Large In-Frame Deletions of the Rod-Shaped Domain of the Dystrophin Gene Resulting in Severe Phenotype

Yoram Nevo MD[1], Francesco Muntoni MD FRCPCH[3], Caroline Sewry PhD[3], Cyril Legum MD[2], Miriam Kutai MD[1], Shaul Harel MD[1] and Victor Dubowitz MA PhD FRCP FRCPCH[3]

[1] Pediatric Neuromuscular Service, Institute for Child Development and Pediatric Neurology Unit, and [2]Genetic Institute, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel
Affiliated to Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel
[3] Department of Paediatrics and Dubowitz Neuromuscular Center, Hammersmith Hospital, London, UK
Affiliated to Imperial College School of Medicine, London, United Kingdom

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Abstract

Background: The prediction that Duchenne muscular dystrophy patients have out-of-frame deletions and Becker muscular dystrophy patients have in-frame deletions of the dystrophin gene holds well in the vast majority of cases. Large in-frame deletions involving the rod domain only have usually been associated with mild (BMD) phenotype.

Objectives: To describe unusual cases with large in-frame deletions of the rod-shaped domain of the dystrophin gene associated with severe (Duchenne) clinical phenotype

Methods: Screening for dystrophin gene deletion was performed on genomic DNA by using multiplex polymerase chain reaction. Needle muscle biopsies from the quadriceps were obtained using a Bergström needle. The biopsies were stained with histologic and histochemical techniques as well as monoclonal antibodies to dystrophin 1, 2 and 3.

Results: In three children with large in-frame deletions of the rod domain (exons 10–44, 13–40 and 3–41), early-onset weakness and a disease course suggested the DMD phenotype.

Conclusions: This observation emphasizes the uncertainty in predicting the Becker phenotype in a young patient based on laboratory evaluation, and that the clinical picture should always be considered.

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Duchenne and Becker muscular dystrophies are allelic conditions associated with mutations in the dystrophin gene located on the X chromosome at Xp21 [1]. Their clinical spectrum varies from the severe phenotype of DMD – where the early onset of the disease and the progressive loss of muscle function result in wheelchair dependency by the age of 13 and death in the late teens or early twenties – to mild BMD, where a wheelchair may never be needed and a normal life span is frequently attained [1,2].

About 65% of Xp21 muscular dystrophy patients have intragenic deletions or duplications causing gross rearrangements of the dystrophin gene [2,3]. Dystrophin is a 427 kd protein, localized to the sarcolemma [4,5] and consisting of four domains. The N-terminal domain has a putative actin-binding function [6]. The large, second rod-shaped domain is formed by 24 repeat sequences with a predicted helical conformation similar to repeats found in both alpha-actinin and spectrin (the rod-shaped domain). The third domain is cysteine-rich and bears some homology to alpha actinin, and the fourth is the carboxyl-terminal region [7,8].

The majority of deletions is clustered in two hot spots: one is the distal rod domain (exons 45–53), and the other is a cluster at the N-terminus [9]. However, the size and location of the deletions do not correlate with the severity of the clinical condition, since very small deletions can give rise to a DMD phenotype and relatively large deletions can result in a BMD phenotype [9,10].

To explain these phenotypic differences, Monaco et al. [11] proposed the "reading frame theory," according to which DMD is generally associated with mutations that disrupt the reading frame, leading to production of a truncated and presumably unstable protein. In contrast, the milder phenotype (BMD) is usually associated with mutations that do not affect the reading frame, leading to the presence of a more functional dystrophin [11]. The prediction that DMD patients have out-of-frame deletions and BMD patients have in-frame deletions is true for over 90% of patients [12]. There are, however, exceptions to this rule [3,10,13–15].

