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Merosin-deficient congenital muscular dystrophy with mental retardation and cerebellar cysts unlinked to the LAMA2, FCMD and MEB loci

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Abstract

We report a case of congenital muscular dystrophy with secondary merosin deficiency, structural involvement of the central nervous system and mental retardation in an 8-year-old girl from a consanguineous family. She had early-onset hypotonia, generalized muscle wasting, with weakness especially of the neck muscles, joint contractures, mental retardation and high creatine kinase. Muscle biopsy showed dystrophic changes with partial deficiency of the laminin α_2 chain. Cranial magnetic resonance imaging revealed multiple small cysts in the cerebellum, without cerebral cortical dysplasia or white matter changes. The laminin α_2 chain (6q2), Fukuyama type congenital muscular dystrophy (9q31–q33) and muscle–eye–brain disease (1p32–p34) loci were all excluded by linkage analysis. We suggest that this case represents a new entity in the nosology of congenital muscular dystrophy.

Keywords: Congenital muscular dystrophy; Secondary merosin deficiency; Cerebellar cyst; Mental retardation

1. Introduction

Congenital muscular dystrophies (CMD) are a heterogeneous group of autosomal recessive neuromuscular disorders characterized by early-onset muscle weakness, hypotonia, joint contractures and a dystrophic pattern at muscle biopsy. Mental retardation and structural abnormalities of the central nervous system occur in some forms, particularly the Fukuyama type CMD (FCMD), muscle–eye–brain disease (MEB) and Walker–Warburg syndrome (WWS). Severe visual failure with structural involvement of the eyes is characteristic for the two latter syndromes. In the two other well-known groups, merosin-positive and merosin-deficient CMD, no major structural brain abnormalities are present although white matter hyperlucency in cranial magnetic resonance imaging (MRI) is a consistent finding in merosin-deficient CMD cases [1–3].

Primary merosin deficiency is more frequently complete than partial and is associated with a normal intellectual development. It is caused by mutations in the gene encoding the laminin α_2 chain, LAMA2, located on chromosome 6q2 [1,4–6].

Secondary partial merosin deficiency has been reported in some CMD forms, usually associated with mental retardation. Associated structural brain abnormalities were first reported in FCMD, which is due to mutations in the gene coding for fukutin on 9q31 [7–9], and in MEB, recently localized to 1p32–p34 [10,11]. Four cases with severe mental retardation, microcephaly, brain abnormalities and calf hypertrophy, one of them previously reported by De Stefano et al. [12], have been recently fully characterized as a novel entity by Villanova et al. [13]. This last entity shares several features (mental retardation, secondary merosin deficiency and calf hypertrophy) with the two siblings that we had previously reported, but these cases had no brain abnormalities [14].

A novel and unique CMD case that we had previously presented in a workshop [15] is reported here: an 8 year-old girl with a severe phenotype, mental retardation, cerebellar cysts and partial merosin deficiency but unlinked to any

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known loci for merosin deficiency or CMD with central nervous system involvement.

2. Case report

The patient was the first child of first degree consanguineous parents. At initial admission she was 1.5 years old. She was noticed to have decreased movements and activity at the age of 3 months, sat with support at 7 months, but was still unable to stand at 1.5 years. On physical examination she was hypotonic, had facial weakness, decreased deep tendon reflexes and could only sit without support. Her head circumference was between the 25th and 50th centile. EMG showed myopathic abnormalities and the creatine kinase was 4515 units/l. Muscle biopsy at the age of 1.5 years revealed prominent endomysial and perimysial fibrosis, adiposis and variation in fibre size, with few necrotic and regenerative fibers, features compatible with congenital muscular dystrophy.

During follow-up she presented a progressive evolution, developing prominent weakness of the neck muscles, bilateral contractures of the knees, ankles and elbows. She never walked or stood alone. At the age of 8 years she was severely hypotonic with extreme weakness of the head and neck muscles. She also presented muscle wasting without any hypertrophy, rigidity of the spine, scoliosis and increased lordosis and could barely sit without support (Fig. 1). She was mentally retarded, with an IQ of 59 by Stanford–Binet test at the age of 6 years. There were no signs of ocular manifestations, and formal ophthalmologic examination was normal.

