

## SHORT REPORT

## Spanish family with myalgia and cramps syndrome

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A Spanish family is reported with dystrophinopathy of myalgia and cramps syndrome type. There were five affected males and three females, and also six asymptomatic carriers. Muscle biopsy showed a dystrophic pattern, but immunohistochemistry carried out with three anti-dystrophin antibodies was normal. Dystrophin analysis by western blot revealed a dystrophin of reduced quantity and molecular weight. DNA analysis showed a deletion of the dystrophin gene involving exons 45–52. The natural history of this disorder and the large intrafamilial clinical variability are discussed.

Exercise intolerance associated with myalgia, muscle cramps, or myoglobinuria may be associated with a dystrophinopathy. Gospe *et al* in 1989<sup>1</sup> were the first to describe a family with an X linked recessive disorder of childhood onset characterised by muscle cramps, myalgias, and exercise intolerance. On examination patients had pseudohypertrophic calves but no muscle weakness. Analyses revealed raised serum creatine kinase (CK) levels and myoglobinuria. Muscle biopsy showed myopathic changes and the immunohistochemical examination and immunoblot revealed an abnormal dystrophin. Several dystrophin gene deletions have been described as the origin of this syndrome.

Since Gospe's description of the disorder in an American family, sporadic cases have been reported.<sup>1–6</sup> Figarella-Branger *et al*<sup>2</sup> found five males with exercise intolerance and abnormal immunohistochemistry. DNA analysis revealed a deletion in the dystrophin gene in two of these patients, affecting the proximal part of the rod domain in one and the distal part of this domain (exons 45–52) in the other. Ishigaki *et al*<sup>3</sup> reported a four year old Japanese boy with myalgia and cramps which developed at the age of 28 months; there were persistently raised CK values but normal physical examination and muscle biopsy. DNA analysis revealed a deletion of exons 13–18 in the dystrophin gene. Kleinstueber *et al*<sup>4</sup> reported an eight year old boy with this syndrome and deletions of exons 45–51 of the dystrophin gene. Doriguzzi *et al*<sup>5</sup> described a nine year old boy with exertional myalgia; he had episodes of myoglobinuria and was found to have a deletion of exons 45–48 of the dystrophin gene.

In this paper we report a Spanish family with myalgia and cramps showing deletion of exons 45–52 of the dystrophin gene. The natural history of the disorder can be studied in this large family. The intrafamilial clinical variability is remarkable.

## FAMILY STUDY

The Spanish family reported (fig 1) contains no consanguineous relationships. The proband (II,4) complained of muscular pain and cramps in the extensor muscles of the lower limbs provoked by both normal activity and physical exercise from the age of four years. The patient could not run and became easily tired when walking long distances or

going upstairs. At the age of 45, the symptoms extended to the muscles of the forearm and hand. He also developed myoglobinuria with fever and after physical exercise. He had never experienced chest pain. Neurological examination revealed only hypertrophic calves. Analysis showed persistently high serum CK levels (2000 to 4000 U/l at rest in different evaluations). At age 50, the patient remains the same and no muscle weakness has developed.

The proband's mother, aged 76 (I,2), had suffered from leg cramps during sleep since she was a child. She had hypertrophic calves and her serum CK was 384 U/l (normal <185).

Two brothers, aged 47 (II,5) and 40 (II,7) respectively, complained of similar symptoms and were affected to a variable degree. They first experienced myalgias and cramps in the first decade of life. Neither of them had ever had cardiac symptoms. Patient II,5 had no limitation on physical activity. Patient II,7 had reported some difficulty in going up stairs or standing up from a chair in the past year. Examination revealed hypertrophic calves and mild bilateral paresis in both quadriceps (4/5 strength) with amyotrophy of the distal part. Serum CK was persistently high, between 953 and 1315 U/l. Four years after the first evaluation, muscular strength remains the same.

One sister of 52 (II,3) had complained of leg cramps after physical exercise since she was a child. She also had hypertrophic calves and a serum CK of 327 U/l. Her youngest child of 17 (III,8) had suffered from cramps and myalgias since he was four years old. His symptoms improved in adolescence and he even participated in sport. Examination revealed hypertrophy in the quadriceps muscles and the calves. He had myoglobinuria and CK levels of around 4000 U/l on several determinations. Two years later he is a very good football player and shows diffuse muscular hypertrophy.

One grandson (IV,1) of the proband's sister (II,1), aged seven, had had cramps and muscular pain in all four limbs and myoglobinuria since he was three years old.

