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Becker muscular dystrophy with marked divergence between clinical and molecular genetic findings: case series

G. P. Ramelli[a], *F. Joncourt*[b], *J. Luetschg*[c], *J. Weis*[d], *M. Tolnay*[e], *J. M. Burgunder*[f]

[a] Department of Paediatrics, Ospedale San Giovanni, Bellinzona, Switzerland
[b] Human Genetics, Children's University Hospital, Berne, Switzerland
[c] Division of Neuropaediatrics, University Children's Hospital Basel, Switzerland
[d] Division of Neuropathology, Institute of Pathology, University of Berne, Switzerland
[e] Department of Pathology, University of Basel, Switzerland
[f] Department of Neurology, University of Berne, Switzerland

Summary

Both Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are caused by mutations of the X-linked dystrophin gene. BMD patients are less affected clinically than DMD patients. We present five patients with a diagnosis of BMD. First, two identical twins, with a deletion of exon 48 of the dystrophin gene, who experienced prominent muscle cramps from the age of three. The histopathological examination of muscle biopsies of these two twins revealed only very slight muscle fiber alterations. Second, two brothers who displayed marked, unusual intrafamilial variability of the clinical picture as well as showing a new point mutation in the dystrophin gene. And finally, a fifth boy who displayed a new point mutation in the dystrophin gene. Although he was clinically asymptomatic at the age of 15 and muscle biopsy only showed very minor myopathic signs, serum Creatine Kinase (CK) levels had been considerably elevated for years. Taken together, these cases add to the spectrum of marked discrepancies in clinical, histopathological and molecular genetic findings in BMD.

Key words: Becker muscular dystrophy; phenotype-genotype correlation; intrafamilial variations

Introduction

X-linked dystrophinopathy, resulting from mutations in the dystrophin gene, is the most common cause of inherited myopathy in males and shows varying degrees of severity, ranging from asymptomatic CK elevation to the mild Becker muscular dystrophy (BMD) and to the severe Duchenne muscular dystrophy (DMD) phenotype. DMD is a rapidly progressive disease and an affected boy may lose the ability to walk independently before the age of 12. The course of BMD is more benign and the disease has a slower rate of progression. In BMD, the mean age of the onset of symptoms, such as muscle weakness and poor walking, is reported to be around 12 years of age. Time of ambulation loss also varies from adolescence onward to adulthood [1]. Unlike DMD, for which the clinical phenotype and morphological results are relatively homogeneous, BMD shows a more heterogeneous profile, with little correlation between clinical pictures and laboratory findings.

We describe the clinical features, intrafamilial variation and association with the histopathological features in 5 patients with BMD.

Abbreviations

BMD	Becker Muscular Dystrophy
DMD	Duchenne Muscular Dystrophy
CK	Creatine Kinase
EMG	Electromyography
MRC	Medical Research Council

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Case reports

Patients 1 and 2 were identical twins. They developed normally until the age of three, when they started to complain about cramps and myalgia during routine activity. The family history was negative for neuromuscular diseases. Serum CK levels of the parents and a sister were normal. The neurological examination at the age of three was normal. Serum CK levels were consistently elevated to values of around 2500 IU. Electromyography (EMG) showed myopathic features. Electrocardiography and echocardiography were normal. Results of glucose and glucagon tolerance tests and an ischaemic exercise test were normal. Muscle biopsies from the quadriceps muscle were taken from both boys and disclosed a normal picture, except for a minor variability in sarcolemmal labelling with the antiserum against the dystrophin rod domain. There was no evidence of a glycogen storage disorder. Cramps and myalgia occurred more often in the following years without episodes of myoglobinuria. At the follow-up 6 years after the first observation, slight difficulties in running fast were noted. This, and the persistently high CK values and the slight abnormalities in dystrophin immunostaining, led us to examine the dystrophin gene for deletion. The dystrophin gene was screened for deletions by multiplex polymerase chain reaction. Twenty-six exons were screened simultaneously (exons 1, 3, 4, 6, 8, 12, 13, 16, 17, 19, 32, 34, 41–53, 60, muscle- and brain-promotor); a method, with which more than 98% of all known deletions in the dystrophin gene are detected. Deletion of exon 48 was diagnosed. A western blot was performed and a very small size difference in dystrophin bands between the index cases and a control case was noted.

