Merosin Expression in Muscle of Western Cases with Fukuyama-Like Congenital Muscular Dystrophy

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Abstract

The Fukuyama type of Congenital Muscular Dystrophy (CMD) is characterized by the association of a progressive muscular deficit with a severe brain involvement. In muscle of the Japanese patients with this form of CMD, the amount of the laminin-α2 chain (merosin) is severely reduced. Besides the usual cases with the Classical form, CMD patients with severe CNS involvement and no ocular alterations may also be found in Western countries; however, these often differ from the subjects with typical Fukuyama CMD for some clinical or neuroimaging aspects and are, thus, considered cases of Fukuyama-like CMD. Laminin subunits were studied by immunocytochemistry in muscle of two such cases. They were two girls, respectively 13 and 14-year-old, who suffered from a congenital and non-progressive muscular disorder of dystrophic type, associated with epilepsy, mental retardation and clear evidence of pachygyria, ventricular dilatation and white matter alterations on neuroimaging. Differently from the cases with Fukuyama CMD, neither of the two patients showed a partial reduction of merosin in the basement membrane of muscle fibers: actually, in the former muscle merosin was undetectable, while in the latter its immunolabelling was normal. These biochemical data indicate that Western cases with Fukuyama-like CMD could represent an heterogeneous group of CMD: ongoing linkage genetic analysis will clarify their possibility of being phenotypical variants of the already identified forms of CMD.

Key words: Congenital Muscular Dystrophy, Fukuyama-like type, merosin.

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In the Fukuyama type of Congenital Muscular Dystrophy (CMD), a progressive muscular deficit with neonatal onset is characteristically associated with major brain abnormalities [2, 5, 6, 11]. They are mainly represented by gyral malformations, ventricular dilatation and white matter alterations [5, 11]. Severe CNS involvement is characteristic not only of the Japanese form of CMD but also of the Muscle-Eye-Brain Disease and of the Walker-Warburg syndrome, the two variants of CMD in which the muscle disorder is furthermore associated with severe ocular abnormalities [2, 6, 12]. In Western countries, the most frequently diagnosed form of CMD is the Classical one [10], in which minor and asymptomatic neuroradiological signs of CNS involvement are often detected [15, 18]. On the other hand, rare Western cases with CMD, major brain alterations and no overt eye abnormalities have also been reported [2, 3, 6]. Often they resemble very closely the Japanese patients with Fukuyama CMD, but differ from these for some clinical or neuropathological aspects. These Western patients have been considered cases of Fukuyamα-like CMD [3, 10, 17, 18].

Recently, immunochemical investigations on laminin subunits of the muscle basement membrane of patients with CMD have pointed out a reduction of laminin-α-2 chain (merosin) to about 25% of control value in Fukuyama CMD cases [7] and a complete deficiency of the same protein in a subgroup of cases with Classical CMD [4, 14]. We here report the heterogeneous findings about the merosin expression in the muscle fibers of two of four cases with Fukuyamα-like CMD studied by us. These two patients, one merosin-deficient and the other merosin-non-deficient, presented a very similar CNS involvement on both clinical and neuroradiological grounds.

Cases Reports and Methods

Case 1

This 14-year-old girl of Caucasian ancestry was the third child of non consanguineous parents. Her older sister suffered from a severe neonatal hypotonia and died at two months of age because of acute respiratory failure. The patient was born by normal delivery, at the end of an uneventful pregnancy. Her neonatal weight was 3200 g. At birth, she was greatly hypotonic and presented joint contractures (foot and hip bilaterally). Her serum CK was 950 IU/l (normal range: 0-160), the EMG showed myopathic features and the biopsy of her right vastus lateralis muscle gave evidence of clear dystrophic alterations.

Her psychomotor development was delayed: she gained head control at seven months and sat without support at two years of age; since six years of age, she has been able to walk a few steps with bilateral aid, but never walked unassisted. When two-year-old, she uttered single words and was toilet trained by her third year. At the age of seven she started having absences, sometimes associated with clonic movements of the eyelids or myoclonic jerks of the upper limbs. Interictal EEG showed focal spike or spike-wave complex activity in the inferior right temporal region. Valproate therapy reduced seizures to three or four a week, but they never completely disappeared, in spite of the treatment with other anti-epileptic drugs, used by monotherapy or variously combined. At the age of nine, her neurological examination showed: severe and generalized muscular weakness, extended to the facial muscles, evident hypotonia and reduced muscle trophism at the four limbs, multiple joint contractures (elbow and ankle bilaterally and right knee), absent deep tendon reflexes. She showed a good head control and the ability to use hands and raise arms over her head, although with some difficulty. Psychological examination pointed out a mild mental retardation (Raven PM47 test: level 5). Serum CK was twice the upper normal value. EMG showed myopathic alterations. The brain CT scan showed ventricular dilatation and diffuse, severe white matter alterations, mainly in the anterior supratentorial regions. Brain MRI (Fig. 1) revealed tetraventricular dilatation, mainly in lateral ventricules, severe and widespread white matter alterations, more evident in the frontal lobes, pachygyria of the occipital cortex and some small areas of cerebellar polymicrogyria. Subsequently, her muscular deficit continued a static clinical course.