Cranial MRI, at the age of 6 years, revealed multiple small cysts in the cerebellar cortical and subcortical areas, but was otherwise unremarkable (Fig. 2). There were no signs of cerebral cortical dysplasia or changes in white matter signal intensity. Brain stem was normal.

A second muscle biopsy was performed at the age of 6 years and an overt increase in fibrosis and adiposis was observed. The variation in fiber size was still prominent, while necrotic and regenerative changes were completely absent.

3. Methods

3.1. Morphological analysis

Open muscle biopsy was performed from the quadriceps muscle after written consent. The tissue was immediately frozen in isopentane cooled in liquid nitrogen. Six-micrometer cryosections were used for conventional histological stains and immunohistochemical studies. Antibodies against spectrin, laminin α_2 chain (80 kDa), laminin α_5 chain, dystrophins (dys1, dys2, dys3), α-sarcoglycan and β-dystroglycan were used according to previously described procedures [16].

3.2. Genetic analysis

DNA was extracted from blood lymphocytes by standard techniques after obtaining informed consent. The following microsatellite markers were studied to determine potential linkage to known CMD loci: D6S407, D6S1705 and D6S1620 for LAMA2; D9S306, D9S2105, D9S2171 and D9S2107 for FCMD; D1S211, D1S2677, D1S197 and D1S200 for MEB [5,9,11,17].

In all cases the forward primer was labeled at its 5′end by a 6-FAM, NED (or TET), or HEX fluorochrome. Polymerase chain reactions (PCRs) were performed under the following conditions: 40 ng genomic DNA, 1 × buffer supplied by the manufacturer (Perkin–Elmer), 0.15 mM of each dNTPs, 5 pmol of each primer, and 0.5 units AmpliTaq Gold polymerase (Perkin–Elmer) in a final volume of 15 μl. The amplification conditions were 10 min denaturation at 94°C; then 30 cycles with 30 s at 94°C, 30 s at 55°C, 1 min at 72°C; and finally an extension step at 72°C for 3 min. Amplified PCR products were separated by electrophoresis on a 4% acrylamide/bisacrylamide 19:1, and 6 M urea gel, using a 377 DNA sequencer apparatus (Applied Biosystems). Results were analyzed by GENSCAN (version 3.1) and GENOTYPER (version 2.1) software.