Patient 3 was 10 years old and his younger brother *(Patient 4)* was 8 years old at the time of the evaluation. The family history was negative for neuromuscular diseases. The mother had normal pregnancies and births with both boys. The older brother showed a normal psychomotor development. He could sit at the age of 7 months and could walk independently at the age of 13 months. There were no signs of weakness. He rarely complained of myalgia. He could perform gymnastics at school like the other children, but ran slowly. The neurological examination showed mild calf hypertrophy. In contrast, the younger brother showed retarded motor milestones and was not able to sit before the age of 12 months and could walk at 18 months. He had episodes of frequent falling and was a slow runner. At clinical examination he showed a mild proximal muscle wasting, with MRC grade 4 power in his biceps, triceps and deltoid muscles, as well as calf hypertrophy. He could not walk on his heels and had positive Gower's signs. Both boys had myopathic changes in the EMG and elevated serum CK levels (Patient 3 with 8480 IU and patient 4 with 12 070 IU). A quadriceps muscle biopsy of patient 3 revealed prominent dystrophic features (figure 1), whereas signs of dystrophy in the quadriceps muscle of his younger brother were only minimal. Immunohistochemical analysis demonstrated a reduced staining with antibodies against dystrophin (Dys 1–3, Novocastra, UK). Molecular genetic analysis of the dystrophin gene revealed a novel splice-site mutation c357+2T>A in intron 5 (figure 2).

Patient 5 had a normal antenatal and birth history. His psychomotor development was normal and independent walking was achieved at 13 months. At the age of 6, he was