At the age of 14, her seizures became of the complex-partial type. She kept having two or three attacks per week, when treated with valproate and ethosuxymide in the plasma therapeutical range. In the interictal EEG there was evidence of plurifocal, brief paroxysmal discharges of spikes or polyspike-wave complexes. At the same age, repeated psychological evaluation gave evidence of a mild to moderate mental retardation, not quantified by intelligence tests because of the worsening of her previous affective and attention disorders. Her motor abilities were the same as those at 10: she was wheelchair bound (even though able to make few steps with bilateral assistance) and had good hand-ability and head control. The neurological examination, besides the severe and diffuse muscular weakness, with moderate facial involvement, detected clear-cut generalized muscle wasting and hypotonia, joint contractures at elbows, ankles and knees and absent deep tendon reflexes. Serum CK evaluation was 405 IU/l (normal range: 0-160). Ophtalmological examination was normal, whereas the visual evoked potentials and the electroretinography were not evaluated.

Case 2

The clinical and neuroimaging data of this patient have been extensively reported elsewhere [17] and are briefly summarized hereafter.

At birth, this 13-year-old girl of Caucasian ancestry presented evident weakness and hypotonia with high level of serum CK (23 times the normal value). EMG showed a myopathic pattern and in muscle biopsy there were dystrophic alterations. Her motor and mental development was delayed. In this patient, repeated neuromuscular and psychological examinations showed a moderate and non-progressive muscle involvement associated with a moderate to severe mental retardation.

As case 1, she suffered from epilepsy (frequent atonic seizures and complex absences). Repeated CT scans showed severe alterations of the central white matter, that improved in time, and wide ventricular dilatation, mainly of the occipital horns. MRI added evidence of bilateral pachygyria in the posterior cerebral regions, without clear signs of polymicrogyria of the cerebellar cortex.

Immunocytochemistry of laminin subunits

Laminin-α1 (A) and –α2 (merosin) chains were studied in cryostat muscle sections from biopsies of our two cases, by indirect immunofluorescence microscopy. The methods of this investigation were as those described by Tomè et al. in 1994 [14]. Other than by monoclonal antibodies anti-merosin (Chemicon) and anti-laminin –α1 chain (Gibco BRL), the muscle cryosections were also investigated by monoclonal antibodies anti-dystrophin C-terminal, anti-43 kDa and anti-50 kDa dystrophin-associated glycoproteins (Novocastra). The same immunocytochemical study was also carried out in muscle preparations of other two cases of Fukuyama-like CMD, here not reported, and in muscle cryosections of five non neuromuscular controls.

Results and Discussion

The immunocytochemical investigation of the muscle biopsy of case 1 showed a normal staining of the cryostat muscle sections by all the antibodies used, except for the antibodies anti-laminin -α1 (A) and -α2 (merosin) chains. The fiber surfaces of this case showed absence of merosin (Fig. 2), differently from control muscle where specific immunolabelling determined a uniform staining around the fibers. Moreover, while in control cryosections laminin