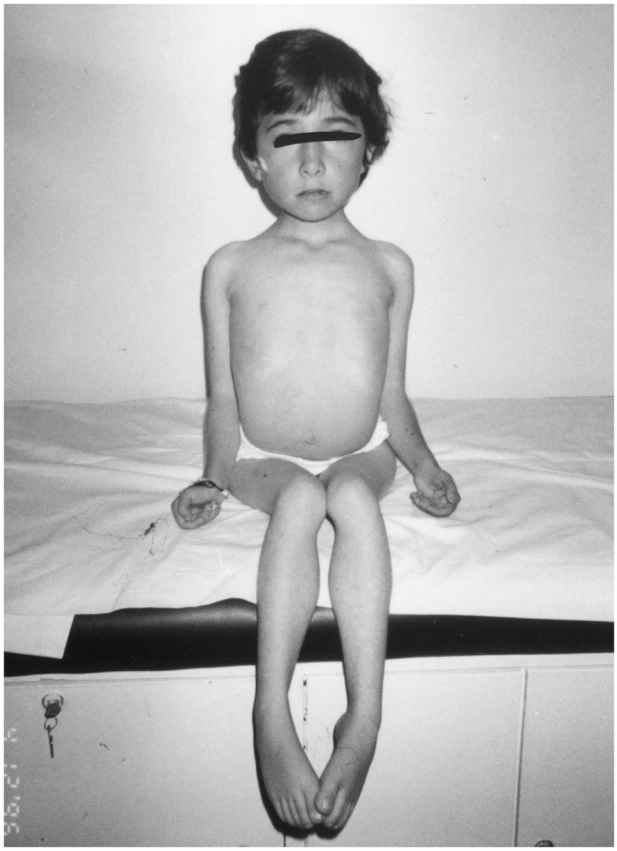
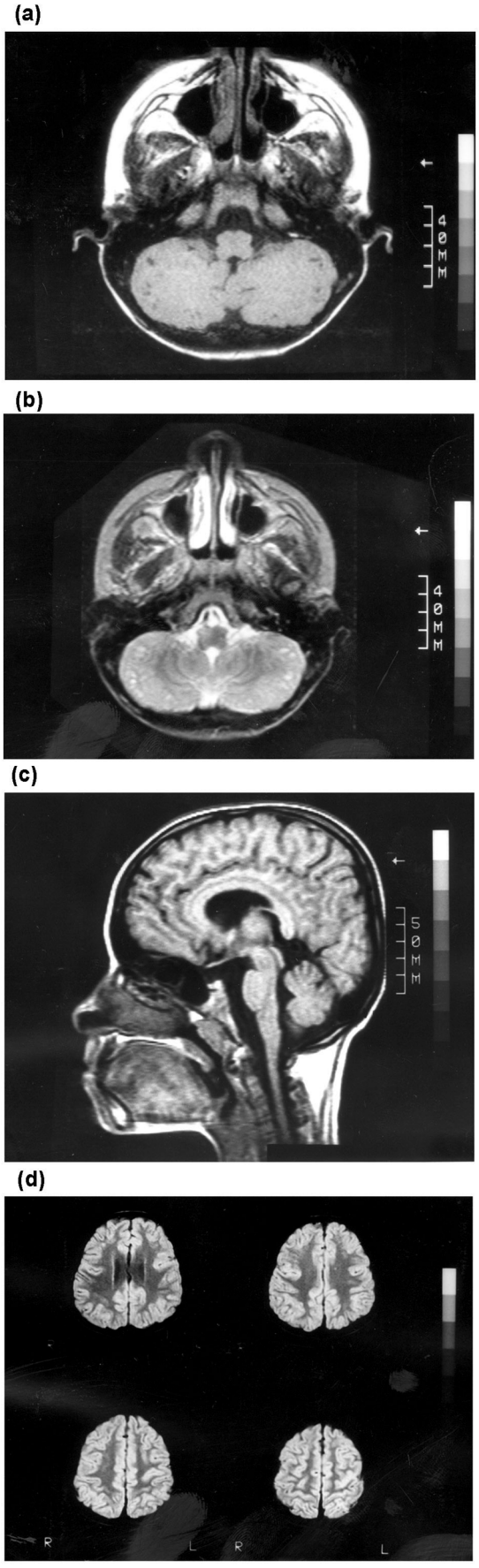


Fig. 1. The patient at 6 years of age, showing severe muscle wasting, facial weakness and increased lumbar lordosis.



4. Results

4.1. Immunohistochemistry

Immunohistochemical study of the second biopsy showed moderate to severe reduction of the laminin α_2 (Fig. 3) and upregulation of the laminin α_5 chains. Spectrin, dystrophin-1, -2, -3, α-sarcoglycan and β-dystroglycan were normal.

4.2. Genetic analysis

Several polymorphic markers spanning the LAMA2 (6q2), FCMD (9q31–q33) and MEB (1p32–p34) loci were used to assess linkage in this consanguineous family to these genes, using the homozygosity mapping principle [18]. As shown on Fig. 4, the genetic analysis of the family members ruled out the three loci as the ones responsible for the disease as far as possible in this small family with a single index patient. In other words, linkage analysis showed a high probability that the disease was not related to any of the three loci since the affected child was heterozygous for all the informative markers from the three critical genomic regions. Furthermore, she shared identical haplotypes with her unaffected brother in FCMD and MEB loci.

