 45, and 60 have been previously cloned by genomic walking or jumping (Monaco et al. 1987; van Ommen 1987; Wapenaar et al. 1988; den Dunnen et al., in press). Intron sequences flanking exon 41 were obtained from a partially spliced cDNA clone (Koenig et al. 1988). Phages containing the other exons (table 1) were isolated from genomic libraries screened with the dystrophin cDNA probes 5b, 8, and 9a. The exon-intron boundaries were determined as described elsewhere (Koenig et al. 1988). The flanking intron sequences are freely available on request.

### *Deletion Analysis*

Deletion breakpoints in the dystrophin gene were mapped relative to exons by Southern blot analysis, using cDNA probes spanning the entire 14-kb transcript (Koenig et al. 1987). Data are presented for deletions in which all missing exons could be established unambiguously. We excluded from the study any deletions that were incompletely characterized when all data were pooled, as well as the deletions for which an abnormal sized fragment ("junction fragment") was detected with the cDNA probe. Cases with junction fragments were excluded because it is not always possible to identify which exon(s) is/are present in the junction fragment and because the breakpoint in the junction fragment might occur within an exon or in the flanking splicing sequence and thus have unpredictable consequences on the reading frame. However, it is possible that we failed to detect—and thus to exclude—very small junction fragments or junction fragments that contain only a portion of an exon. We assumed that the presence of an intact genomic fragment indicated that the exon and its flanking splicing sequences were intact, since the restriction sites that define the genomic fragment are located at a distance from these sequences. In most cases, the extent of deletion was established from *Hin*dIII digests of genomic DNA. In some cases, however, the *Eco*RI pattern (Burghes et al. 1987) or the *Bgl*II pattern (Darras and Francke 1988*b*) was concomitantly analyzed to confirm the results obtained from *Hin*dIII digestion.

## Results

The consequence of a deletion on the reading frame was determined by examining the type of exon-intron borders of the remaining two exons that flanked the deletion. The exon borders were classified as one of three types (1, 2, or 3) depending on their position in the coding triplets (table 1). A deletion that juxtaposes two exons with borders of the same type maintains the reading frame. In contrast, a deletion that juxtaposes two exons with borders of a different type disrupts the reading frame, leading to early termination of protein translation. Figure 1 illustrates the analysis of three deletions, one that maintains and two that disrupt the