Two types of in-frame deletions have been associated with early onset of disease and severe (DMD) phenotype. In the first type, extremely large in-frame mutations exceeding 36 exons seem to be associated with a severe clinical phenotype [14,16,17]. In the other type there are large in-frame deletions which involve the 5' region. These typically start at exon 3 or 4 and extend to the mid-rod domain [7,14,17–19]. Patients with small deletions removing exons 3 and/or 5 were also reported to have a DMD or intermediate phenotype [18]. On the other hand, patients with large in-frame deletions within the rod domain have usually been associated with the mild phenotype, indicating that the absence of a large majority of this region does not fully abolish dystrophin function [8].

We report three children with large in-frame deletions of the rod domain. Two of these deletions are within the rod domain (exons 10–44 and 13–40) and the third extends from the 5' region to the rod domain (exons 3–41). These children presented with early onset of weakness during their first years of life and a disease course that suggests a DMD phenotype, despite evidence of high residual expression of a truncated dystrophin on muscle biopsy.

BMD = Becker muscular dystrophy
DMD = Duchenne muscular dystrophy

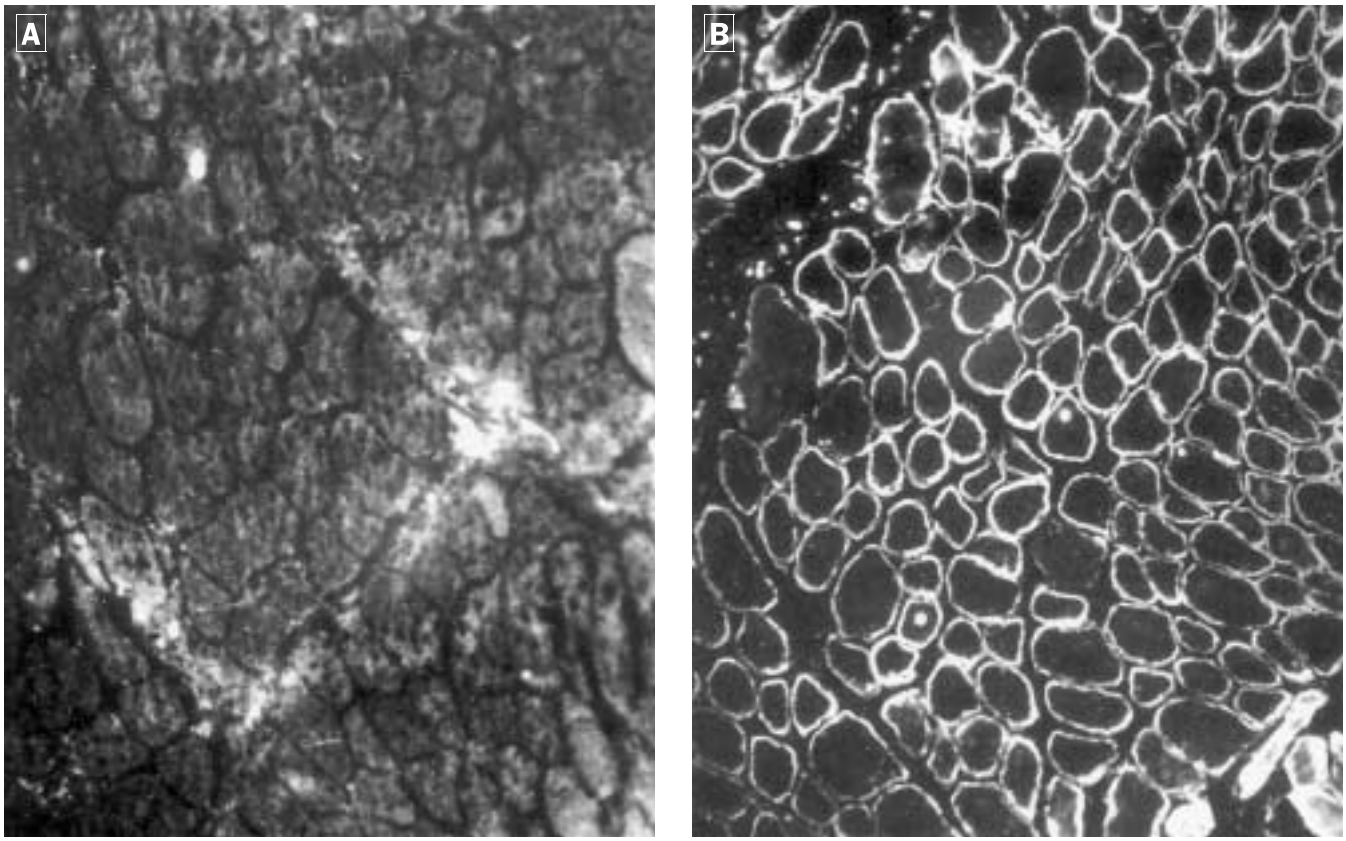

Figure 1. Immunohistochemistry in patient 1. Note the negative Dys 3 stain **[A]** and the positive Dys 2 stain **[B].**

Patient Descriptions

Patient 1

This child was admitted to the pediatric department of the Tel Aviv Sourasky Medical Center at the age of 5 months with a febrile illness and leukocytosis. During evaluation of his intercurrent disease, a serum creatine kinase level of 13,000 IU/ml was found.

He was born at term following an uneventful pregnancy and was delivered by cesarean section because of prolonged labor. His birth weight was 4,120 g. The perinatal period was normal. He smiled at 2 months, sat unsupported at the age of 5 months, though he had a degree of head lag at that time. A first muscle biopsy at the age of 7 months was interpreted as suggestive of a myositis. Dystrophin stains were inconclusive. He was treated with prednisone for 2 months with no clinical response.

He stood without support at 10 months and started walking unsupported at 13 months. At the age of 16 months his parents reported that he had difficulty in getting up from the floor without support. Examination at age 4 revealed prominence of the calves, deltoid and biceps muscles. He stood with exaggerated lordosis and walked with a waddling gait. He could get up from the floor with a Gowers' maneuver. At age 5 the child could not get up from the floor without assistance. At age 7 he could not walk independently and was wheelchair-bound.