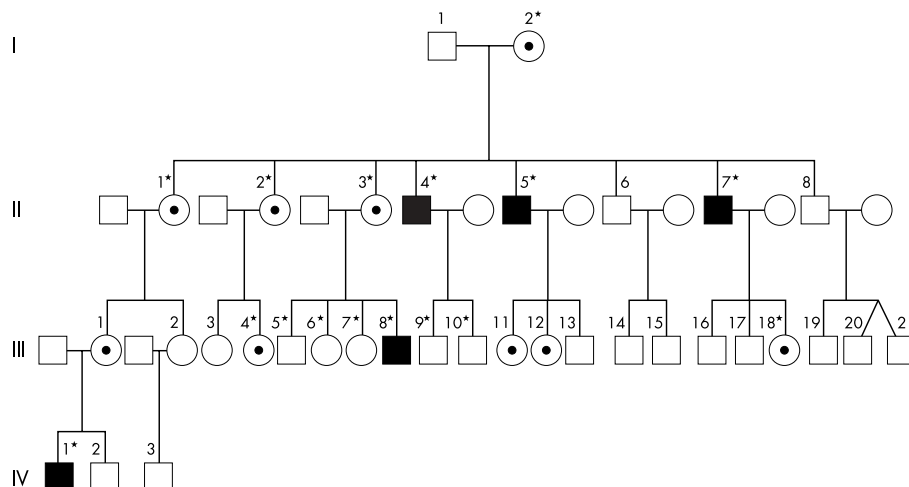
Other members of the family, who were asymptomatic, also participated in the genetic study (table 1).

## Complementary tests

All affected males had raised serum CK at rest on repeated evaluations. Three females (I,2; II,1, and II,3), one of them asymptomatic, had a slightly raised serum CK. The proband had normal results in the ischaemic exercise lactate and ammonium test. Electromyography and cardiac investigations (ECG and echocardiography) were also normal.

Biceps muscle biopsy was carried out in patients II,3, II,4, and II,7. Haematoxylin and eosin staining showed similar results: a myopathic pattern with variation in fibre size, basophilia, necrosis, ring fibres and clefts, internal nuclei, and fibrosis. Biochemical tests (lipid, glycogen, and mitochondrial metabolism) were all negative. The immunohistochemical

**Abbreviations:** BMD, Becker muscular dystrophy



**Figure 1** Pedigree of the kindred. Conventional symbols are used. Family members who were examined are marked with an asterisk.

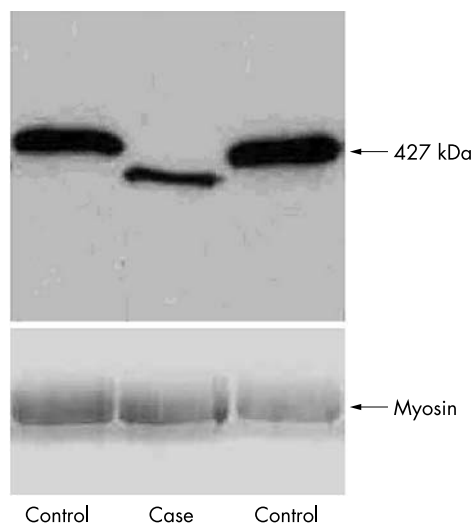
procedure was carried out with three antibodies (Novocastra®) against different protein domains—anti-dys1, rod domain; anti-dys2, C terminus; and anti-dys3, N terminus—with normal results. Spectrin immunohistochemical labelling, immunoreactivity for  $\alpha$ -,  $\beta$ -,  $\delta$ -, and  $\gamma$ -sarcoglycans, and a laminin test were also carried out, with normal results.

A dystrophin western blot was undertaken on SDS-PAGE, using goat polyclonal anti-rod (30 kDa) or mouse monoclonal anti-C-terminus dystrophin antibodies (Novocastra), as described elsewhere.<sup>7</sup> This was done on the muscle biopsy from patient II.4. A non-dystrophic muscle was used as a control. The post-blot gel was stained with Coomassie blue to estimate the amount of muscle proteins present in each sample to obtain the relative quantity of dystrophin. We found a band of dystrophin corresponding to the carboxyl domain, of lower molecular weight and lesser intensity (fig 2)

### Genetic analyses

Molecular analysis was carried out using multiplex polymerase chain reaction (PCR). Genomic DNA was extracted from peripheral blood following the protocol described by Dracopoli *et al.*<sup>8</sup> We analysed 23 exons and two gene promoters located in the main deletion hot spot of the gene in three different PCRs. Deletions of exons 45–52 were identified in affected male patients.