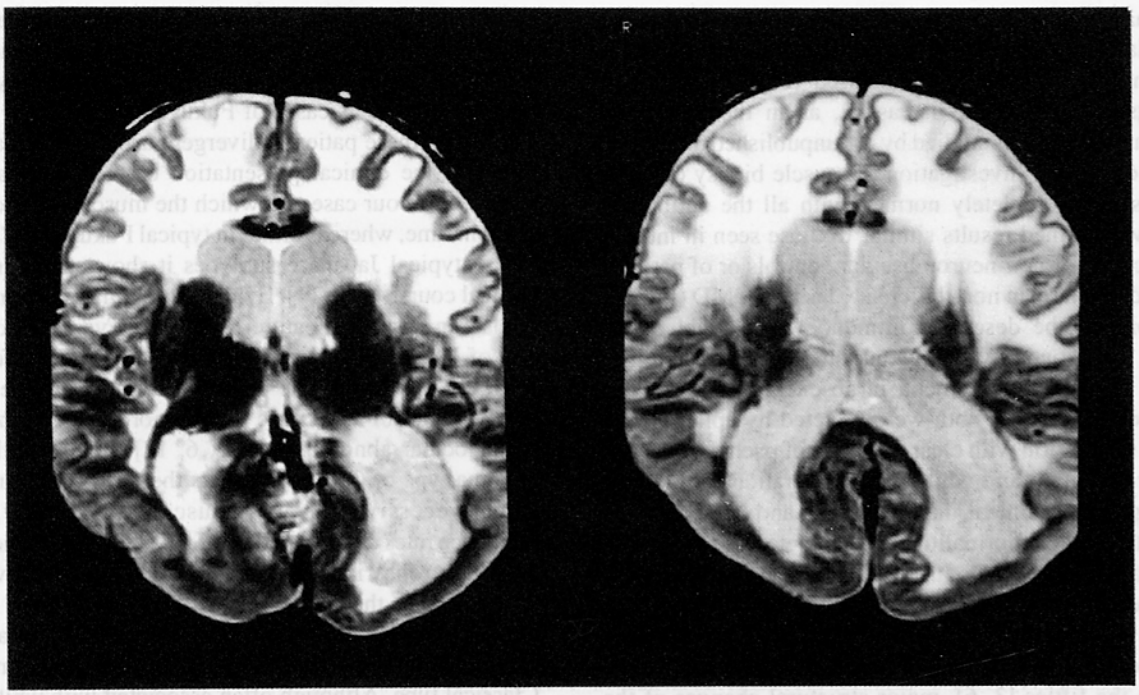

Figure 1. Brain MRI of case 1. In these two axial T2 images (TR 2000; TE 90) there is clear evidence of pachygyria in the occipital lobes, widely enlarged ventricles and severe white matter abnormalities, mainly in the anterior regions.

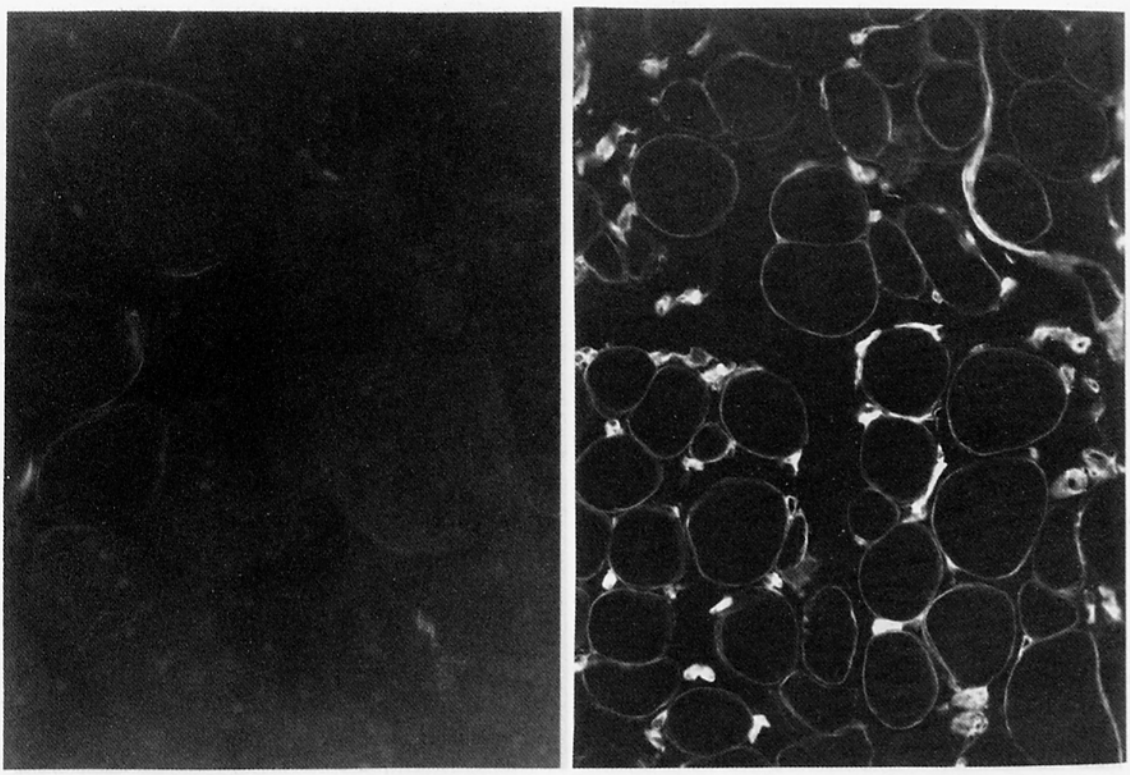

Figure 2. Immunolabelling of laminin subunits in muscle biopsy of case 1: the absence of merosin (left) and the overexpression of laminin-α1 chain (right) are shown.