A repeat quadriceps muscle biopsy at the age of 16 months showed abnormal dystrophin expression, as staining was absent with N-terminal and mid-rod antibodies (Dys1 and 3) but positive with C-terminal anti-dystrophin antibodies (Dys 2) [Figure 1]. DNA analysis revealed a large deletion of exons 10–44.

Patient 2

This young child presented at 10 months of age with infantile spasms. The pregnancy and delivery had been uneventful, but his mother felt that he had always been "floppy" and less active than her other child.

At age 10 months he was found to have repetitive flexion myoclonic spasms of short duration. A clinical diagnosis of West syndrome was supported by the finding of a hypsarrythmic electroencepahalogram. He was extensively investigated for metabolic disease, and magnetic resonance imaging of the brain was performed. All results were normal, with the exception of CK levels that were grossly elevated. Treatment with adrenocorticotropic hormone caused a cessation of the infantile spasms within 3 days and improved his motor and, to a lesser extent, cognitive function. His EEG normalized after a few months. By 15 months however, he was noted to have significant global developmental delay. He was unable to sit or express any word. He walked without support at the age of 3 years and began to use single words at age 3½ years. A repeat EEG at the age of 3 did not show any epileptiform activity,

CK = creatine kinase
EEG = electroencephalogram

but only a slowing of the background. At the age of 5.5 years he used only three words and showed autistic features. He had a waddling gait, could not run freely, and rose from the floor with a Gowers' sign.

A quadriceps muscle biopsy at 19 months showed abnormal dystrophin (absent staining with Dys 1 and Dys 3) but strong staining with C terminal anti-dystrophin antibodies (Dys 2). These results indicated a large in-frame deletion. DNA analysis revealed a deletion affecting exons 13–40.

Patient 3

This boy presented at the age of 11 months when he started to walk with an unsteady gait. He was the product of a normal full-term pregnancy. He ate well, and his early motor milestone had been normal. He acquired head control between the age of 2 and 3 months, sat at 5 months and started to walk at 11 months.