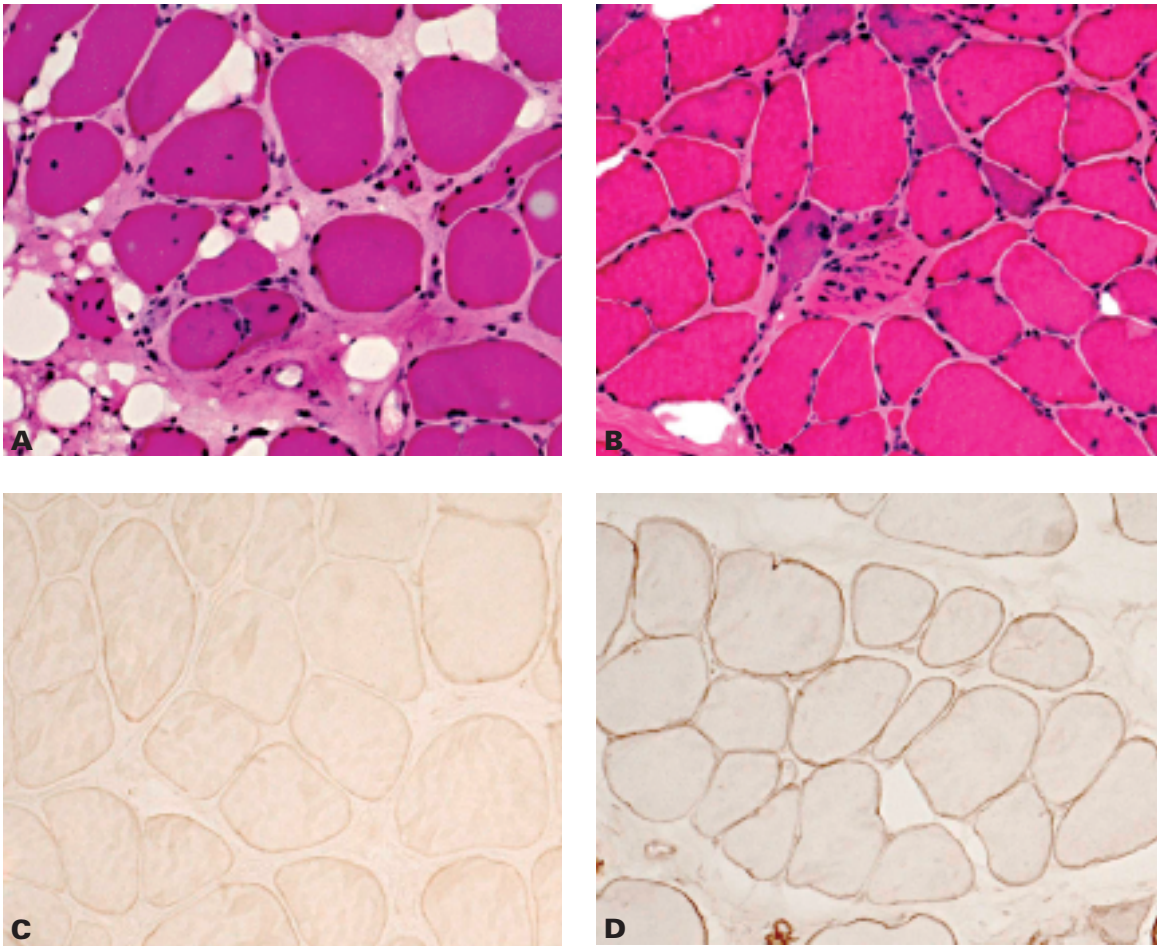


Figure 1

Becker dystrophy (patient 3). HE stain revealed marked fibre size variation, internalised nuclei and interstitial fibro-fatty replacement of muscle (A). In some areas muscle fibres were necrotic and regenerating (B). Immunohistochemistry with an antibody against the rod domain of dystrophin (antibody NCL-Dys1, Novocastra, UK, undiluted) revealed almost total absence or irregular subsarcolemmal staining of muscle fibres (C). Strong, upregulated staining for utrophin (antibody against N-terminus of utrophin, Novocastra, UK, dilution 1:10) around the periphery of most fibres (D).
A–D: original magnification ×200.

Figure 2

Molecular genetic analysis of the dystrophin gene, patient 4. DMD Exon/Intron 5, Base-change c357+2T>A leading to a novel splice site mutation in intron 5.

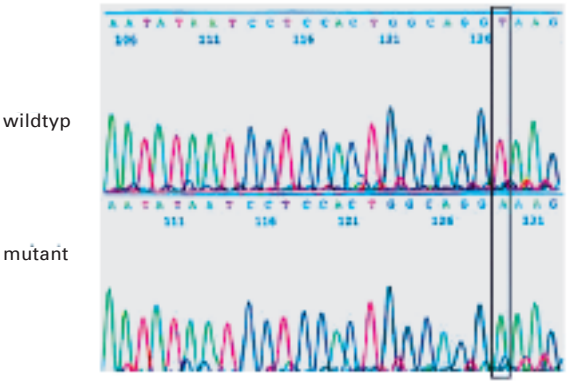
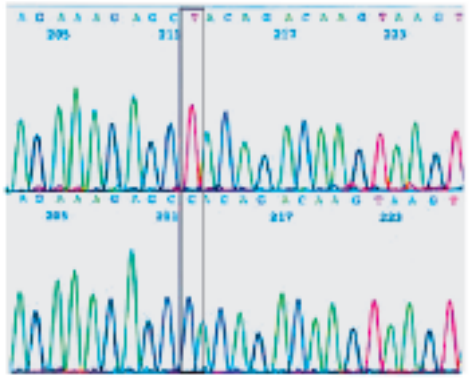


Figure 3

Molecular genetic analysis of the dystrophin gene, patient 5. DMD Exon 21, base change c2795T>C leading to a novel missense mutation p. L932P.



seen by the general practitioner because he had fever and joint pains. Serum analysis showed consistently elevated CK levels of around 2200 IU. His biopsy specimen revealed mild myopathic features including variation in fibre size and a slightly increased number of centralised muscle fibre nuclei. Immunohistochemical analysis demonstrated no reduction in staining with antibodies against dystrophin (Dys 1–3, Novocastra, UK), as well as against alpha-, beta-, gamma- and delta-sarcoglycan (Novocastra, UK). His subsequent development was normal. At the age of 10, he was able to participate in all sports activities without any difficulty. Molecular genetic analysis of the dystrophin gene revealed a novel missense mutation in exon 21, resulting in an exchange of leucin at position 932 by prolin (figure 3).

Discussion

We report on clinical variability and laboratory findings in five children with BMD. Two were twins, two were brothers and one was a sporadic case. Elevated serum CK levels ranging from 2000 to 12 000 IU/L were found in all patients. In patient 5 the high CK was found during a routine screening. Clinical phenotype, muscular dystrophin expression, and results of the molecular genetic investigations of these cases were compared.