A study of female carriers was carried out by indirect molecular analysis with microsatellite markers. The entire



**Figure 2** Western blot analysis of dystrophin in affected and normal samples: the band of dystrophin corresponding to the carboxyl domain is of lower molecular weight and lesser intensity. Myosin bands are similar in both cases.

nuclear family was included in the study and the segregation analysis served to identify female carriers.

**Table 1** Clinical and genetic features in affected family members

Patient	Age (years)	Sex	Clinical features	Serum CK (IU)	Biopsy	Dystrophin gene deletion
I,2	76	F	Cramps, calf hypertrophy	384	–	del 45–52
II,1	55	F	Calf hypertrophy	314	–	del 45–52
II,2	54	F	None	120	–	del 45–52
II,3	52	F	Cramps, calf hypertrophy	327	Yes	del 45–52
II,4	49	M	Myalgia, cramps, calf hypertrophy, myoglobinuria	2560	Yes	del 45–52
II,5	45	M	Myalgia, cramps, calf hypertrophy	1250	–	del 45–52
II,7	40	M	Myalgia, cramps, weakness, calf hypertrophy, myoglobinuria	953	Yes	del 45–52
III,1	23	F	None	–	–	del 45–52
III,4	18	F	None	–	–	del 45–52
III,8	17	M	Myalgia, cramps, calf hypertrophy, myoglobinuria	4000	–	del 45–52
III,11	20	F	–	–	–	del 45–52
III,12	21	F	–	–	–	del 45–52
III,18	15	F	None	100	–	del 45–52
IV,1	7	M	Myalgia, cramps, calf hypertrophy, myoglobinuria	3212	–	del 45–52

CK, creatine kinase; F, female; M, male.

### Summary of features

The patients examined (fig 1, table 1) can be divided into two groups: group 1 with five affected males, and group 2 with three affected females and six asymptomatic carriers. Group 1 had clinical symptoms including myalgia and cramps after effort (100%), calf hypertrophy (100%), episodes of myoglobinuria (60%), difficulty running or climbing stairs (40%), and quadriceps weakness (20%). None had cardiac involvement. Raised CK levels (>1000 U/l) were present in all patients. The age of onset ranged from three to 10 years.

In the group 2, nocturnal and exertional cramps were present in two of the patients (22%), calf hypertrophy in three (33%), and slightly high CK levels in three (33%). Neither myalgia nor myoglobinuria was present. None of these individuals experienced cardiac disease or weakness. The age of onset of the symptoms was in the first decade of life.

Histological, biochemical, and genetic data have already been described and are included in table 1.

### DISCUSSION

The study of this family with a muscle disease—characterised by myalgias, cramps, lack of muscular weakness, calf hypertrophy, hyperCKaemia, dystrophic pattern on muscle biopsy, and deletion of the dystrophin gene—is compatible with dystrophinopathy. Similar clinical symptoms characterise several metabolic myopathies such as phosphofructokinase deficiency, myophosphorylase deficiency, and exercise intolerance caused by mitochondrial myopathy. There is also a myalgia and cramps syndrome with a neurogenic cause and an autosomal dominant transmission that develops in infancy and improves after adolescence.<sup>9</sup> Thus dystrophin analysis should be included in the evaluation of patients with childhood onset of exercise intolerance or recurrent myoglobinuria.<sup>10</sup>

Hypertrophied calves, predominant male involvement, and dystrophic features on muscle biopsy in patients with exercise intolerance may suggest a dystrophinopathy. However, calf hypertrophy is not always present in this disorder.<sup>3</sup> Moreover, muscle biopsy may be normal.<sup>3 5 6</sup> For instance Ishigaki *et al* reported a four year old Japanese boy with myalgia and cramps and high serum CK values in whom physical examination, muscle biopsy, and dystrophin immunostaining were all normal. DNA analysis revealed a deletion of exons 13–18 in the dystrophin gene.<sup>3</sup>

In our family all the symptomatic patients had calf hypertrophy but females as well as males seemed to be affected. Three females (I,2, II,1, II,3) presented with cramps after exercise, nocturnal cramps, calf hypertrophy, and raised serum CK. The second female was clinically asymptomatic but had calf hypertrophy and raised serum CK. The third female underwent biceps muscle biopsy which had a dystrophic pattern similar to that found in her brothers. Molecular work up showed that the three women were carriers of a deletion mutation of exons 45–52. These women were considered to be symptomatic carriers, although they had only a mild clinical phenotype. There were six more women in the family who were asymptomatic carriers (II,2; III,1; III,4; III,11; III,12; and III,18).