Interestingly, his parents had always noted his difficulty from sitting to standing. There were no other concerns regarding his health. The family noted frequent falls and difficulty in getting up from the floor. The parents were reassured and it was not until the child was 5 years old that they sought a second medical opinion, since the child could not run and had increasing difficulty walking long distances or ascending stairs. At that age the child walked with a waddle but could cover relatively long distances on a flat area (about 3/4 mile). He was unable to run, would fall frequently (two to three times a day) and got up from the floor with a Gowers' sign.

He attended a regular school where he kept up with his peers. A cognitive assessment showed a verbal I.Q. of 100 and a performance I.Q. of 90.

The family history was negative. A muscle biopsy at the age of 8 years showed a dystrophic picture, and the immunocytochemistry showed expression of an internally truncated protein (no staining with Dys 1 and 3, but residual expression of dystrophin using Dys 2 antibodies). The result was confirmed by the finding of an intragenic deletion of exon 3–41.

Methods

DNA analysis

Screening for dystrophin gene deletion was performed on genomic DNA by using multiplex PCR as described by Hodgson et al. [20]

Antibodies and immunohistochemistry

Needle muscle biopsies from the quadriceps were obtained under local anesthesia using a Bergström needle. The biopsies were mounted transversely and frozen in isopentane precooled in liquid nitrogen. Serial sections were stained with a variety of histologic and histochemical techniques [21]. Unfixed frozen sections (5 μm) were incubated with the three dystrophin antisera for 30 minutes. Bound antibodies were detected using a biotinylated secondary antibody and Texas-red streptavidin as described by Sewry et al. [22], or peroxidase.

Monoclonal antibodies were obtained from Novocastra Laboratories (Newcastle upon Tyne, UK). These included Dys 1 (central rod amino acids 1181–1388), Dys 2 (carboxy-terminal amino acids 3669–3685), and Dys 3 (proximal rod amino acids 321–494).

Discussion

We have described three children with onset of significant weakness during the first years of life and large in-frame deletions of the rod domain of dystrophin. In all of them the preservation of the reading frame was deduced by the strong staining in all muscle fibers with C-terminus anti-dystrophin antibodies. Indeed, the presence of the intact distal part of dystrophin supports the maintaining of the reading frame distal to the deletion.

Koenig et al. [12] found that the correlation between deletion and phenotype fit the reading-frame model in 92% of their 238 dystrophinopathy patients. Almost all the unusual cases with in-frame deletions and DMD phenotype belong to one of two subtypes: a) huge (>36 exons) in-frame mutations associated with a severe clinical phenotype [14,16,17]; and b) relatively large deletions in the 5' region extending into the mid-rod domain [18], such as deletions removing exons 4–18, 4–41 and 3–42 [14], 3–25 [13], 3–31 and 3–25 [7,19], 3–34 [23] , 4–41 and 3–42 [24], or shorter deletions of exons 3–13 [18] and 3–15 [3]. Exons 3 and 5 may be important to dystrophin function since this region contains at least one putative actin-binding site [2], and their deletion has been associated with a relatively severe phenotype, despite these being small exons whose removal does not affect the reading frame [18]. Patient 3 has a deletion of more than 36 exons that involves the actin-binding site (exons 3–41), and therefore his severe phenotype is predictable.

On the other hand, patients 1 and 2 are unusual in having large in-frame deletions within the rod domain (exons 10–44, 13–40) with early onset and severe weakness, which is inconsistent with a mild (BMD) phenotype usually ascribed to deletions in this domain [24]. Love and co-workers [8] reported such large in-frame DNA deletions (starting at exons 8,9,13 and 16 with endpoints at exons 32,33,36,44 or 48), corresponding to up to 46% of the coding region of dystrophin, associated with a mild clinical course. They concluded that up to 63% of the rod domain appears dispensable, resulting in a BMD phenotype, as long as the N-and C-terminus of dystrophin are maintained. Another six patients with large in-frame deletions in the rod domain have been described (exons 10–41, 13–40, 13–44, 13–48, and two patients with deletion in exons 13–41) [25]. They had only mild reductions of dystrophin-associated proteins. Five of them had Becker phenotype and one patient could not be classified [24]. A few other patients with in-frame mutations of the rod domain (involving exons 6–13, 10–13, 45–46 and 52–55) and severe Duchenne phenotype have been reported [9,12,13,19,23]. It is interesting that two of these patients had deletions of exon 13, which was deleted in our patients.