## Table 1

**Exon Boundaries of 17 Exons Distributed over the Rod Domain of the Dystrophin Gene**

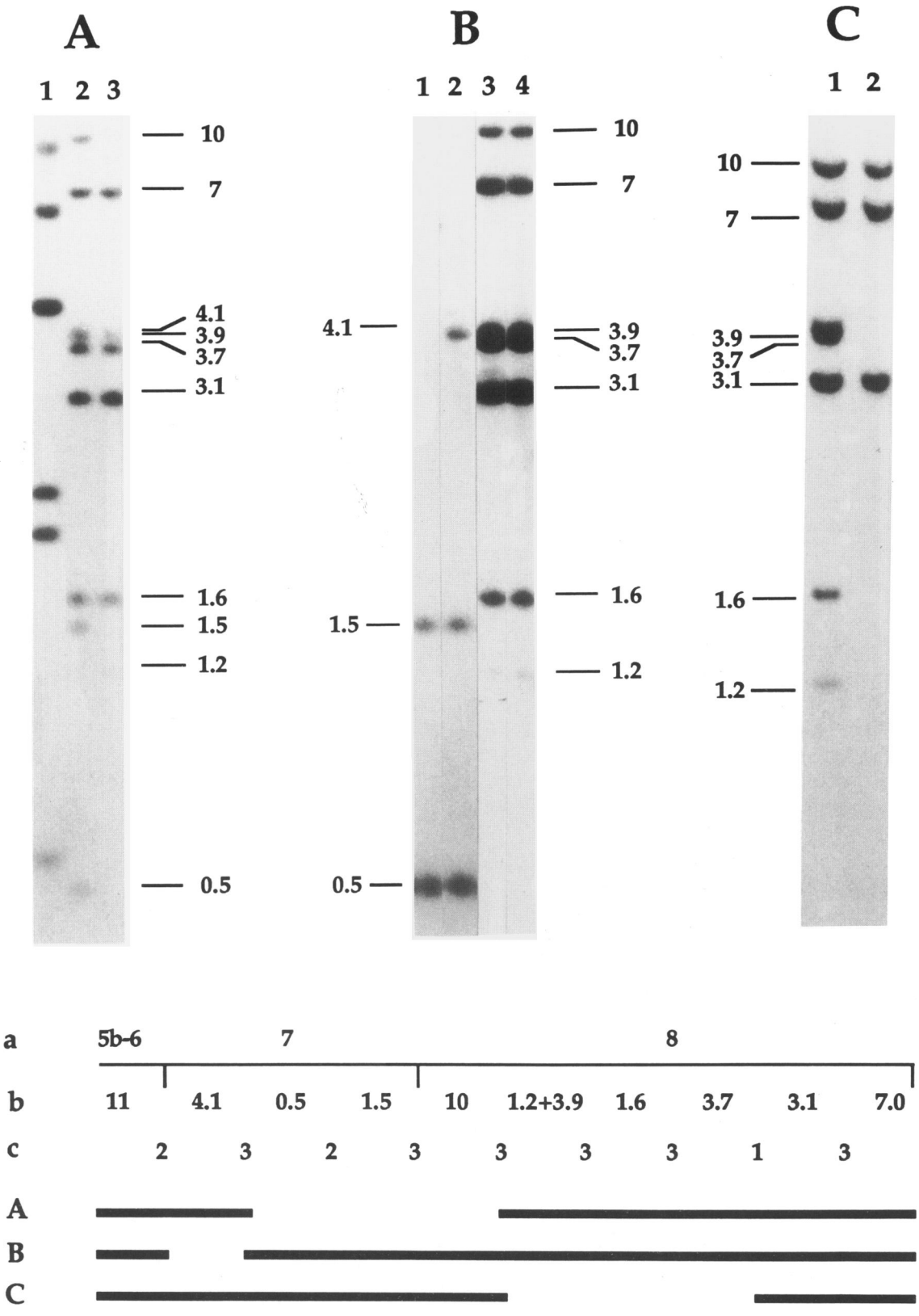
| *Hin*dIII Genomic Fragment | Exon | 5′ Border Type | Starts | Ends | 3′ Border Type |
|---|---|---|---|---|---|
| 20 | 22 | 1 | 3012 | 3157 | 3 |
| | 31 | 3 | 4442 | 4552 | 3 |
| 18 | 32 | 3 | 4553 | 4726 | 3 |
| | 33 | 3 | 4727 | 4882 | 3 |
| .4 | 35 | 3 | 5054 | 5233 | 3 |
| 1.3 | 36 | 3 | 5234 | 5362 | 3 |
| 1.5 | 37 | 3 | 5363 | 5533 | 3 |
| 6.2 | 41 | 3 | 5948 | 6130 | 3 |
| 11 | 43 | 3 | 6326 | 6498 | 2 |
| .5 | 45 | 3 | 6647 | 6822 | 2 |
| 10 | 47 | 3 | 6971 | 7120 | 3 |
| 1.25 + 3.9 | 48 | 3 | 7121 | 7306 | 3 |
| 1.6 | 49 | 3 | 7307 | 7408 | 3 |
| 3.7 | 50 | 3 | 7409 | 7517 | 1 |
| 3.1 | 51 | 1 | 7518 | 7750 | 3 |
| 7.8 + 1.0 | 53 | 1 | 7869 | 8080 | 3 |
| 8.3 | 54 | 3 | 8081 | 8235 | 2 |
| 2.3 | 55 | 2 | 8236 | 8425 | 3 |
| 3.5 | 60 | 3 | 9146 | 9292 | 3 |