The twins showed unusual clinical symptoms with exercise-induced cramps after the age of 3. Although they were symptomatic, the histology was normal except for a variability in the sarcolemmal staining with the antibody against the dystrophin rod domain. A very small size difference between the dystrophin bands of the patients and of a control case was noted, and an exon 48 deletion confirmed the diagnosis. Two previous studies reported three cases of patients with only elevated serum CK without signs of weakness, and the same deletion of exon 48 and similar histological results [2, 3]. The isolated deletion of exon 48 seems to be correlated with a mild course of the disease. Interestingly Beggs et al. found a high incidence of severe cramps and myalgia among patients with deletions and duplications in the proximal rod domain suggesting that this region is functionally different from the distal portion of the rod [2]. Patients 3 and 4 differed greatly in their clinical manifestations, although they were brothers. The younger brother, with classical features of BMD and positive Gower's signs, showed very mild histopathological alterations, whereas his brother was clinically asymptomatic, but showed marked dystrophic changes in his muscle biopsy. Thus, the two brothers showed a different clinical course and histopathological alterations despite having the same dystrophin gene mutation. The different clinical courses may be due to differences in the way the mutation affects splicing. These differences may be quantitative and/or qualitative in nature. The mutation c357+2T>A destroys the donor splice site. Its effect at the level of the gene product, however, is difficult to predict, since splicing is a complicated process involving a variety of components. It is conceivable that the mutation results in exon 5 skipping, a change that would leave the reading frame intact. It is also possible that a different gene product, longer or shorter than the wildtype dystrophin is formed. The use of a splice site different from normal may also render splicing less efficient, additionally leading to a reduction in the quantity of protein formed. Furthermore intrinsic muscle factors or environmental phenomena may play a role [4]. Only the analysis on mRNA-level will yield the answer. These studies are ongoing. Patient 5 was asymptomatic and histopathological examinations revealed only minor muscle fiber alterations.

Unlike patients with DMD, for whom the clinical phenotype and the morphological findings are relatively homogeneous, BMD patients display a more heterogeneous profile. Clinical phenotype

Table 1

Clinical features in 5 consecutive patients with BMD.

Patients No	1	2	3	4	5
Age at diagnosis	9 years	9 years	10 years	8 years	8 years
Walking independently	15 months	15 months	13 months	18 months	13 months
Presenting symptoms	Cramps	Cramps	Running slowly	Poor climbing and running	No symptoms
Clinical status	No weakness	No weakness	Minimal proximal limb girdle weakness	Proximal limb girdle hypostenia	normal
Gower's signs	Negative	Negative	Negative	Positive	Negative
Calf hypertrophy	Present	Present	Present	Present	Present
CK	2387 IU	2655 IU	8480 IU	12070 IU	2203 IU
Biopsy	Minor abnormalities	Minor abnormalities	Prominent dystrophic features	Moderate dystrophic features	Mild myopatic
Immuno histo-chemistry (Dystrophin)	Slight abnormalities	Slight abnormalities	reduced	reduced	No reduction
Gene mutation	Deletion exon 48	Deletion exon 48	Splice-site mutation c357+2T>A in intron 5	Splice-site mutation c357+2T>A in intron 5	Missense mutation in Exon 21

CK: Creatine Kinase (normal value <170 IU)

as well as the morphological changes may differ greatly. At the clinical level, the pattern of muscle involvement mimics that of DMD in the majority of patients, albeit with later age of onset and slow progression [5–7]. As in our patients 1, 2 and 5, atypical manifestations of the disease are increasingly reported, such as exercise-induced cramps and myalgia, dilated cardiomyopathy, asymptomatic elevation of CK, and myoglobinuria. Exercise induced myalgia found in BMD patients is considered secondary to the increased mechanical fragility of the muscle fibres. Occasional myalgia and cramps can occur even during normal activity [8, 9].

