Review article

The expanding phenotype of laminin α2 chain (merosin) abnormalities: case series and review

Kristi J Jones, Graeme Morgan, Heather Johnston, Vivienne Tobias, Robert A Ouvrier, Ian Wilkinson, Kathryn N North

Abstract

Initial reports of patients with laminin α2 chain (merosin) deficiency had a relatively homogeneous phenotype, with classical congenital muscular dystrophy (CMD) characterised by severe muscle weakness, inability to achieve independent ambulation, markedly raised creatine kinase, and characteristic white matter hypodensity on cerebral magnetic resonance imaging. We report a series of five patients with laminin α2 deficiency, only one of whom has this severe classical CMD phenotype, and review published reports to characterise the expanded phenotype of laminin α2 deficiency, as illustrated by this case series. While classical congenital muscular dystrophy with white matter abnormality is the commonest phenotype associated with laminin α2 deficiency, 12% of reported cases have later onset, slowly progressive weakness more accurately designated limb-girdle muscular dystrophy. In addition, the following clinical features are reported with increased frequency: mental retardation (~6%), seizures (~8%), subclinical cardiac involvement (3-35%), and neuronal migration defects (4%). At least 25% of patients achieve independent ambulation. Notably, three patients with laminin α2 deficiency were asymptomatic, 10 patients had normal MRI (four with *LAMA2* mutations reported), and between 10-20% of cases had maximum recorded creatine kinase of less than 1000 U/l. *LAMA2* mutations have been identified in 25% of cases. Sixty eight percent of these have the classical congenital muscular dystrophy, but this figure is likely to be affected by ascertainment bias. We conclude that all dystrophic muscle biopsies, regardless of clinical phenotype, should be studied with antibodies to laminin α2. In addition, the use of multiple antibodies to different regions of laminin α2 may increase the diagnostic yield and provide some correlation with severity of clinical phenotype.
(*J Med Genet* 2001;**38**:649–657)

Keywords: congenital muscular dystrophy; laminin α2 chain; merosin; skeletal muscle

Institute for Neuromuscular Research, The Children's Hospital at Westmead, PO Box 3515, Parramatta, Sydney, NSW 2124, Australia and the Department of Paediatrics and Child Health, University of Sydney, Australia
K J Jones
K N North

Department of Medical Genetics, Sydney Children's Hospital, Randwick, Australia
G Morgan

Department of Neurology, Sydney Children's Hospital, Randwick, Australia
H Johnston

Division of Paediatric Anatomical Pathology, SEALS, Sydney, Australia
V Tobias

Institute for Neuromuscular Research, The Children's Hospital at Westmead, Sydney, Australia
R A Ouvrier

Department of Paediatrics, John Hunter Hospital, Newcastle, Australia
I Wilkinson

Correspondence to:
Professor North,
kathryn@chw.edu.au

The muscular dystrophies are a subgroup of the primary myopathies with genetic aetiology, which characteristically have "dystrophic" features on muscle biopsy, defined as increased fibre size variability, increased connective tissue, and the presence of degenerating and regenerating fibres.[1] The muscular dystrophies are broadly classified on the basis of age of onset and pattern of weakness into those presenting with weakness at birth or within the first few months of life (congenital muscular dystrophy (CMD)), and those with later onset, progressive weakness (for example, limb-girdle weakness as in the X linked dystrophinopathies and limb-girdle muscular dystrophies (LGMD)). The congenital muscular dystrophies are traditionally further subdivided on the basis of the presence or absence of clinical central nervous system (CNS) involvement. Patients with classical congenital muscular dystrophy have "muscle weakness with hypotonia or arthrogryposis, normal or moderately raised serum creatine kinase (CK), usually normal intellect, and brain imaging which may show a normal picture or evidence of changes in the white matter on CT or magnetic resonance imaging".[2]

Mutations in *LAMA2*, the gene encoding the laminin α2 chain of merosin, were originally identified in a subset of patients with congenital muscular dystrophy.[3 4] The clinical phenotype first described in the laminin α2 negative patients was relatively homogeneous and satisfied the ENMC diagnostic criteria for classical CMD.[2] The phenotype of these patients was characterised by onset at birth or in the first six months of life, severe muscle weakness, and contractures. They rarely achieved independent ambulation and had creatine kinase (CK) levels >1000 U/l. Characteristic white matter hypodensity was evident on cerebral magnetic resonance imaging (MRI), with abnormally high T2 signal in the periventricular and subcortical white matter; however, in most cases there was no clinical evidence of central nervous system involvement.[5–7] The original studies of laminin α2 were limited by clinical ascertainment bias, as patients with classical severe CMD were preferentially selected for study.[8–12]