NOTE. — The position of the exon boundaries is given relative to the cDNA nucleotide sequence (Koenig et al. 1988). The exon-intron boarders are classified as 1, 2, or 3 if they are after the first, second, or third nucleotide of a coding triplet, respectively. The intron-exon borders are thus also classified as 1, 2, or 3 if they are before the second, third, or first nucleotide of a coding triplet, respectively. The exon-containing *Hin*dIII genomic fragments are designated by their size in kbp. The numbering of the exons as indicated in table 1 and fig. 1 is still preliminary, since some exons have not been sequenced. The numbering of these latter exons is based on the repetitive nature of this portion of the dystrophin gene and on genomic mapping analysis. The exon numbers are used in the text for the sake of clarity.

reading frame. The boundaries of the first 21 exons of the DMD gene have been described elsewhere (Koenig et al. 1988; Malhotra et al. 1988; Monaco et al. 1988). In the present study, we cloned and sequenced 17 additional exons. The position of the exon borders relative to the cDNA nucleotide sequence is given in table 1.

Of the 420 independent deletions available (Koenig et al. 1987; Darras et al. 1988; Liechti-Gallati et al. 1989; Lindlöf et al. 1989; Gilgenkrantz et al., in press; and present study), 273 deletions (fig. 2) fulfilled the criteria outlined in Material and Methods. For 10 of the 273 deletions, the consequence of the deletion on the translational reading frame could not be determined because the type of one of the two relevant exon borders was not determined. Deletions in five DMD patients included the first exon, and therefore no protein is expected to be produced, because of the lack of promoter and initiation site for translation. For the remaining 258 deletions, we obtained the following data: 60 of 70 BMD and intermediate patients had a deletion that

**Figure 1** Examples of three frequent types of deletion in the dystrophin gene. Panels *A–C;* Southern blot analysis of *Hin*dIII-digested genomic DNA from DMD and BMD patients. Lane *A*1, *Hin*dIII-digested lambda molecular-weight marker DNA; lanes *A*2, *B*1, *B*2, *B*3, *B*4, C1, and C2, DMD patients (lanes *B*1 and *B*3 and lanes *B*2 and *B*4 are from the same patients, respectively); lane *A*3, BMD patient; lanes *A*2 and *A*3 simultaneous hybridization with probes 7 and 8; lanes *B*1 and *B*2, hybridization with probe 7 alone; lanes *B*3, *B*4, C1, and C2, hybridization with probe 8 alone. Exon deletions in the dystrophin gene can be identified in lanes *A*3, *B*1, and C2 with other patients not exhibiting a deletion in this region. All exons detectable with probe 5b-6 were present in the DNA of patient *B*1/*B*3 (data not shown). The 1.2-kb fragment in panel *A* was clearly visible on a longer exposure. A diagram of the extent of the deletions shown on panels *A–C* is represented below the panels. *a,* Dystrophin cDNA probes; *b,* size of *Hin*dIII exon-containing fragments detected with dystrophin cDNA probes; *c,* exon border type. Interruptions in the black bars represent the extent of the deletions. *A, B,* and *C* refer to the deletions shown on panels *A, B,* and *C,* respectively. Deletion *A* (BMD patient) maintains the reading frame, while deletions *B* and *C* (DMD patients) disrupt the reading frame.


A
1 2 3
10
7
4.1
3.9
3.7
3.1
1.6
1.5
1.2
0.5
B
1 2 3 4
10
7
4.1
3.9
3.7
3.1
1.6
1.5
1.2
0.5
C
1 2
10
7
3.9
3.7
3.1
1.6
1.2
a
5b-6
7
8
b
11
4.1
0.5
1.5
10
1.2+3.9
1.6
3.7
3.1
7.0
c
2
3
2
3
3
3
3
1
3
A
B
C