–α1 chain was poorly detected by specific antibodies, in muscular fibers of our case 1 immunostaining of the same laminin subunit was over-expressed (Fig. 2). Opposite findings were obtained in case 2, as in the other two Fukuyama-like cases studied by us (unpublished data). The immunolabelling investigation of muscle biopsy of these patients was completely normal: with all the antibodies used, we obtained results similar to those seen in muscle cryosections of non-neuromuscular controls or of patients affected by merosin non-deficient Classical CMD (Fig. 3).

In spite of the described immunocytochemical divergence, these two CMD patients shared a quite similar pattern of CNS involvement, in absence of overt ocular alterations. Actually, both were affected by epilepsy and mental retardation with clear evidence of pachygyria, ventricular dilatation and white matter alterations on neuroimaging. These clinical manifestations and MRI signs of brain structural abnormalities are characteristic of the Fukuyama type of CMD. In the Japanese cases, however, other neuroimaging changes can be detected, as the cystic lesions of the cerebellar cortex associated with diffuse polymicrogyria, recently studied by Aida et al. [1].

Since the fifties [3, 6], major structural changes of the brain, similar to those described in our two cases, have also been found in other Western patients. Although presenting a CNS involvement with characteristics of the type reported for the Fukuyama form of the disease, these CMD cases were different in some clinical or pathological aspects from the Japanese patients [5, 6, 11] and they were, thus, referred to as cases of Fukuyama-like CMD [3, 10, 17, 18]. In these patients, divergent aspects could also be found in the clinical presentation of the muscular dystrophy, as in our cases, in which the muscular deficit was static in time, whereas either in typical Fukuyama CMD or in its atypical Japanese subtypes it shows a progressive clinical course [1, 5, 11]. Evidence of brain malformations is also an essential feature of Muscle-Eye-Brain disease and of Walker-Warburg syndrome: however, differently from the Fukuyama-like expression of the disease, these two variants of CMD are furthermore characterized by major ocular abnormalities [2, 6, 12]. In Japan, the Fukuyama type of CMD represents the most common autosomal recessive form of muscular dystrophy, with incidence rates close to those observed for Duchenne Muscular Dystrophy in the same population [5]. As shown in a recent survey that we conducted on a sample of Italian population [10], in Western countries, CMD appears less frequently than in Japan and its most common form is the Classical type. Although often associated with white matter alterations on neuroimaging [15, 18], this CMD form usually does not present clinical manifestations of CNS

