Merosin deficient congenital muscle dystrophy in children – clinical features and retrospective immunohistochemical study of own muscle biopsy material

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Abstract

Congenital muscle dystrophy (CMD) is a heterogenous group of muscle disorders characterised by early clinical presentation, dystrophic myopathic pattern of skeletal muscle in biopsy, weakness, hypotonia, atrophy of muscles, sometimes joint contractures. Nearly half of CMD cases in humans is associated with deficiency of laminin alpha 2 subunit (merosin). The gene LAMA2 responsible for the production of laminin 2 is located on chromosome 6q2. Pattern of inheritance is autosomal recessive. The most widely used diagnostic procedure of merosin deficient CMD (MDCMD) is immunohistochemical confirmation in skeletal muscle biopsy specimen. The aim of the study was retrospective immunohistochemical examination of merosin in frozen archival skeletal muscle biopsy specimens. In available samples of 18 children selected for the study on the basis of age, skeletal muscle histology and clinical presentation, the study revealed merosin deficiency in 4 children, two boys and two girls aged 4–16 months at the time of biopsy. Authors analyse and discuss clinical presentation, muscle histology, spectrophotometric analysis of respiratory chain complexes in retrospectively revealed children with MD CMD.

Key words: congenital muscular dystrophy, merosin, merosin deficient congenital muscular dystrophy, merosin immunohistochemistry

Introduction

Congenital muscle dystrophy (CMD) is a heterogenous group of muscle disorders characterised by early clinical presentation, dystrophic myopathic pattern of skeletal muscle in biopsy, weakness, hypotonia, atrophy of muscles, sometimes joint contractures. Typically muscle shows extensive muscle fibre damage with intense interstitial and replacement fibrosis. Clinically CMD is characterised by weakness, hypotonia, atrophy of muscles, sometimes joint contractures [8, 3].

Nearly half of CMD cases in humans is associated with deficiency of laminin alpha 2 subunit (called merosin) in muscle. Those cases are frequently associated with central nervous system involvement with cerebral white matter lesions which may be observed in MR imaging [12, 6]. Laminins are proteins located in basement membranes of diverse tissues. Merosin deficient CMD (MDCMD) is the first of congenital dystrophies in which the biochemical defect and genetic background has been identified. [11]. The gene LAMA2 responsible for the production of laminin α2 is located on chromosome 6q2 [4]. Inheritance is autosomal recessive. Secondary merosin deficiency was also described in a form of CMD with gene mutation localised on chromosome 1q42 [2]. The degree of merosin reduction may differ in individual patients what is reflected by different degree of clinical severity of the disease [1, 9]. Some children may even attain the ability to walk with or without support. In mo-
severe cases myopathy is early and profound leading to death due to hypoventilation and infection. As in other muscular dystrophies there is still no specific cure for MDCMD but physiotherapy and diverse supportive measures or even surgical intervention may alleviate the symptoms and delay the progression of the disease.

Since introduction of anti-merosin monoclonal antibodies to laboratory practice, the diagnosis is mainly based on immunohistochemical studies confirming the absence or reduction of merosin in muscle or in skin. Skin biopsies may reveal lack of merosin present in neural elements, but are not suitable for detection of proteins involved in pathogenesis of other myopathies so are not useful in differential diagnosis in patients in whom the merosin is not lacking [7]. Some laboratories perform genetic tests which ultimately prove the diagnosis, but this method is not widely available. As is the case for most other proteins studied immunohistochemically in diagnosis of myopathies the normal expression of merosin is sarcolemmal as shown on Fig. 1 [10].

Aim of the study

Our study consists of retrospective immunohistochemical examination of merosin in frozen archival skeletal muscle biopsy specimens obtained during the years 1996–2004, when this method had not been used in routine biopsy assessment. Selection of patients was based on clinical symptoms, early presentation and dystrophic pattern of muscle damage on light microscopy.

Patients and methods

Initially the group of 34 patients (15 boys and 19 girls) was selected for the study from the whole pediatric biopsy material (n=320) using the above mentioned criteria. Their age ranged from 3 weeks to 6 years at the time of biopsy. Assessment of archival muscle samples revealed that only 18 frozen tissue blocks obtained from 9 girls and 9 boys are suitable for further investigation due to technical reasons. Clinical presentation or suspicion at the time of biopsy comprised: floppy child; spinal muscular atrophy; congenital dystrophy; congenital myopathy; mitochondrial myopathy/respiratory chain disorder. Archived histopathology reports concluded the following: congenital muscle dystrophy, muscle dystrophy, nonspecific myopathy, miosisitis.

Apart from controlled merosin immunohistochemistry the muscle was reassessed in routine myopathology panel of stains and reactions comprising: hematoxylin and eosin; modified Gomori trichrome; oil red O; succinate dehydrogenase; NADH dehydrogenase; cytochrome c oxidase; acid phosphatase; myosin ATP-ase at pH 4,3/4,6/9.4. Spectrophotometric assay of respiratory chain complexes was also included into the study. Methods of biochemical investigation were described earlier [5].