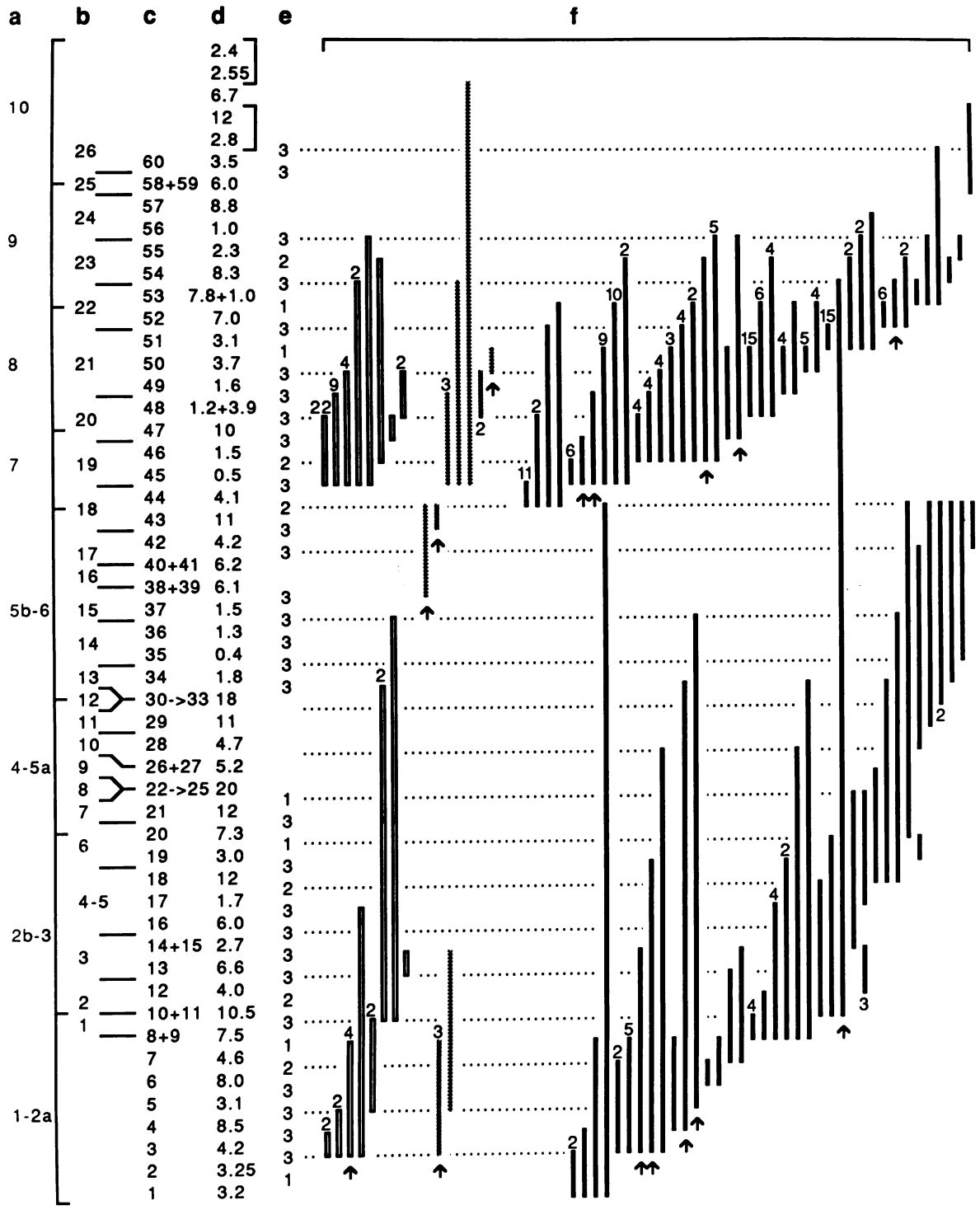



**Figure 2** Extent of 273 independent DMD/BMD deletions relative to the dystrophin exon map. *a*, Dystrophin cDNA probes used for the detection and delineation of the deletions: other combinations of cDNA probes were also occasionally used. The most distal probes (11–14) are not shown, since no deletion was detected with them; *b*, distribution of the repeat units (Koenig et al. 1988) relative to exons 9–60; *c*, exon numbers; *d*, exon-containing *Hin*dIII genomic fragments (sizes in kbp); *e*, exon border type as defined in the legend of table 1; *f*, extent of the deletions found in 273 DMD/BMD patients. Empty bars denote BMD patients; black bars denote DMD patients; hatched bars denote young patients with conditions clearly milder than those of typical DMD and intermediate patients. The numbers above or below the bars indicate the number of independent patients sharing deletions of the same exons; absence of such a number indicates that the deletion was unique to the present study. The arrows indicate the deletions that do not follow the reading-frame rule.

