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# Differentiation of Duchenne and Becker Muscular Dystrophy Phenotypes with Amino- and Carboxy-Terminal Antisera Specific for Dystrophin

Dennis E. Bulman,*'‡ E. Gordon Murphy,† Elizabeth E. Zubrzycka-Gaarn,* Ronald G. Worton,*'‡ and Peter N. Ray*'‡

*Genetics Department and Research Institute and †Division of Neurology, Hospital for Sick Children; and ‡Department of Medical Genetics and Medical Biophysics, University of Toronto, Toronto

## Summary

**Antibodies directed against the amino- and carboxy-terminal regions of dystrophin have been used to characterize 25 Duchenne muscular dystrophy (DMD), two intermediate, and two Becker muscular dystrophy (BMD) patients. Western blot analysis revealed an altered-size (truncated) immunoreactive dystrophin band in 11 of the 25 DMD patients, in one of the two intermediate patients, and in both BMD patients, when immunostained with antiserum raised against the amino terminus of dystrophin. None of the DMD or intermediate patients demonstrated an immunoreactive dystrophin band when immunostained with an antiserum specific for the carboxy terminus of the protein. In contrast, dystrophin was detected in both BMD patients by the antiserum specific for the carboxy terminus. Quantitative studies indicated that the relative abundance of dystrophin in patients with a severe (DMD), intermediate, or mild (BMD) phenotype may overlap, therefore suggesting that differential diagnosis of disease severity based entirely on dystrophin quantitation may be unsatisfactory. Our results suggest that a differential diagnosis between DMD and BMD would benefit from examination of both the N terminus and C terminus of the protein, in addition to measurements of the relative abundance of the protein.**

## Introduction

Duchenne muscular dystrophy (DMD), an X-linked recessive disorder affecting approximately 1/3,000 newborn males, is characterized by progressive skeletal muscle weakness resulting in death usually in the second decade of life (Emery 1987). Becker muscular dystrophy (BMD) is a milder and less prevalent clinical variant of DMD and is known to be caused by a subset of mutations in the DMD gene. Identification of the DMD gene at Xp21 (Monaco et al. 1986; Burghes et al. 1987) and cloning and sequencing of the entire cDNA (Koenig et al. 1987, 1988) have been important milestones in the understanding of the disease. The cloning of the gene has led directly to the identification of dystrophin, the protein product of the DMD gene (Hoffman et al. 1987*a;* Zubrzycka-Gaarn et al. 1988). This protein, missing or altered in boys with DMD or BMD, is a large cytoskeletal protein (Hammonds 1987; Davison and Critchley 1988) with a predicted molecular weight of 427 kD. While subcellular fractionation had initially localized dystrophin to the triads of skeletal muscle (Hoffman et al. 1987*b;* Knudson et al. 1988), immunohistochemistry provided a more definitive localization of dystrophin at the sarcolemma (Arahata et al. 1988; Bonilla et al. 1988; Sugita et al. 1988; Watkins et al. 1988; Zubrzycka-Gaarn et al. 1988; Carpenter et al. 1990; Cullen et al. 1990). This surface localization was subsequently confirmed in further subcellular fractionation (Salviati et al. 1989). The function of dystrophin has not yet been clarified, but a role in preserving the integrity of the sarcolemmal membrane during muscle

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Address for correspondence and reprints: Dr. P. N. Ray, Department of Genetics, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada.

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contraction is an attractive idea that is consistent with our knowledge of the disease pathology (Rowland 1980).

Recent advances in diagnostic methodology and neonatal screening programs are allowing identification of DMD/BMD patients at an early age (Jacobs et al. 1989). However, prognostic indicators for DMD/BMD at these stages have been lacking. Clinically, there is a continuous spectrum of severity, and the definitions of DMD (wheelchair bound before age 12 years) and BMD (still ambulatory at age 16 years) are arbitrary. Genetic studies are beginning to yield important new prognostic information. Deletions are the most common form of mutation in both DMD and BMD (den Dunnen et al. 1987; Forrest et al. 1987; Koenig et al. 1987), and the nature of the mutation appears to be a determining factor of severity in approximately 90% of cases. As expected on theoretical grounds, deletions that are predicted to result in an mRNA transcript with a disrupted reading frame are most frequent in the DMD group, whereas deletions that do not disrupt the reading frame are found more often in the BMD group (Monaco et al. 1988; Baumbach et al. 1989; Gillard et al. 1989; Koenig et al. 1989). Noteworthy exceptions have been reported (Malhotra et al. 1988; Baumbach et al. 1989).