The previously reported cases of dystrophinopathy–myalgia and cramps syndrome are characterised by lack of muscular weakness. Such reports have included young individuals, children, and adolescents.<sup>1–6 10</sup> The oldest individual included in these studies was 33. We do not know the natural evolution of the disease in these patients. Becker muscular dystrophy (BMD) with adult onset, mild phenotype, and dystrophin deletion exons of the rod domain has been reported.<sup>11–14</sup> Deletions around exons 45–53 are the most common and generally cause typical BMD, while deletions in the middle of the rod domain cause a very mild phenotype

and deletions of the proximal portion are associated with myalgia and cramps. These data suggest that the rod domain is not completely homogeneous with respect to function, as deletions in three different regions of this domain cause generally different phenotypes.<sup>15</sup> However, phenotypic variability among patients with similar mutations is observed and this may reflect the contribution of epigenetic or environmental factors.<sup>15</sup> In our family, patient II,7 began complaining of muscular weakness in his quadriceps at 40 years of age, and this symptom has been stable for four years. Other older members of the family did not present with weakness, and two of them (II,5 and III,8) were able to carry out significant physical activity. The propositus (II,4) was 50 years old at examination and he had no muscle weakness, although he started to have exercise intolerance when he was a child. This intrafamilial clinical variability suggests that other factors that are still unknown may play a role in determining the severity of the disease.

The morphological findings on biopsy revealed a dystrophic pattern. However, the immunohistochemistry was normal in spite of staining with three anti-dystrophin antibodies (anti-dys1, rod domain; anti-dys2, C terminus; and anti-dys3, N terminus), which was done to avoid diagnostic errors. These data coincide with other reported cases of dystrophinopathy–myalgia and cramps syndrome.<sup>1 3 4 16</sup> Most of these cases had abnormal immunoblotting, like patient II,4 who had dystrophin of reduced quantity and molecular weight. It is known that a significant decrease in dystrophin abundance is present in severe clinical phenotypes of BMD, while mild phenotypes are characterised by near normal amounts of dystrophin on blots.<sup>14 15 17</sup> Angelini *et al* observed a normal pattern of dystrophin immunohistochemical reaction in 11% of 125 patients with BMD. This percentage corresponds to subclinical, benign, and moderate phenotypes.<sup>14</sup> Probably the dystrophin deletion in this family may produce a mildly abnormal dystrophin which explains a normal immunohistochemical reaction. In cases with myopathic muscle biopsy and normal dystrophin immunostaining, immunoblotting can be the key to identifying the abnormal protein.

The first described genetic defects associated with this type of dystrophinopathy were deletions in the proximal third of the rod domain of the dystrophin gene (exons 10–22, exons 13–17).<sup>1 3</sup> However, other deletions have been described, occurring in the distal third of the rod domain: exons 45–52,<sup>2</sup> exons 45–51,<sup>4</sup> and exons 45–48.<sup>5</sup> These mutations are in-frame deletions, which probably affect a portion of the dystrophin molecule that does not significantly alter its function.<sup>14 15</sup> This could explain the benign nature of the phenotype and also the normal dystrophin immunostaining. Such deletions have already been described in mild phenotypes of BMD.<sup>10–16</sup> However, these patients who manifest myalgia, exertional cramps, and myoglobinuria develop late onset muscular weakness (up to the age of 20 or later), and many of them have cardiomyopathy.<sup>14</sup>

BMD and myalgia-cramps syndrome seem to represent different phenotypic expressions of the same disease. This confirms the extremely variable presentation and progression of dystrophinopathies. A better understanding of the molecular basis of this syndrome may provide useful strategies to minimise or eliminate the symptoms of dystrophinopathies.

### Conclusions

The clinical, histopathological, biochemical, and genetic data of the family we describe correspond to a myalgia and cramps syndrome. The natural history of the disease in this family suggests that there is large intrafamilial clinical variability, with some mildly affected patients and others with more definite symptoms, even to the extent of muscle weakness.

Further cases need to be reported so that the natural history of this supposedly benign dystrophinopathy can be clarified.

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