maintained the reading frame (in-frame deletions), and 178 of 188 DMD patients had a deletion that disrupted the reading frame (out-of-frame or frameshift deletions). Thus, the correlation between deletion and phenotype fit the reading-frame model in 238 (92%) of the 258 cases. Twenty cases were in contradiction with the model. Ten patients with BMD or intermediate muscular dystrophy had an out-of-frame deletion, seven of them having deletions of exons 3–7. Interestingly, five DMD patients had a similar deletion. The other 10 exceptions were DMD patients who had deletions that did not disrupt the reading frame. In 3 of these 10 cases the deletion was especially large (30, 32, and 44 exons), probably resulting in a protein too small to be functional. For the seven other DMD exceptions, it is unlikely that the deletions involve domains crucial for the function of the protein, since larger in-frame deletions involving the same domains have been found in BMD patients. It is possible that some of the exceptions to the reading-frame rule are due to limitations of the Southern blot technique used in the present study or that the clinical progression of some patients less than 13 years old may be different from what was predicted at initial diagnosis.

For the BMD cases due to an in-frame deletion, we analyzed the age at loss of ambulation with respect to both the size of protein deletion and the domains deleted (table 2). Of 57 patients with small deletions (1–13 exons), 25 were still ambulatory at age 24 years (some were still ambulatory beyond age 64 years), and three became wheelchair bound at ages 16, 18, and 21 years, respectively. The 29 other patients were less than 23 years old and still ambulatory. On the other hand, three patients had large in-frame deletions (removing 24–27 exons in the rod domain); two were in wheelchairs by age 16–17 years, and one was ambulatory at age 26 years. Another large deletion, for which the effect on the reading frame could not be determined, involved at least 19 exons and extended slightly beyond the rod domain; that patient became wheelchair bound at age 14 years. Those four large BMD deletions were smaller than the three large in-frame deletions found in DMD patients (30, 32, and 44 exons deleted, respectively), indicating that, for the cases with large in-frame deletions, the severity of the disease correlates with the size of the deletion. The clinical expression of the small deletions showed an unexpectedly high variability, even for deletions of the same exons. For example, 22 pa-

**Table 2**

**Ambulatory Status of BMD and Intermediate Patients with In-frame Deletions**

| Exons Deleted | Age When Wheelchair Bound (years) | | | | | | |
|---|---|---|---|---|---|---|---|
| 3 | >29 | 18 | | | | | |
| 3–4 | ≈30 | >20 | | | | | |
| 3–16 | >29 | | | | | | |
| 5–9 | >19 | >17 | | | | | |
| 5–13 | >10 | | | | | | |
| 10–33 | >26 | 16/17 | | | | | |
| 10–36 | 16 | | | | | | |
| 13 | >17 | | | | | | |
| 45–47 | >64 | >52 | >40/>29 | >35 | >35 | >34 | >33 |
| | 30/>15 | 30/>10 | >28 | >27 | >25 | >22 | 21 |
| | >19 | >19 | >18 | >18 | >16 | >16 | >15 |
| | >15 | | | | | | |
| 45–48 | >64/>15 | >42 | >41 | >28 | 26 | >22 | >15 |
| | >15 | >15 | >12 | >9 | >8 | | |
| 45–49 | >31/>21 | ≈25 | >22 | >20 | | | |
| 45–53 | >27 | >8 | | | | | |
| 45–55 | >16 | | | | | | |
| 46–54 | ≈25 | | | | | | |
| 47 | 16 | | | | | | |
| 48–49 | 53 | >18 | >14 | >11 | | | |

NOTE.—The extent of deletions is indicated by the number of the first and last exons deleted. The ages when patients became wheelchair bound is indicated. Patients still ambulatory are indicated by ">[age at last clinical examination]" (e.g., >29). Related patients are separated by a slash (e.g., 16/17).

tients had deletion of exons 45–47; one of these patients became wheelchair bound at age 21 years, and two were still ambulatory at ages 52 and 64 years, respectively (table 2).