Study of the protein has also provided prognostic information, as western blot analysis has detected dystrophin of altered molecular weight in BMD patients but has failed to detect dystrophin in DMD patients (Hoffman et al. 1988, 1989; Patel et al. 1988; Arahata et al. 1989). The report of a BMD patient with no detectable dystrophin but an apparently normal DMD gene locus appears to be an exceptional case (Patel et al. 1989). For the majority of DMD patients, it has been suggested that truncated dystrophin molecules resulting from apparent frameshifting mutations may be too unstable to be observed on western blots (Hoffman and Kunkel 1989).

In our own studies aimed at developing prognostic indicators for DMD and BMD patients (Bulman et al. 1989), we have used Southern blot analysis and quantitative western blot analysis with two anti-dystrophin antibodies. The two specific antisera, directed against opposite ends of dystrophin, have been used to estimate both the relative abundance and the size of dystrophin molecules in DMD and BMD patients. The detection of truncated dystrophin molecules in DMD patients as reported here and elsewhere (Nicholson et al. 1989) contrasts with results of earlier studies and may be related, in part, to the higher specificity and avidity of these newer amino-terminal antibodies.

## Material and Methods

### *Antisera*

Antisera 9219 was produced in sheep through immunization with a TrpE-DMD fusion protein, synthesized in *Escherichia coli,* from a gene construct containing exons 4–16 of the dystrophin gene. Plasmid construction and fusion-protein induction have been described elsewhere (Zubrzycka-Gaarn et al. 1988). Bacterial proteins were size fractionated on a preparative SDS-polyacrylamide gel (Laemmli 1970). The gels were rinsed with distilled water and then were immersed in cold 0.25 M KCl, in order to visualize the fusion protein. A band containing approximately 1 mg of the precipitated fusion protein was excised from the gel and was crushed and mixed with either Freund's complete adjuvant, for the initial immunization, or Freund's incomplete adjuvant, for subsequent injections. The sheep was injected at six separate sites. The resulting antisera were partially purified according to a method described elsewhere (Hoffman et al. 1987*a*). Production, purification, and characterization of serum 1461, which was raised in a rabbit and recognizes the last 17 amino acids of dystrophin, has been described in detail elsewhere (Zubrzycka-Gaarn et al., in press).

### *Clinical Classification of Patients*

All patients in the present study attended the muscular dystrophy clinic at The Hospital for Sick Children, Toronto. The age at which patients became permanently wheelchair bound was the main clinical parameter used to differentiate DMD, intermediate, and BMD phenotypes. Patients who became permanently wheelchair bound before the age of 12 years were classified as DMD, whereas individuals who were ambulatory at the age of 16 years were classified as BMD patients. Patients who became wheelchair bound between the ages of 12 and 16 years were classified as intermediate. Those patients who were too young to be differentially classified according to the above scheme were assessed using clinical, histopathological, genetic, and biochemical data.

### *Sample Preparation*

Muscle biopsies of patients were taken under local

anesthetic, placed on ice, and snap-frozen within 20 min. All biopsies were stored in liquid nitrogen. Samples were crushed in a precooled mortar embedded in dry ice. Approximately 50 mg of crushed muscle was added to 0.2 ml of a homogenate solution (1% SDS, 1 mM PMSF, 1 mM benzamidine, 5 mM EGTA, 0.5 µg leupeptin/ml and 0.2 units trasylol/ml). The samples were vortexed for 30 s, incubated at 100°C for 2 min, and centrifuged at 12,000 *g* for 1 min. An aliquot of the supernatant was removed, and the protein concentration was determined using BSA as the protein standard (Lowry et al. 1951). The remaining supernatant was divided into 20-µl aliquots and put into precooled (−20°C) tubes and stored at −70°C. Each aliquot was thawed only once, in order to minimize artifacts due to repeated freezing and thawing.

### *Gel Electrophoresis*

Samples were heated to 100°C for 2 min in Laemmli (1970) sample buffer and were electrophoresed on 25-well, 6% (1% crosslinker) SDS-polyacrylamide gels (1.5 mm × 16 cm × 20 cm) with a 4% stacking gel (1% crosslinker). All gels were run at 50 V until the dye front reached the bottom of the gel.