Table 1 Cases with laminin α2 abnormality

Case/sex	Age of onset/ presentation	Current age/ maximum motor milestone (age)	Immunocytochemistry (laminin α2)		Immunoblot (laminin α2 Chemicon MAB1922)	Highest serum CK (U/l) (normal <200U/l) (age)	Pattern of weakness at presentation	Contractures	CNS involvement/ intellect/seizures	Cerebral MRI scan (T2 weighted images)
			Chemicon MAB1922	NCL-merosin						
Case 1 F	Birth	9 y/stood with support (4 y)	Negative	Negative	Negative	7121 (1/12)	Marked neonatal hypotonia	Yes	Normal intellect No seizures	"Typical" white matter changes
Case 2 F	Birth	5 y/sat unsupported (3 y)	↓	↓	↓ amount N size	2009 (11/12)	Marked neonatal hypotonia	Yes	Focal seizures Moderate intellectual delay	"Typical" white matter changes
Case 3 M	4 y	7 y/walked (18 mth)	Negative	Negative	Negative	3554 (4 y)	Asymmetrical limb-girdle weakness	Yes	Normal intellect No seizures	"Typical" white matter changes*
Case 4 F	Presented at 2 y	6 y/walked (2 y)	+	Insufficient muscle	Negative	2500 (2 y)	Limb-girdle weakness	No	Normal intellect No seizures	"Typical" white matter changes
Case 5 F	Presented at 2 y	7 y/sat unsupported (18 mth)	↓	↓↓	↓ amount N size	3371 (18/12)	Generalised weakness and hypotonia	Yes	No seizures Severe intellectual delay	Focal cortical dysplasia Patchy increased white matter signals†

+ = patchy (discontinuous) membrane staining, ↓ = decreased (continuous) membrane staining, ↓ amount = decreased amount, N = normal.
*Fig 1. †Fig 2.

Over the past few years there have been a number of case reports showing variability in the clinical phenotype associated with laminin α2 deficiency. In contrast to the idea that laminin α2 deficiency is associated with severe classical CMD without clinical CNS involvement, these reports have indicated: onset of weakness and/or presentation in childhood or adulthood[13–15] with some patients remaining asymptomatic at the time of diagnosis[15 16]; mild, non-progressive proximal weakness[13 17–20]; achievement of ambulation[12 13 21]; creatine kinase less than 1000 U/l[5 12 14 16 21–29]; neuronal migration defects on MRI[30–33] or normal MRI with no white matter changes[15–16 27 34–36]; symptomatic central nervous system involvement, that is, mental retardation[12 16 31–38] and seizures.[5 9 12 15 21 27 28 31–33]

Patients with classical CMD and laminin α2 deficiency may have additional disease features including altered visual and somatosensory evoked potentials with minor neurological and perceptuomotor deficits,[39 40] electrical evidence of peripheral demyelinating neuropathy,[41 42] and subclinical cardiac involvement.[34 43–46]

Individual case reports do not accurately reflect the relative frequency of a particular clinical feature in association with laminin α2 deficiency, and there have been few systematic studies without clinical ascertainment bias. To address these methodological issues, we have analysed a group of 202 Australian patients with primary muscle disease ascertained on the basis of dystrophic or myopathic features on muscle biopsy.[47] We have identified 5/202 patients with laminin α2 deficiency and, surprisingly, only one of these patients has the features of the typical CMD found in classical laminin α2 deficiency. We report this case series to illustrate the expanding clinical phenotype of patients with laminin α2 deficiency. In addition, we have reviewed the reported cases of laminin α2 deficiency to provide an overview and to estimate the frequency of "typical" and "atypical" phenotypes.

Methods

Patients were ascertained retrospectively and prospectively through archived muscle biopsy material (1979-1999) at the major reference laboratories in the state of New South Wales for muscle disease. Biopsies were divided into two groups: (1) "dystrophic" biopsies (as defined above) and (2) biopsies with non-specific myopathic changes, excluding those with an alternative clinical or pathological diagnosis such as facioscapulohumeral dystrophy, nemaline myopathy, or central core disease. Immunocytochemical studies were performed using antibodies to the 80 kDa fragment of merosin (MAB1922 (1:2000) Chemicon), and NCL-merosin ((1:200) Novocastra), dystrophin (NCL-DYS1 (1:1), NCL-DYS2 (1:2), NCL-DYS3 (1:1), Novocastra), α-, β-, and γ-sarcoglycan (monoclonal α-sarcoglycan (NCL-50DAG (1:50) Novocastra), and affinity purified β- and γ-sarcoglycan (β-SG (1:50), γ-SG (1:1000)). Abnormalities of dystrophin and laminin α2 were confirmed by immunoblot. The study method is detailed in Jones *et al*.[47] We reviewed the patient records of all patients and personally examined all five patients with laminin α2 abnormalities and reviewed their cerebral MRI scans.