DMD patients with deletions beginning with exons 54, 55, or 58 could potentially synthesize the first three-quarters of the dystrophin protein, including the N-terminal domain and the first 22 or 24 repeat units of the rod domain. These truncated molecules are predicted to lack only the last 800–1,000 amino acids, including the cysteine-rich and C-terminal domains, and are either nonfunctional or unstable, because they result in DMD. Therefore, at least a part of the last two domains of dystrophin seems to be essential for the function or stability of dystrophin. In contrast, the first two domains can be entirely missing (N-terminal domain) or partially missing (up to 1,400 amino acids or 13 repeats of the rod domain) and still result in the milder BMD.

Interestingly, we found no small in-frame BMD or intermediate deletions involving exons 31–44, despite the numerous possibilities to create in-frame deletions in this region. In contrast, several out-of-frame deletions in this region were found in DMD patients and in two intermediate patients. This anomaly becomes more striking when one considers the frequency of deletions that have one breakpoint between exons 44–45. The intron between exons 44 and 45 is the one most frequently involved in deletion breakpoints, at least in part because of its extremely large size (den Dunnen et al., in press; Gilgenkrantz et al., in press). Eighty-three deletions (fig. 2) had one breakpoint in that intron: 43 patients with either BMD or intermediate myopathy had in-frame deletions, and 29 DMD patients had out-of-frame deletions that extended toward the 3′ end of the gene, while 11 DMD out-of-frame deletions extended toward the 5′ end of the gene (these latter 11 deletions are deletions of exon 44 alone). It is particularly puzzling that no BMD deletions starting in that very large intron and extending 5′ to exon 44 have been found, since such deletions should be in-frame. Perhaps some internal deletions, particularly between exons 31 and 44, yield either no clinical manifestations or very mild limb weakness. We also found no deletions in the region encoding the cysteine-rich and C-terminal domains, but both BMD and DMD deletions are underrepresented in those regions.

## Discussion

The present data help to define BMD and DMD at the molecular level and should be useful in establishing the prognosis for individual patients even in sporadic cases with no affected relatives. Clinical/molecular correlations based on the alteration of the reading frame are valid in 92% of cases. Deletions of exons 3–7 (Malhotra et al. 1988; present study) have no consistent clinical pattern, while either deletions of the first exon or large in-frame deletions (30 or more exons) were associated with DMD. After exclusion of (*a*) deletions of exons 3–7 and (*b*) large in-frame deletions, the correlation between deletion and clinical severity was as predicted in 96% of cases. As such, prognosis based on the extent of deletion has already reached a relatively high level of accuracy, and understanding the molecular mechanism underlying the exceptions to the reading-frame rule is likely to improve the accuracy of this approach. Malhotra et al. (1988) and, more recently, Baumbach et al. (1989) have reached opposite conclusions; however, both studies were limited to a smaller subset of deletions for which the reading frame could be predicted. The combination of genomic deletion analysis and direct dystrophin analysis (Hoffman et al. 1988) should give maximal diagnostic and prognostic accuracy, even before the onset of any clinical symptoms.