In order to compensate for the variable amounts of muscle tissue per sample, all samples were first normalized for their myosin content. A control sample consisting of 25 µg of normal male muscle protein was loaded on every gel along with 25 µg of patient muscle protein and was subjected to SDS-PAGE. After electrophoresis, the gels were stained with Coomassie brilliant blue (Bio-Rad) and were destained (7.5% acetic acid, 5% methanol). The gels were subjected to computerized scanning laser densitometry (Molecular Dynamics 300A), and the levels of myosin for each sample were compared with that of the normal control sample from the same gel. Previous analysis demonstrated that the densitometric response to myosin content in Coomassie-stained polyacrylamide gels remained linear beyond that observed in 100 µg of total muscle protein (data not shown).

### *Quantitative Western Blot Analysis*

Duplicate 6% polyacrylamide gels (1% crosslinker), each containing an amount of myosin equivalent to approximately 50 µg of normal muscle protein/well, were subjected to SDS-PAGE. All gels contained normal control samples in addition to patient samples. After gel electrophoresis, the proteins were transferred to nitrocellulose (Towbin et al. 1979) at 500 mA for 18 h at 15°C by using Towbin buffer without methanol. After the western transfer, the nitrocellulose was dried, and the efficiency of the transfer was checked by staining the blots with Ponceau S (Sigma). The blots were subsequently destained with PBS. The membranes were blocked for 2 h at room temperature with 5% nonfat milk in TBST (10 mM Tris, HCl, pH 8.0; 500 mM NaCl; 0.05% (v/v) Tween-20) and 1% normal donkey serum and then were incubated with affinity-purified antiserum 9219 or 1461 at a dilution of 1:5,000 or 1:1,000, respectively, for 2 h. Blots were then washed (3 × 10 min) with TBST and incubated with an alkaline phosphatase–conjugated donkey anti-sheep antibody (serum 9219) or with a goat anti-rabbit (serum 1461) secondary antibody. Blots were washed with TBST (4 × 10 min), were rinsed with TBS, and were developed with nitroblue tetrazolium and bromo-chloro-indoylphoshate. All antibodies were diluted in TBST and 1% normal donkey serum.

Black-and-white photographs of all western blots were subjected to computerized scanning laser densitometry (Molecular Dynamics 300A). Photographs of all blots were taken with Technical Pan film (Kodak) and were printed on Polycontrast 3 resin-coated paper (Kodak). The entire photograph was scanned, and each immunoreactive dystrophin band was analyzed in three dimensions (X, Y, density). Volume integration was performed after first subtracting the background surrounding the band to be quantitated. Standard curves of western blots containing a dilution series, ranging from 1 to 50 µg of normal skeletal muscle protein, were analyzed in order to ensure that all densitometric measurements were obtained in the linear range.

The absorbance of the dystrophin band from the normal control was compared with that of each patient, on the same blot. The relative abundance of patient dystrophin was recorded as a percentage of the absorbance in the normal control.

## Results and Discussion

Both the amino-terminal specific and carboxy-terminal specific antisera used in the present study identify dystrophin in human and mouse skeletal muscle but do not react with skeletal muscle protein from the *mdx* mouse (fig. 1). The *mdx* mouse has in the DMD gene a nonsense mutation which would be expected to truncate dystrophin to approximately 115 kD (Sicinski et al. 1989).

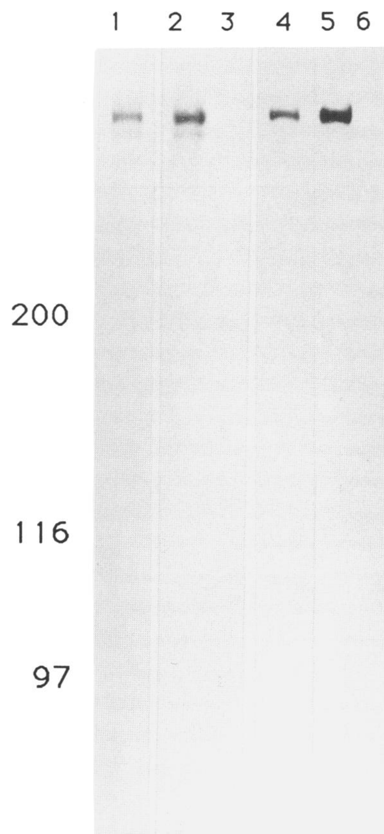


**Figure 1** Western blot of 50 μg normal human muscle (lanes 1 and 4), 50 μg normal mouse muscle (lanes 2 and 5), and 50 μg *mdx* mouse muscle (lanes 3 and 6) immunostained with antiserum 9219 (lanes 1–3) or 1461 (lanes 4–6). The positions of molecular-weight markers are given.