The function of the rod domain is still uncertain. Several alternative explanations may account for the presence of severe phenotypes with in-frame deletions of the rod-shaped domain, which do not involve the N-terminal actin-binding site and the C-terminal binding sites of dystrophin to the dystroglycan complex. Dysfunction of dystrophin may result from removing a large portion

PCR = polymerase chain reaction

of the molecule essential for its function [18], production of a protein too small to be functional [12], alternative splicing [12], and disruption of the organization of the dystrophin-associated proteins [24]. The presence of patients with similar in-frame deletions and protein levels, but with significantly different clinical progressions, suggests that epigenetic and environmental factors may play a significant role in determining the severity of a patient's disease [9].

Defining the range of mutations in genes causing human disease is important for determining the mechanisms of genotype-phenotype correlation and gene function for precise carrier detection and prenatal diagnosis [25]. Studying the rare exceptions of in-frame mutations within the rod domain that result in severe phenotype may become important for designing minigenes for gene therapy, as well as for highlighting the pitfalls in giving a prognosis [18]. In our patients the combination of genetic testing and immunohistochemistry suggested in-frame deletions of the rod domain, usually associated with mild phenotype. However, the clinical course was associated with onset of significant weakness and disability during the first years of life – more appropriate to the diagnosis of DMD. This observation once again emphasizes the fact that predicting the prognosis of Becker phenotype based on laboratory evaluation in a young patient requires caution, and that the clinical picture should always be considered.