Histopathological findings ranging from no change to severe dystrophic changes can be noted in BMD. Most interestingly, we saw patients with a severe clinical involvement but without major alteration of the muscular structure. On the other hand in asymptomatic patients morphological examination of the muscle biopsy specimens may reveal severe dystrophic changes. This might be due to a selective involvement of certain muscles. Whereas both twins showed a similar clinical picture and the same morphological results, the other two brothers showed a marked intrafamilial variability. This suggests that epigenetic and environmental factors play a significant role in determining the severity of a BMD patient's disease [3, 10]. Patient 5 was a special case since his elevated creatinine kinase level was discovered during routine examination. The patient showed no signs of weakness until now. He participated in intensive sports activities although his CK levels were already considerably elevated at the age of eight and histopathological analysis revealed mild myopathic changes already at that time.

On the molecular level, BMD is caused by deletions, point-mutations or duplications in the dystrophin gene. Deletions account for the majority of cases [11]. They may vary greatly in their extent and location but, generally, it is observed that BMD is caused by deletions that do not disrupt the reading frame, thus leading to a shortened gene product. Such a protein may still be partially functional as long as it retains the essential domains. Patients with mutations in domain I of dystrophin, the actin-binding domain, tend to be quite severely affected and are often classified as severe BMD. Deletion of domain I is expected to reduce protein stability by disrupting interaction with components of the cytoskeleton. This is compatible with the generally lower protein level and, hence, the rather severe progression seen in these patients. Domain II is the rod domain of the protein, which was shown not to be essential for protein function. A previous study presented two patients with domain II deletions, who were asymptomatic [9]. Others in the presence of very large deletions in this domain have a mild clinical progression, still being able to walk in their sixties [12]. The clinical course, as well as the histopathological and immunochemical findings observed in our twins diagnosed with deletion of exon 48, fits well with the above-mentioned reading-frame concept: the deletion was within the rod domain and did not disrupt the reading frame. Dystrophin expression was nearly normal in quantity. The mutation L932P identified in patient 5 affects a single amino acid in the rod domain. The assumption that it is a true but mild missense mutation rather than a polymorphism, is based on the fact that no other sequence change was identified in this patient so far. Moreover, to our knowledge L932P has never been described in any healthy individual. The absence of symptoms in this patient also fits well with the general concept. Other studies showed that domain III and the proximal half of domain IV, the cystein-rich and carboxy-terminal domain respectively, were functionally essential.Patients with frame shifting deletions, that result in the loss of these domains, invariably had no detectable dystrophin and suffered from DMD. Early studies already suggested that these domains were essential

to dystrophin stability [13]. The loss of just the most terminal portion of domain IV was associated with mild, non-progressive BMD, indicating that this region was not essential to dystrophin function.

Although a majority of cases fit well with the above-mentioned reading-frame concept, a growing number of exceptions exist. In contrast to our findings, other patients with a deletion of exon 48 have been diagnosed as sufferring from DMD. Several studies suggested that reliable genotype-phenotype predictions are hard to make due to the large variation among patients with identical deletions. This is further illustrated by the great variability found in the 5 patients described in the present paper.

Correspondence:
G.P. Ramelli
Department of Paediatrics
Ospedale San Giovanni
CH-6500 Bellinzona
Switzerland
E-Mail: gianpaolo.ramelli@eoc.ch

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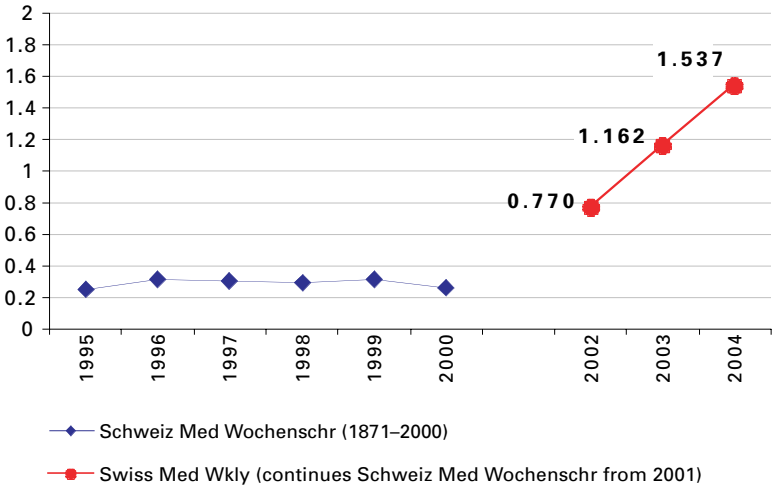

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