5. Discussion

Our CMD case with secondary merosin deficiency, cerebellar cysts and mental retardation cannot be classified among the previously known entities. In several CMD patients with merosin deficiency, brain abnormalities have been repeatedly reported. In primary merosin deficient CMD patients, white matter hyperlucency is a typical finding but, in general, it is not associated with structural brain abnormalities or mental retardation. Nevertheless, several cases showing polymicrogyria [19–21] and focal cortical dysplasia [22] have been described, and all of these cases were associated with complete merosin deficiency. Also, cerebellar involvement in the form of hypoplasia of cerebellar vermis and/or hemispheres has been reported in primary merosin deficiency as well as pontine hypoplasia and occipital agyria by Philpot et al. [23].

Among the CMD forms with secondary merosin deficiency, the best known are FCMD and MEB with characteristic brain malformations [2,24]. Mild cases of FCMD have been described with moderate mental retardation, ability to walk, with or without muscle pseudohypertrophy, but they still present gyral abnormalities [25]. In cases of MEB, confirmed by linkage analysis, myopia is a constant feature in addition to brain abnormalities [24].

Fig. 2. T_1-weighted (a) and T_2-weighted (b) axial MRI slices from the level of cerebellum demonstrates subcentimeter cysts which are hypointense on T_1 and hyperintense on T_2-weighted images in the cortical and subcortical regions. Mid-sagittal T_1 (c) and periventricular axial T_2 (d) images reveal no pathological findings.

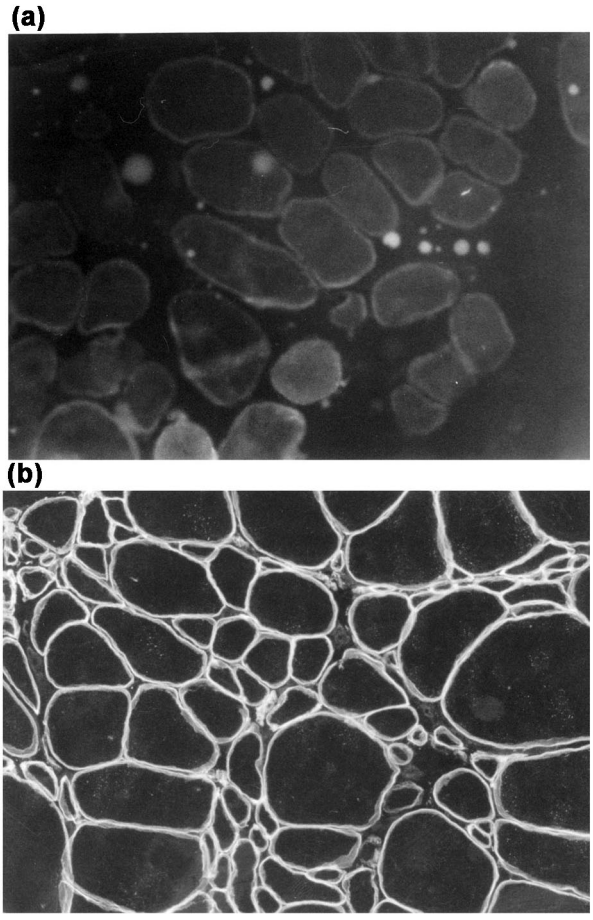


Fig. 3. Muscle biopsy of the patient (a) showing reduced fluorescence for laminin α_2 chain, compared with a normal control (b).