Results

Abnormalities of laminin α2 were present in 5/131 (4%) dystrophic muscle biopsies and 0/71 non-specific myopathic biopsies. Patient findings are summarised in table 1. In 2/5 patients, laminin α2 was negative on immunocytochemistry and immunoblot, and three patients had partial deficiency of laminin α2. Case 4, with absence of laminin α2, also had abnormal staining with dystrophin and α-sarcoglycan. Only one patient (case 1) had typical classical CMD, two had seizures and intellectual impairment, and two presented after infancy with non-progressive weakness. All were born to non-consanguineous white parents, with no other affected family members. Creatine kinase was more markedly raised in our patients with abnormal laminin α2 (range 2009-7121 U/l, normal <200 U/l, average 3750 U/l) than in the laminin α2 positive CMD patients (range 22-1710 U/l, average 324 U/l). Cases 1-4 had "typical" white matter

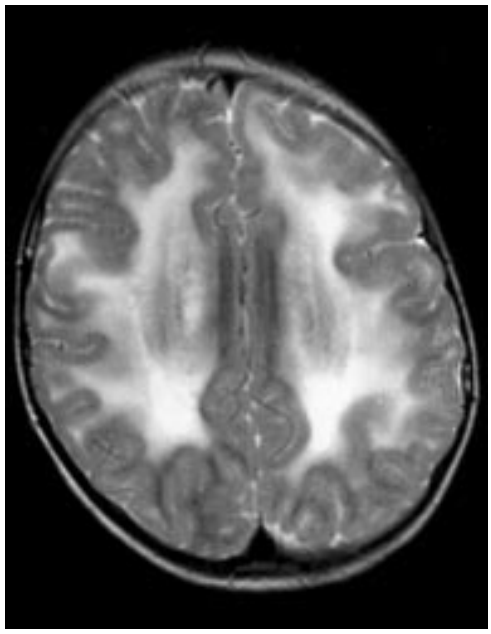

Figure 1 Cerebral MRI scan (T2 weighted image) of case 3 showing widespread T2 hyperintensity of white matter.

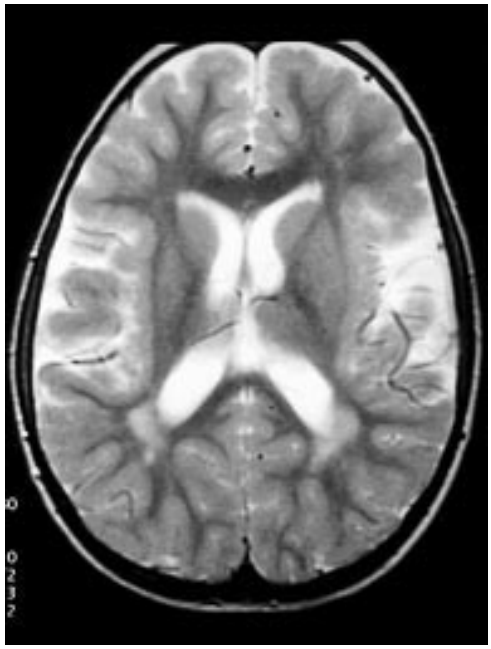

Figure 2 Cerebral MRI scan (T2 weighted image) of case 5 showing patchy areas of T2 hyperintensity of white matter, with focal cortical dysplasia.

hypodensity on cerebral MRI (T2 weighted images) (fig 1) and the changes in case 5 were atypical (described below) (fig 2).

Case reports

Case 1 was born following a pregnancy complicated by reduced fetal movements. She required ventilation for 24 hours and moderate hypotonia and contractures were noted at birth. She sat at 15 months, stood with support at 4 years, and does not walk at 9 years. Weakness is generalised and non-progressive, more marked proximally, and she is unable to rise from the floor. She has facial weakness and her eyes are structurally normal. Cardiovascular examination is normal. Intellect is normal and she does not have seizures.

Case 2 was hypotonic and hyporeflexic from birth, with little antigravity movement. Focal seizures began on day 2 and have been difficult to control. Cerebral CT scan in the neonatal period showed free subdural blood in the falx. Developmental milestones were delayed, she sat unsupported at 3 years, and does not walk at 5 years. Weakness has been static and nerve conduction velocities are normal, although sural nerve responses are unobtainable. She has moderate intellectual delay. Cardiac and ophthalmological examination is normal.