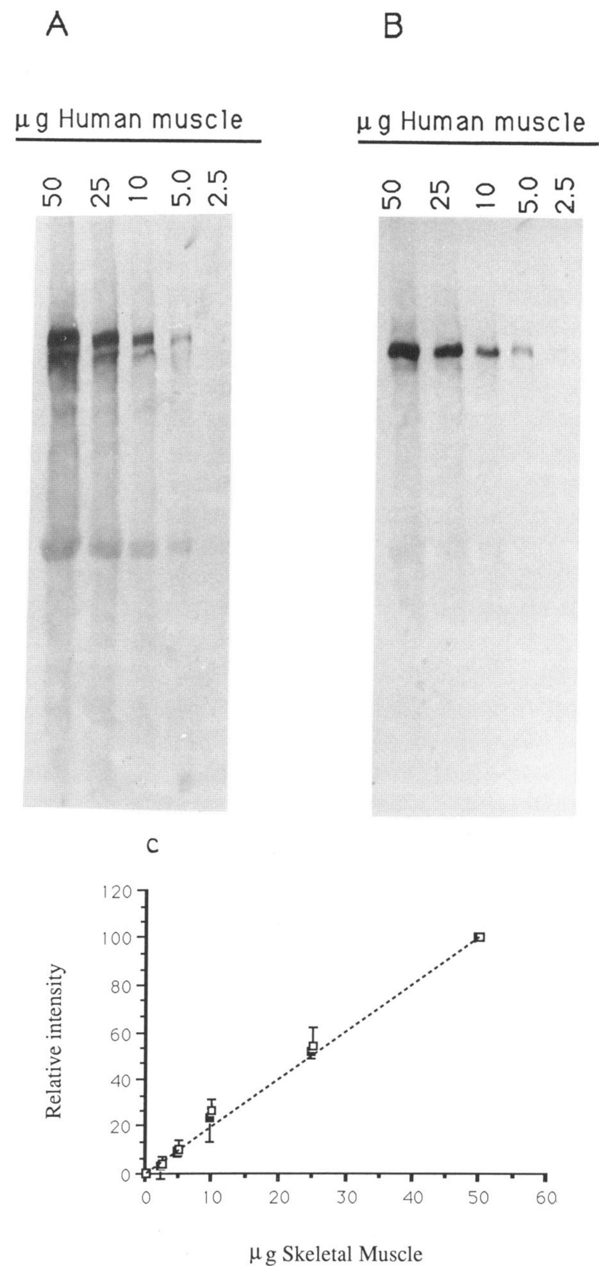


**Figure 2** Western blot containing limiting dilution of total human muscle immunostained with (*A*) serum 9219 and serum 1461 (*B*). Lane 1, 50 μg. Lane 2, 25 μg. Lane 4, 5 μg. Lane 5, 2.5 μg. *C*, Densitometric analysis of western blot. Photographs of western blots were subjected to computerized laser scanning densitometry (Molecular Dynamics Model 300A). Absorbance units of immunoreactive dystrophin were normalized to 50 μg. Photographs from three independent blots were analyzed for each antisera. The mean of the normalized values was plotted against total amount of human muscle applied per well (serum 1461 [□] and serum 9219 [■]). The vertical bars at tops of open boxes and at bottoms of closed boxes represent 3 SD. The broken line (– – –) represents expected results when a linear response of absorbance versus total protein loaded is given.