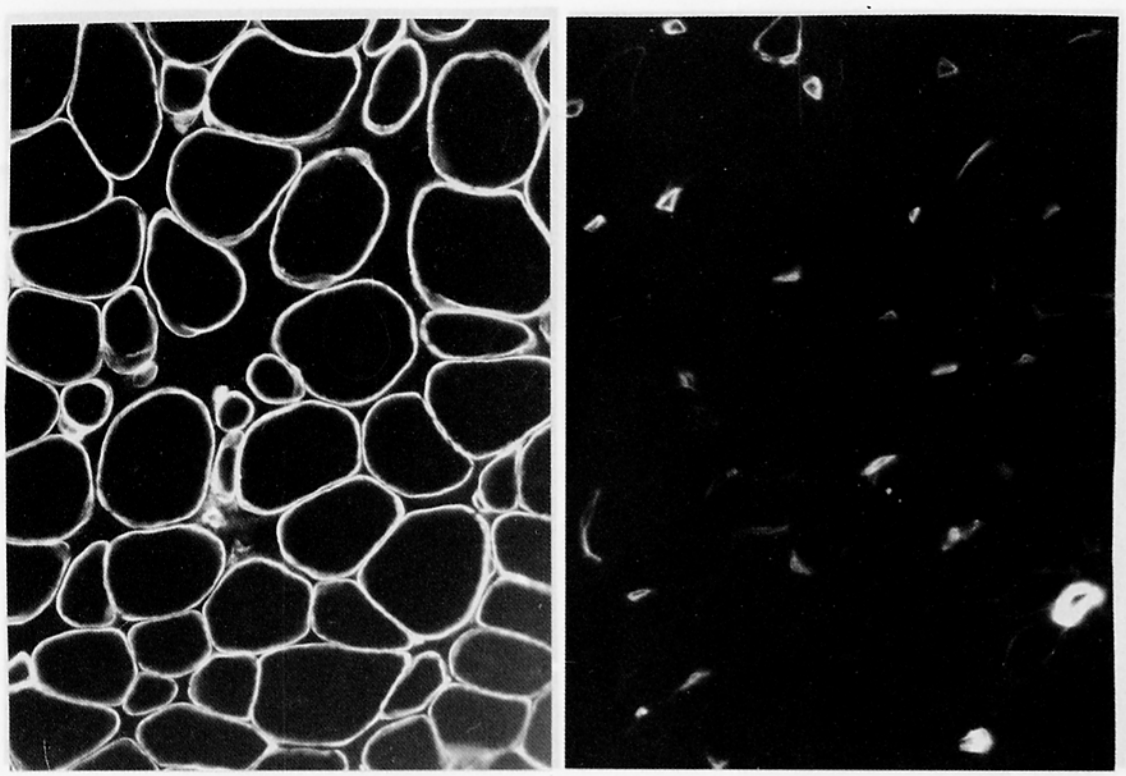

Figure 3. Immunolabelling of laminin subunits in muscle of one of our merosin-non-deficient patients: normal amount of merosin (left) and lamin-α1 chain (right) were found in all of the examined cryosections.

involvement. In the same populations the Fukuyama-like form is very rare [10].

In 1993 Hayashi et al. studied the laminin subunits on the basement membrane of muscle fibers from patients with Fukuyama CMD [7]: they found a consistent partial deficiency of merosin in muscle of all the cases considered. Merosin is the α2 chain of laminin, an extracellular matrix protein linked to the dystrophin-associated glycoproteins; its deficiency, although incomplete, was considered by the same Authors [7] of relevance in the muscle pathophysiology of Fukuyama CMD. Moreover, since merosin influences migration of neurons and neurite growth, they also assumed that its deficiency could play a role in the pathogenesis of the brain alterations of the disease [7]. Subsequent linkage genetic analysis mapped the Fukuyama CMD gene to chromosome 9q31-33 [13] and not to chromosome 6q22-23, the region of the merosinlocus: thus, the deficiency of this protein was considered a secondary abnormality of the disease. In the following year, a complete deficiency of merosin was detected by Tomé et al.[14] in cryosections from muscle biopsies of 13 out of 20 patients with Classical CMD. These merosin-deficient cases formed a fairly homogeneous clinical group, showing severe muscle involvement and, on neuroimaging, asymptomatic cerebral white matter abnormalities, without gyral malformations [4, 8]. This leukoencephalopathy, that exceptionally has also been found in merosin-non-deficient cases [16], may show a progressive clinical course [18]. Molecular genetic investigations subsequently carried out in merosin-deficient Classical CMD, mapped its gene to chromosome 6q22-23, in the region of the laminin α2 locus [9], with evidence of splice site and nonsense mutations [8]. These studies confirmed the primary role of merosin deficiency in pathogenesis of the muscular alterations in this subtype of Classical CMD.

The immunochemical investigation of muscle merosin in our two cases with Fukuyama-like CMD found a normal amount of the protein in one and absence of the same in the other: these data indicate that a biochemical heterogeneity may underly this clinical phenotype of CMD. To our knowledge, there are no available studies of the laminin subunits in other cases of Fukuyama-like CMD, with which to compare our data. However, it seems noteworthy that both the merosin-deficient case and the merosin-non-deficient one shared the same major brain malformations, suggesting that these are not correlated to the amount of merosin in muscle. Our merosin-deficient case may represent a phenotypical variant of the Classical CMD with merosin deficiency linked to chromosome 6q22-23; however, a phenotypical variant of the Fukuyama type of CMD, with absence of merosin instead of the characteristic partial deficiency reported by the Japanese Authors [7], can not be ruled out. Genetic analysis could clarify this issue, but it is not available yet either for our cases or for others with Fukuyamα-like CMD.

On the whole, our study identified both a merosin-deficient and a merosin-non-deficient case of Fukuyama-like CMD, with evidence of a biochemical divergence between this clinical expression of CMD and the Fukuyama type of the disease. Moreover, it suggests that the Fukuyama-like CMD of Western countries may represent a possible heterogeneous confluence of phenotypical variants of other well defined forms of CMD.

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