In the future, routine detection of deletions could be carried out by the powerful technique of polymerase-chain-reaction (PCR) amplification (Saiki et al. 1988). The distribution of the deletions (fig. 2) indicates that PCR amplification of the nine exons 3, 8, 13, 43, 44, 47, 50, 51, and 52 should allow detection of 90% of all deletions. Once a deletion has been identified, further delineation of the exact exons lost could also be better carried out by PCR amplification of appropriate sets of exons. This technique should overcome several limitations of the Southern blot technique, namely, problems with comigrating fragments, weakly hybridizing fragments, and characterization of the exons in junction fragments. Our determination of the sequences flanking 35 exons (Koenig et al. 1988; Monaco et al. 1988; and present study) should be helpful in designing oligonucleotide primers for multiplex PCR amplification (Chamberlain et al. 1988) of these exons.

Correlation between phenotype and molecular deletion for a given patient strongly suggests that the two exons directly flanking the deletion are indeed spliced together in that patient, resulting in the synthesis of altered mRNA and protein, as predicted. It is possible, however, that rearrangements due to deletion breakpoints in the intron may alter, in some instances, the splicing process or that the donor and acceptor splice sites flanking the deletion may not be compatible. Such

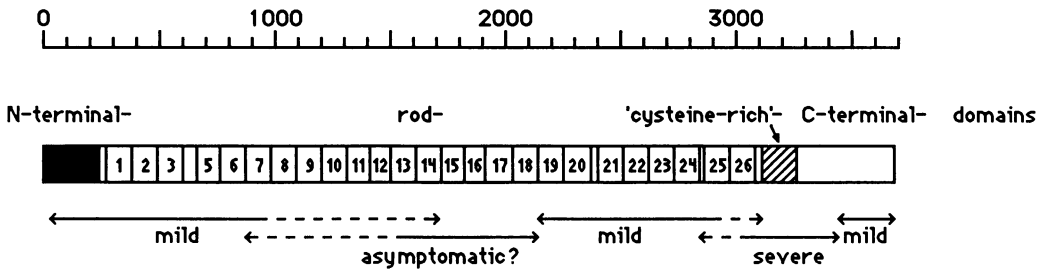



**Figure 3** Correlation of symptomatology with dystrophin domain alterations. Dystrophin domains are as reported by Koenig et al. (1988). The amino acid scale is given on top. The symptomatology expected for small domain alterations (see Discussion) is indicated below the domain diagram. Large alterations, such as large deletions and out-of-frame deletions, result in more severe symptoms. Mild = mild muscular dystrophy, Becker type; severe = severe muscular dystrophy, Duchenne type.

alterations of the splicing specificity might explain some cases of discrepancy between phenotype and molecular deletion. All the BMD and intermediate cases with out-of-frame deletions would produce an in-frame mRNA if the first exon beyond the distal deletion breakpoint is skipped. Position of the distal deletion breakpoint within intron 7 (between exons 7 and 8) might determine whether exon skipping will occur or not and might thus explain the wide range of phenotypes associated with deletions of exons 3–7 (Malhotra et al. 1988; present study). A similar mechanism might apply to the out-of-frame deletion of exon 50 (3.7-kb *Hin*dIII fragment); two intermediate patients (one described in Baumbach et al. 1989 and one described in the present study) and six DMD patients (one described in Baumbach et al. 1989 and five described in the present study) have this deletion.

The present data are also useful in analyzing the relationship of the different dystrophin domains to the pathophysiology of DMD and BMD. We assumed that, for the cases where the phenotype is in agreement with the reading frame, the dystrophin protein is synthesized as predicted by the deletion analysis. Interstitial deletions in the N-terminal domain and in both the first 13 and the last 8 repeats result in BMD, while terminal deletions of both the cysteine-rich and C-terminal domains result in DMD. Darras and Francke (1988*a*) and McCabe et al. (1989) have reported a terminal deletion involving part of the C-terminal domain in a patient with mild, nonprogressive, Becker-like muscular dystrophy. Thus, the region that, when missing, results in DMD is limited to the cysteine-rich domain and to the first half of the C-terminal domain. The high degree of conservation of these domains during evolution (Lemaire et al. 1988) also indicates that they are essential for the proper function of dystrophin. Furthermore, analysis of the distribution of deletions in relation to exon border types suggests that the frequency of in-frame deletions may be underestimated. Asymptomatic individuals or those with no muscle weakness might have deletions in the dystrophin gene, and we predict that such deletions are more likely to occur in the region of exons 31–44 (repeats 12–18). A tentative "functional" map of dystrophin (fig. 3) can be constructed from this deletion analysis. This map illustrates that alterations of the cysteine-rich and/or C-terminal domains appear to be more deleterious than do alterations of other parts of dystrophin and that alterations of the middle section of the rod domain might be less deleterious or asymptomatic.