Sera 9219 and 1461 have a high affinity for dystrophin on western blots, detecting this protein in 2.5 μg of normal human skeletal muscle protein. Photographs of western blots subjected to laser scanning densitometry gave an absorbance of dystrophin that was linearly related to the amount of protein loaded, up to approximately 60 μg of normal human muscle extract. Within this range, therefore, an accurate determination of the relative amount of dystrophin could be made (fig. 2). To generate figure 2*C*, three independent dilution-series blots were analyzed for each antibody. Data from each blot were normalized against 50 μg of skeletal muscle protein. Data were averaged and plotted, with error bars representing 3 SD about the mean (fig. 2*C*). This technique of quantitation was found to be highly reproducible, with the sensitivity of the two antisera (sera 9219 and 1461) appearing to be equal.

**Table I**

**Summary of Quantitative Western Blot Analysis**

| Patient | Age[a] (years) | Age When WCB[b] (years) | % of Normal Dystrophin | | MW of Dystrophin | | Exons Deleted[d] |
|---|---|---|---|---|---|---|---|
| | | | Serum 9219 | Serum 1461 | Observed | Predicted[c] | |
| DMD: | | | | | | | |
| 43 | 16 | 7 | 15 | <5 | 232 | 245 | 44 |
| 45 | 14 | 11 | 6 | <5 | 246 | 256 | 37–41 |
| 49 | 17 | 11 | 11 | <5 | 292 | 286 | 51 |
| 64 | 13 | 10 | 17 | <5 | 126 | ... | |
| 65 | 10 | 8 | 11 | <5 | 420 | ... | |
| 59 | 9 | 8 | <5 | <5 | ... | ... | |
| 66 | 25 | 10 | <5 | <5 | ... | ... | |
| 35 | 10 | amb | 6 | <5 | 269 | 270 | 48–52 |
| 39 | 7 | amb | 9 | <5 | 293 | 297 | 50–52[e] |
| 55 | 8 | amb | 7 | <5 | 233 | 237 | 43 |
| 56 | 9 | amb | 9 | <5 | 240 | ... | |
| 60 | 8 | amb | 6 | <5 | 367 | ... | |
| 34 | 9 | amb | 6 | <5 | 269 | 277 | 49–52 |
| 46 | 8 | amb | <5 | <5 | ... | 268 | 48–50 |
| 26 | 8 | amb | <5 | <5 | ... | 268 | 48–50 |
| 42 | 10 | amb | <5[f] | <5 | ... | 25 | 8–9 |
| 41 | 11 | amb | <5[f] | <5 | ... | 11 | 5–7 |
| 68 | 7 | amb | <5[f] | <5 | ... | 0 | 1 |
| 30 | 9 | amb | <5 | <5 | ... | ... | |
| 38 | 9 | amb | <5 | <5 | ... | ... | |
| 51 | 9 | amb | <5 | <5 | ... | ... | |
| 29 | 7 | amb | <5 | <5 | ... | ... | |
| 25 | 9 | amb | <5 | <5 | ... | ... | |
| 32 | 7 | amb | <5 | <5 | ... | ... | |
| 63 | 10 | amb | <5 | <5 | ... | unknown[g] | |
| Intermediate: | | | | | | | |
| 53 | 16 | 14 | 16 | <5 | 209 | 207 | 20–37[e] |
| 48 | 17 | 14 | <5 | <5 | | 251 | 37 |
| BMD: | | | | | | | |
| 54 | 17 | amb | 21 | 25 | 390 | 401 | 45–48 |
| 40 | 9 | amb | 14 | 17 | 390 | 396 | 45–49 |

[a] As of 1990.
[b] Age when patient became permanently wheel chair bound; amb = still ambulatory.
[c] Molecular weight of truncated dystrophin molecule, determined from the translation of the cDNA sequence in those patients with detectable deletions or duplications.
[d] Exon number, based on that designated by Koenig et al. (1989).
[e] Duplication.
[f] Not expected to react with 9219 because of a dystrophin deletion in the region recognized by the antisera.
[g] Deletion status unknown.

Since muscle derived from DMD and BMD patients may contain large amounts of fat and connective tissue, depending on the age and the clinical condition of the patient, dystrophin quantitation based on total protein content of the muscle extract would underestimate the relative amount of dystrophin in dystrophic muscle fibers. Dystrophin measurements were therefore made on western blots from gels in which the sample loaded was normalized to the amount of myosin in 50 μg of normal skeletal muscle protein.