References

1. Dubowitz V. Muscle Disorders in Childhood. 2nd edn. London: WB Saunders, 1995:325–69.
2. Nicholson LV, Johnson MA, Bushby KM, et al. Integrated study of 100 patients with Xp21 linked muscular dystrophy using clinical, genetic, immunochemical, and histopathological data. Part 1: Trends across clinical groups. *J Med Genet* 1993;30:728–36.
3. Den Dunnen JT, Grootscholten PM, Bakker E, et al. Topography of the Duchenne muscular dystrophy (DMD) gene: FIGE and cDNA an analysis of 194 cases reveal 115 deletions and 13 duplications. *Am J Hum Genet* 1989;45:835–47.
4. Zubrzycka-Gaarn EE, Bulman DE, Karpati G, et al. The Duchenne muscular dystrophy gene product is localised to the sarcolemma of human skeletal muscle. *Nature* 1988;333:466–9.
5. Bonilla E, Samitt CE, Miranda AF, et al. Duchenne muscular dystrophy: deficiency of dystrophin at the muscle cell surface. *Cell* 1988;54:447–52.
6. Hemmings L, Kuhlman PA, Critchley DR. Analysis of the actin-binding domain of alfa-actinin by mutagenesis and demonstration that dystrophin contains a functionally homologous domain. *J Cell Biol* 1992;116:1369–80.
7. Vainzof M, Takata RI, Passos-Bueno MR, Pavanello RCM, Zatz M. Is the maintenance of the C-terminal domain of dystrophin enough to ensure a milder Becker muscular dystrophy phenotype? *Hum Molec Genet* 1997;2:39–42.
8. Love DR, Flint TJ, Middleton-Price HR, Davies KE. Becker muscular dystrophy patient with a large intragenic dystrophin deletion: implications for functional minigenes and gene therapy. *J Med Genet* 1991;28:860–4.
9. Beggs AH, Hoffman EP, Snyder JR, et al. Exploring the molecular basis for variability among patients with Becker muscular dystrophy: dystrophin gene and protein studies. *Am J Hum Genet* 1991;49:54–67.
10. Malhotra SB, Hart KA, Klamut HJ, et al. Frame-shift deletions in patients with Duchenne and Becker muscular dystrophy *Science* 1988;242:755–8.
11. Monaco AP, Bertelson CJ, Liechti-Gallati S, Moser H, Kunkel LM. An explanation for the phenotypic differences between patients bearing deletions of the DMD locus. *Genomics* 1988;2:90–5.
12. Koenig M, Beggs AH, Moyer M, et al. The molecular basis for Duchenne versus Becker muscular dystrophy: correlation of severity with type of deletion. *Am J Hum Genet* 1989;45:498–506.
13. Nicholson LVB, Bushby KMD, Johnson MA, Gardner-Medwin D, Ginjaar IB. Dystrophin expression in "Duchenne" patients with "in-frame" gene deletions. *Neuropediatrics* 1993;24:93–7.
14. Winnard AV, Klein CJ, Coovert DD, et al. Characterization of translational frame exception patients in Duchenne/Becker muscular dystrophy. *Hum Molec G* 1993;2:737–44.
15. Gillard EF, Chamberlain JS, Murphy EG, et al. Molecular and phenotypic analysis of patients with deletions within the deletion-rich region of the Duchenne muscular dystrophy (DMD) gene. *Am J Hum Genet* 1989;45: 507–20.
16. Fanin M, Freda MP, Vitiello L, Danieli GA, Pegoraro E, Angelini C. Duchenne phenotype with in-frame deletion removing major portion of dystrophin rod: threshold effect for deletion size? *Muscle Nerve* 1996;19:1154–60.
17. Takeshima Y, Nishio H, Narita N, et al. Amino-terminal deletion of 53% of dystrophin results in an intermediate Duchenne-Becker muscular dystrophy phenotype. *Neurology* 1994;44:1648–51.
18. Muntoni F, Gobbi P, Sewry C, et al. Deletions in the 5' region of dystrophin and resulting phenotypes. *J Med Genet* 1994;31:843–7.
19. Nicholson LV, Johnson MA, Bushby KM, et al. Integrated study of 100 patients with Xp21 linked muscular dystrophy using clinical, genetic, immunochemical, and histopathological data. Part 2: Correlation within individual patients. *J Med Genet* 1993;30:737–44.
20. Hodgson SV, Abbs S, Clark S, et al. Correlation of clinical and deletion data in Duchenne and Becker muscular dystrophy, with special reference to mental ability. *Neuromusc Disord* 1992;2:269–76.
21. Dubowitz V. Muscle Biopsy. A Practical Approach. London: Bailliere Tindall, 1985.
22. Sewry CA, Dubowitz V, Abraha A, Luzio JP, Campbell AK. Immuncytochemical localization of complement components C8 and C9 in human diseases muscle: the role of complement in muscle fibre damage. *J Neurol Sci* 1987;81:141–53.
23. Chelly J, Gilgenkrantz H, Lambert M, et al. Effect of dystrophin gene deletions on mRNA levels and processing in Duchenne and Becker muscular dystrophies. *Cell* 1990;63:1239–48.
24. Matsumura K, Burghes AH, Mora M, et al. Immunohistochemical analysis of dystrophin-associated proteins in Becker/Duchenne muscular dystrophy with huge in-frame deletions in the NH2 terminal and rod domains of dystrophin. *J Clin Invest* 1994;93:99–105.
25. Roberts RG, Bobrow M, Bentley DR. Point mutations in the dystrophin gene. *Proc Natl Acad Sci USA* 1992;89:2331–5.

Correspondence: Dr. Y. Nevo, Institute for Child Development, Tel Aviv Sourasky Medical Center, 14 Balfour Street, Tel Aviv 65211, Israel.
Phone: (972-3) 697-3975
Fax: (972-3) 697-3990
email: nevo@tasmc.health.gov.il

Talent is cheaper than table salt. What separates the talented individual from the successful one is a lot of hard work.

Stephen King (1947-), American author, whose prolific output of horror fiction has make his name synonymous with the genre.