## Acknowledgments

We would like to thank the many physicians who contributed to the gathering of blood samples and clinical information; and we would also like to thank E. P. Hoffman, R. Boyce, S. Iwamoto, and L. P. Rowland for discussions and critical reading of the manuscript. L. M. K. is an associate investigator of the Howard Hughes Medical Institute. This work was supported in part by the National Institutes of Health and by the Muscular Dystrophy Association of America.

## References

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

Burghes AHM, Logan C, Hu X, Belfall B, Worton R, Ray PN (1987) Isolation of a cDNA clone from the region of an X:21 translocation that breaks within the Duchenne/Becker muscular dystrophy gene. Nature 328:434–436

Burmeister M, Monaco AP, Gillard EF, van Ommen GJB, Affara NA, Ferguson-Smith MA, Kunkel LM, et al (1988)

A 10-megabase physical 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 16:11141–11156

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

den Dunnen JT, Grootscholten PM, Bakker E, Blonden LAJ, Ginjaar HB, Wapenaar MC, van Paassen HMB, et al. Topography of the DMD gene: FIGE- and cDNA-analysis of 152 cases, involving 85 deletions and 11 duplications (in press)

Engel AG, Banker BQ (eds) (1986) Myology: basic and clinical. Vol 2. McGraw-Hill, New York

Forrest SM, Smith TJ, Gross GS, Read AP, Thomas NST, Mountford RC, Harper PS, et al (1987) Effective strategy for prenatal prediction of Duchenne and Becker muscular dystrophies. Lancet 2:1294–1297

Gilgenkrantz H, Chelly J, Lambert M, Récan D, Barbot JC, van Ommen GJB, Kaplan J-C. Analysis of molecular deletions with cDNA probes in patients with Duchenne and Becker muscular dystrophy. Genomics (in press)

Hoffman EP, Fischbeck KH, Brown RH, Johnson M, Medori R, Loike JD, Harris JB, et al (1988) Dystrophin characterization in muscle biopsies from Duchenne and Becker muscular dystrophy patients. New Engl J Med 318:1363– 1368

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

Lemaire C, Heilig R, Mandel J-L (1988) The chicken dystrophin cDNA: striking conservation of the C-terminal coding and 3′ untranslated regions between man and chicken. EMBO J 7:4157–4162

Liechti-Gallati S, Koenig M, Kunkel LM, Frey D, Boltshauser E, Schneider V, Braga S, et al (1989) Molecular deletion patterns in Duchenne and Becker type muscular dystrophy. Hum Genet 81:343–348

Lindlöf M, Kiuru A, Kääriäinen H, Kalimo H, Lang H, Pihko H, Rapola J, et al (1989) Gene deletions in X-linked muscular dystrophy. Am J Hum Genet 44:496–503

McCabe ERB, Towbin J, Chamberlain J, Baumbach L, Witkowski J, van Ommen GJB, Koenig M, 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 242:756–759

Monaco AP, Bertelson CJ, Colletti-Feener C, Kunkel LM (1987) Localization and cloning of Xp21 deletion breakpoints involved in muscular dystrophy. Hum Genet 75: 221–227

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

Saiki RK, Gelfand DH, Stoffel S, Scharf J, Higuchi R, Horn GT, Mullis KB, et al (1988) Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491

van Ommen GJB, Bertelson C, 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, Abbs S, Blonden LAJ, den Dunnen JT, Grootscholten PM, et al (1988) A deletion hotspot in the Duchenne muscular dystrophy gene. Genomics 2:101–108