Dystrophin analysis was performed on biopsies from 27 patients including 23 DMD, two intermediate, and two BMD patients. A measurable amount of altered-size dystrophin was observed with the amino-terminal specific antiserum (serum 9219) in 11 of the DMD patients, in one of two intermediate patients,

and in both BMD patients. The relative amount of detectable dystrophin ranged from 6% to 17% of normal in the DMD patients, was 16% of normal in the intermediate patient, and ranged from 14% to 21% of normal in the BMD patients (table 1).

In contrast to the results with serum 9219, the carboxy-terminal specific antiserum (serum 1461) did not detect dystrophin in any of the DMD patients. This would be expected if frameshift deletions are causing premature termination of protein synthesis. Relevant to this is the fact that 12 of the DMD patients had detectable deletions or duplications, all of which were predicted to disrupt the translational reading frame. Seven of these 12 patients demonstrated truncated dystrophin, and close agreement was observed in every case between the size of the immunoreactive band detected on western blots and that predicted from the translation of the cDNA sequence (table 1). Patients 64 and 65 produced altered size dystrophin but did not have a detectable deletion or duplication as determined by Southern blot analysis using DMD-cDNA probes. These would be candidates for nonsense mutations or frameshift mutations not detectable by Southern blot analysis.

Both BMD patients (patients 40 and 54) showed positive immunostaining with serum 1461, indicating the presence of the carboxy terminus of dystrophin (fig. 3). For patient 54, clinical evaluation revealed a mild course of disease, and DNA analysis revealed an in-frame deletion of exons 45–48. For patient 40, a similar but distinct deletion of exons 45–49 was detected (Gillard et al. 1989). In both patients, both antisera revealed the expected dystrophin band at 390 kD, but antiserum 9219 revealed an additional immunoreactive band at 238 kD in both samples. This band has not been observed in any of our other patients and may represent a stable breakdown product (Hoffman et al. 1988). An alternative possibility is suggested by the fact that the size of this band corresponds closely to a protein predicted by a translational frameshift at the junction of the deletion, suggesting the possibility of alternative splicing of a subset of transcribed mRNA molecules from the last exon preceding the deletion.

The consistency of dystrophin quantitation with two different antisera is demonstrated in figure 2*C* and in the case of patients 40 and 54. In patient 40 the relative amount of the 390-kD band immunostained with serum 9219 or/and serum 1461 was 14% and 17%, respectively; in patient 54 these amounts were 21% and 25%, respectively. The relative abundance of the 238-kD band observed in these patients, which was only detectable with serum 9219, was 14% and 17%, respectively. Intermediate patient 53 has a well-characterized partial gene duplication predicted to disrupt the translational reading frame (Hu et al. 1990), leading to premature termination of a 207-kD protein. The immunoreactive band observed with antiserum 9219 had a molecular weight of 209 kD, in agreement with this prediction. The absence of immunostaining with antiserum 1461 is also consistent with a truncated protein due to a frameshift duplication (fig. 3).

Of the 14 DMD patients with no detectable immunoreactive dystrophin, three patients had mutations which are not expected to produce a dystrophin recognized by serum 9219. Patient 41 was found to be deleted for all of exon 1. The deletion extended past the region of the promoter responsible for muscle-specific gene expression (Klamut et al. 1988) but did not include the brain-specific DMD promoter (data not shown). The deletions in patient 68 (exons 5–7) and patient 42 (exons 8 and 9) cause frameshifts in the portion of the protein recognized by antibody 9219. Seven patients with no detectable immunoreactive dystrophin had no detectable genomic alteration as determined by Southern blot analysis of patient DNA probed with the entire DMD cDMA. The remaining two patients (patients 46 and 26) had frameshifting deletions which removed the same exons (exons 48–50), apparently resulting in an unstable truncated dystrophin molecule.

In three cases, an altered dystrophin band was detected prior to cDNA analysis. In each case, the predicted site of the disruption in the reading frame, on the basis of the molecular mass of dystrophin, was within 250 bp of the actual site, as determined later by Southern blot analysis with cDNA probes. Western blotting therefore appeared to be an accurate method of determining the site of potential translational frameshifting or nonsense mutations, in those cases where truncated proteins could be detected.