Apart from these two major diseases also defined as distinct genetic entities, additional CMD forms with secondary merosin deficiency have been recognized. Trevisan et al. reported two siblings with different clinical and radiological findings, one of whom showed normal mental development, white matter changes improving by time, mild ventricular dilatation and partial merosin deficiency [26]. Two siblings that we previously reported had severe mental retardation, normal cranial MRI, and calf hypertrophy [14]. Mercuri et al. presented four children from two families, without brain involvement and mental retardation [27]. Muntoni et al. described four siblings also without brain involvement, but with diaphragmatic involvement, and early respiratory failure [28], and they recently mapped the gene of this form [29]. Finally, four other cases who are reported in this issue by Villanova et al. had severe mental retardation, microcephaly, calf hypertrophy, and structural brain changes limited to the subtentorial region [13]. However, none of the cases mentioned above had cerebellar cysts.

Our index patient had severe hypotonia and contractures associated with a merosin deficiency, which was suggestive of a merosin-deficient CMD with an additional feature, the cerebellar cysts. However, white matter was normal on the cranial MRI at the age of 6 years and LAMA2 was excluded since the patient was found to be heterozygous at several markers spanning the LAMA2 locus, contrary to the expectation of homozygosity, as a consequence of a consanguineous mating. Regarding the mental retardation, cerebellar cysts, and secondary merosin deficiency, she could have been a case of FCMD or MEB, but she had no gyral abnormalities and we were also able to exclude these loci by haplotype analysis.

In conclusion, this is a unique case with a severe CMD phenotype associated with secondary merosin deficiency, mental retardation, normal white matter intensity, and cerebellar cysts. No linkage to the known CMD loci with merosin

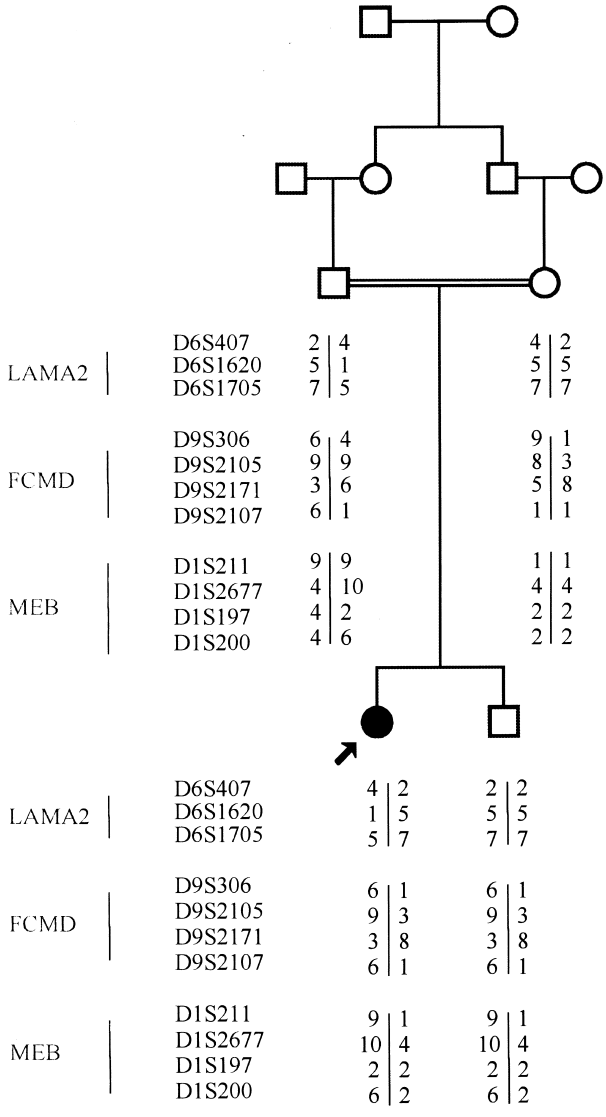


Fig. 4. Haplotypes for 11 polymorphic markers from three genomic regions containing the LAMA2, FCMD and MEB genes. The affected individual, who is the daughter of a first-cousins marriage, is denoted by a blackened symbol. The consanguineous mating is indicated by a double line. The three loci have been excluded as the cause of the disorder by lack of homozygosity of the surrounding markers in the patient.

deficiency or structural central nervous system involvement has been found, which suggests that this case probably constitutes a novel, genuine entity.

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