Results

Immunohistochemical analysis revealed 4 patients (two boys and two girls) with merosin deficiency in muscle. Characteristics of their clinical, pathological, and biochemical features are as follows:

Patient 1
PR. A boy, born after uneventful pregnancy and delivery, as a second child to unrelated parents. Birth weight was 2200 g, length 48 cm, Apgar score was 8. From the beginning muscle hypotonia and poor suckling were observed as well as hypomotility. EMG was myopathic. Serum creatine kinase concentration was markedly elevated – 809 – 1334 u/l. (normal value – to 157 u/l.), and aminotransferases were mildly elevated (ASPAT – 63-95 IU, AlAT- 57-60 IU). During infancy the child was floppy and his motor milestones were delayed. He had slim habitus, dolichocephaly and long and slim fingers. His muscles were atrophic and tendency to contractures were observed. Brain MRI showed abnormal cerebral white matter signal localised mainly in periventricular areas and in anterior and posterior limbs of internal capsule (Fig. 2). Mild ventricular enlargement and mild temporal atrophy were also found. Muscle biopsy was performed at the age of 12 months.

Patient 2
WD. A boy born as a first child of unrelated parents. Pregnancy and delivery was uneventful. Birth weight was 2200 g, length 48 cm, Apgar score was 8. From the beginning muscle hypotonia and poor suckling were observed as well as hypomotility. EMG was myopathic. Serum creatine kinase concentration was markedly elevated – 809 – 1334 u/l. (normal value – to 157 u/l.), and aminotransferases were mildly elevated (ASPAT – 63-95 IU, AlAT- 57-60 IU). During infancy the child was floppy and his motor milestones were delayed. He had slim habitus, dolichocephaly and long and slim fingers. His muscles were atrophic and tendency to contractures were observed. Brain MRI showed abnormal cerebral white matter signal localised mainly in periventricular areas and in anterior and posterior limbs of internal capsule (Fig. 2). Mild ventricular enlargement and mild temporal atrophy were also found. Muscle biopsy was performed at the age of 12 months.

Patient 3
NK. A girl born after first uneventful pregnancy and delivery to nonconsanguineous parents. Birth weight was 3090g,
Apgar score was 10. From the 8th day of life she was markedly hypotonic and had dyspnoe. X-ray examination showed pneumonia and enlargement of the heart. EMG was myopathic. ECG showed partial heart block (RBBB). Serum creatine kinase concentration was markedly elevated – 2751 – 1780 u/l and aminotransferases were slightly elevated AlAT – 117 IU, AspAT – 117 IU. Muscle biopsy was performed at the age of 4 months. Her motor milestones were delayed but her intellectual development is very good at the age of 2,5 years.

Patient 4
	OE, a girl born as a first child to unrelated parents. Pregnancy was complicated by maternal diabetes, delivery was uneventful. Birth weight was 2600g. Apgar score was 10. Since neonatal period she was hypotonic. Progressive muscular weakness was observed during physiotherapy. On physical examination muscular wasting and lack of tendon reflexes were detected. Her motor milestones were markedly delayed, but intellect was spared. Creatine kinase concentration in serum was increased – 894 – 1177 u/l. EMG was myopathic. Brain MRI showed signal abnormality of hemispheric periventricular white matter. U-fibers were also partially involved. Lateral ventricles were mildly widened. Muscle biopsy was performed at the age of 16 months.

Muscle biopsy – histopathology and spectrophotometric assay
Muscle biopsy features of all children displayed the same pattern concluded originally as muscle dystrophy or congenital muscle dystrophy. Replacement fibrosis was most pronounced in patient 4 in whom the biopsy was performed at 16 months. Apart from this finding there were no significant differences in muscle lesions (Fig. 3 and 4). Immunohistochemical examination revealed the same total lack of merosin reactivity in all children (Fig. 5).
Spectrophotometric study was possible to start only in patients 1 and 4 due to lack of muscle tissue in samples obtained during biopsy, resulting from predominance of fibrous and adipose tissue. However, in examined two muscle homogenates, citrate synthase activity appeared below the level of assay reliability for the assessment of respiratory chain complexes activity (Patient 1. – 36 nmol/min/mg and patient 4. – 40 nmol/min/mg, reference value 96,5 – 150,1 nmol/min/mg).

Discussion

Clinical presentation of our MD CMD patients is characterised by relative homogeneity. They were floppy children from birth. Muscle hypotonia that was rather nonprogressive, was observed permanently and motor milestones were markedly delayed. All children had elevated concentration of creatine kinase.

The above clinical picture is however non-specific and it must be emphasised that there are no typical clinical or biochemical features of MD CMD. All children had moderately or markedly elevated creatine kinase concentration. Increase of CK were in range of 1000 – 2700 u/l, what is rather characteristic for all types of CMD. It is not so high as in Duchenne muscular dystrophy where the level is over 10 000 – 20 000 u/l. but is higher than in congenital myopathies where the CK level is mildly elevated or normal. All children had myopathic electromyography what is not characteristic for myopathy or dystrophy.

Typical pitfalls in clinical suspicion / interpretation include differential diagnosis of so called “floppy infant syndrome”. This syndrome is heterogeneous and symptoms are not characteristic so initial clinical suspicions were: spinal muscular atrophy, congenital muscular myopathy, congenital muscular atrophy, Pompe disease and other metabolic diseases, as well as mitochondrial/OXPHOS disorders. MRI findings may suggest other leukodystrophies especially of metabolic origin (MRI of the first patient was described as „metachromatic leucodystrophy or other metabolic leukodystrophy”).

All our patients showed relatively homogenous pattern of skeletal muscle pathology in microscopic examination. Histopathological examination showed dystrophic pattern in routine stains which is not specific, so immunohistochemistry with anti-merosin antibody is needed to confirm the diagnosis.

In MD CMD it appeared difficult to obtain muscle biopsy specimen containing representative fragment of skeletal muscle tissue being suitable for biochemical investigation of respiratory chain complexes activity in muscle homogenate.

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References