As reported elsewhere (Hoffman et al. 1987*b;* Patel et al. 1988), on western blots immunostained with the amino-terminal specific antiserum, dystrophin appeared as a doublet or triplet. The two lower bands were less intense than the upper band and were also absent in DMD and BMD patients (fig. 3). The serum directed against the carboxy terminus of dystrophin detected only the largest of the three bands, and as a result we have used this upper band as our high-molecular-weight marker of 427 kD. Because the lower bands are not detected with serum 1461 and because

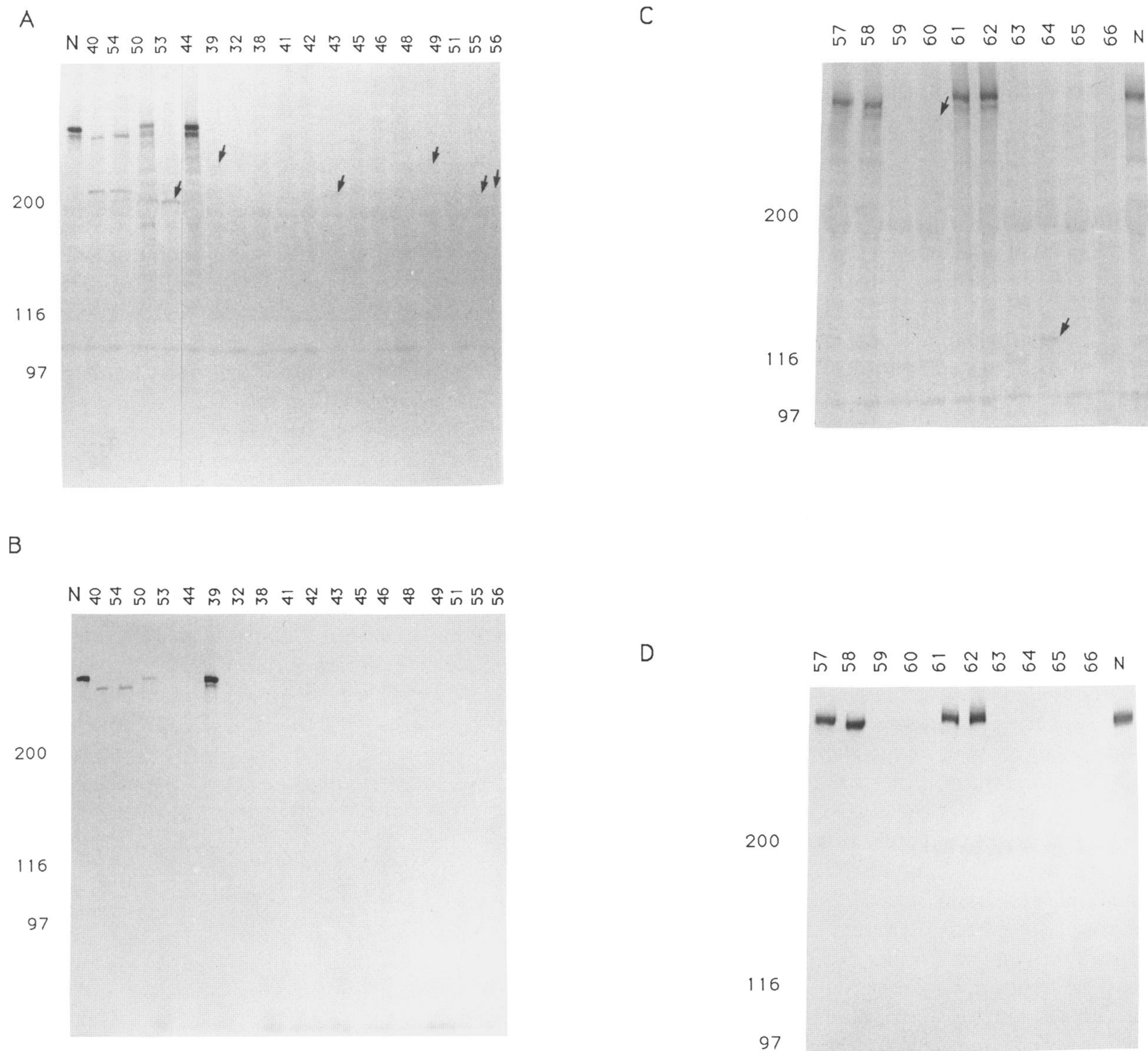


**Figure 3** Western blot of patient muscle immunostained with 9219 (*A* and *C*) and 1461 (*B* and *D*). Sample numbers are given above each lane: N = normal muscle; 40 and 54 = BMD; 53 and 48 = intermediate MD; 39, 32, 38, 41, 42, 45, 46, 49, 51, 55, 56, 59, 60, 63, 64, 65, and 66 = DMD; 50 and 44 = cardiomyopathy; 57 and 58 = juvenile dermatomyositis; 61 = fibromyalgia; 62 = progressive neurodegenerative disease. Arrows indicate faint immunoreactive bands. All samples were normalized, for myosin concentration, to a normal muscle sample, and the equivalent of 50 μg muscle was loaded per lane. Molecular-weight standards (kD) are given in the left margin. Truncated dystrophin seen in the DMD patients is reduced in abundance and may not be readily detectable on photographic reproduction.

both antisera have equal levels of detection, the lower bands are presumed to have a different carboxy terminus. These lower-molecular-weight bands may represent either breakdown products with preferential proteolysis occurring at the carboxy terminus or alternatively spliced isoforms (Fenner et al. 1989).

Although the transfer conditions used in western blotting in both the present study and in others (Hoffman et al. 1987*b;* Patel et al. 1988; Arahata et al. 1989) have been modified to increase the transfer efficiency of large-molecular-weight proteins, the transfer efficiency of different-sized dystrophin molecules (na-

tive vs. truncated) may differ. Such a difference could imply that smaller molecules may transfer more efficiently and could potentially result in an overestimate of the amount of the truncated dystrophin molecules in these patients. While this still remains a possibility, it does not affect the conclusion that many DMD and BMD patients produce detectable levels of truncated dystrophin.

The inability to detect an altered-size dystrophin in the *mdx* mouse or in some of the other patients may be due to the instability of these particular truncated proteins or to instability of mRNA. Indeed, reduced stability of mutant nuclear mRNA is well documented (Humphries et al. 1984; Takeshita et al. 1984).

The spectrum of disease severity among DMD and BMD patients may be due, in part, to the cellular concentration of truncated dystrophin, as well as to the extent to which functional regions of dystrophin have been removed or disrupted. A mutation which removes only a small portion of dystrophin but results in an unstable protein may be as debilitating as a large deletion which removes functional regions but results in a stable molecule. Both stability and functionality of dystrophin probably play a role in phenotypic expression of the disease state. Assessment of the disease status of patients with equivalent amounts of dystrophin but different mutations may allow mapping of functional regions of the dystrophin molecule.

The relatively low level of dystrophin in BMD and intermediate patients implies that the ability to express functional dystrophin at low levels in disease muscle may offer significant therapeutic value to DMD patients. Thus, any proposed attempts to treat DMD by transferring normal myoblasts into dystrophic muscle may have to restore dystrophin levels to no more than 20% of normal in order to dramatically affect the phenotype of the muscle. The ability to quantitate dystrophin in patients before and during such clinical trials will be essential in monitoring the treatment. Preliminary investigations into such an approach that use the *mdx* mouse as a model system have demonstrated detectable and quantifiable levels of dystrophin at the site of myoblast injection (Partridge et al. 1989; Karpati et al. 1990).

It has been suggested that quantitation of dystrophin could be used to predict the severity of the disease. This suggestion was based on visual estimates of the amount of dystrophin found on western blots of a number of BMD and DMD patients (Hoffman et al. 1988, 1989; Arahata et al. 1989). It is difficult to compare the levels of dystrophin in previously reported BMD patients with those of the present study. It has been acknowledged (Hoffman et al. 1988) that no densitometric analysis was performed in these earlier studies; and in the absence of quantitative measures the previously estimated dystrophin levels may be less accurate than those in the present study.

The finding of 11 DMD patients with measurable amounts of truncated dystrophin would suggest that differentiating between DMD and BMD on the basis of immunological staining with only an N-terminal antiserum is inadequate. While further work may be required in order to determine the relative abundance of dystrophin in DMD and BMD patients, our data would suggest that a comparison of N-terminal and C-terminal staining may be of value in differentiating between these two phenotypes. Those patients showing altered dystrophin with both N-terminal and C-terminal antisera would be expected to have a BMD phenotype, while those having no C-terminal staining would be expected to have a DMD phenotype. Such a differential diagnosis will be highly desirable for infants diagnosed in newborn-screening programs (Jacobs et al. 1989), especially when treatments are proposed.

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