Case 3 presented at the age of 4 years with a waddling gait and poor motor skills. No abnormalities were noted at birth, he sat at 8 months, and walked at 18 months. He was mildly hypotonic, with proximal weakness and hyporeflexia. Posture was lordotic with no scoliosis and he had a waddling gait and an awkward run. Aged 9 years, weakness is stable, he is able to rise from the floor without a Gowers' manoeuvre, but tires easily. Facial and extraocular muscles, cardiac examination, electrocardiogram, and echocardiogram are normal. Intellect is normal and he has never had seizures.

Mutations have been detected within the laminin α2 gene of cases 2 and 3 confirming the primary laminin α2 abnormality (P Guicheney, personal communication).[54] Mutation analysis on the other cases has not been performed to date.

Case 4 presented at the age of 2 years with delayed motor milestones. No abnormalities were noted at birth. She sat at 8 months, walked at 2 years with a lordotic waddling gait, and at 6 years falls frequently, has difficulty with stairs, and tires easily. She is hypotonic and hyporeflexic, with proximal weakness, prominent calves, and no contractures. Weakness is non-progressive and intellect is normal. Her eyes are structurally normal. Cerebral MRI showed the typical white matter changes of laminin α2 deficiency. Laminin α2 staining was patchy on immunocytochemistry and absent on immunoblot. Interestingly, this patient also had patchy staining with α-sarcoglycan, DYS1, 2, and 3, and absence of dystrophin on immunoblot. Staining with antibodies to spectrin, β-, and γ-sarcoglycan were normal. The primary abnormality is not certain, as abnormal dystrophin was not noted in any of the other patients studied.

Case 5 presented at 2 years of age with developmental delay. No abnormalities were noted at birth. She sat unsupported at 18 months and at 7 years is able to take a few steps with splints. Hip dislocation was noted at 7 months and there has been no progression of weakness. All growth parameters including head circumference are below the 3rd centile. She is markedly hypotonic with muscle wasting, generalised weakness, and antigravity movement in all limbs. There are contractures of the hips and knees and dystonic posturing of the left hand. There is no facial weakness and

*Table 2 Clinical features of patients with immunohistochemical abnormalities of laminin α2: review of 248 reported cases**

Clinical feature	No of cases (where reported)	Frequency range		Our series	Cases with mutations identified†
		% (n=248)	% (where reported)		
Presentation					
Birth–6 months	219/248	88	88	2/5	50/54
7 months–2 years	19/248	8	8	2/5	2/54
>2 years	7/248	3	3	1/5	2/54
Asymptomatic	3/248	1	1	0/5	0/54
Walking independently					
Not walking by 2 years	95/127	40	75	3/5	49/54
Walking by 2 years	11/127	5	9	2/5	2/54
Learnt to walk after 2 years	16/127	7	12	0/5	3/54
Walk ?age	5/127	2	4	0/5	0/54
Progression of weakness					
Improving	4/25	2	16	0/5	—
Static	8/25	3	32	5/5	—
Slowly progressive	13/25	5	52	0/5	—
Rapidly progressive	0/25	0	0	0/5	—
Creatine kinase					
<1000 U/l	24/124	10	19	0/5	3/27
>1000 U/l	100/124	42	81	5/5	24/27
MRI					
"Typical" white matter changes	156/175	63	90	4/5	33/38
Neuronal migration defect	9/175	4	5	1/5	1/38
Normal	10/175	4	6	0/5	4/38
Mental retardation					
Normal intellect	121/138	49	88	2/5	43/48
Mild	8/138	3	6	1/5	3/48
Moderate	3/138	1	2	1/5	1/48
Severe	6/138	2	4	0/5	1/48
Seizures	19/97	8	20	1/5	5
Cardiac involvement	7/20	3	35	0/1	0

*References 4, 5, 6, 7, 9, 11, 12, 13, 14, 15, 16, 18, 20, 21, 22, 23, 24, 25, 26, 27, 29, 30, 31, 32, 33, 34, 35, 36, 44, 48, 50, 52, 57, 58, 59, 61, 62, 63, 64, 65, 66, 67.
†Figures are expressed as a proportion of those in which the feature is specifically reported. Progression of weakness was not specifically reported.

her eyes are structurally normal. Cardiovascular examination and nerve conduction studies are normal. She has severe intellectual disability, but no seizures. Cerebral MRI showed patchy increased white matter signals (T2 weighted images) and focal cortical dysplasia (fig 2).

Review of published reports

We reviewed clinical, pathological, and molecular genetic data of reported patients with abnormal immunohistochemical staining with antibodies to laminin α2. The clinical features of these patients were summarised in terms of severity of disease and clinical features additional to those usually associated with classical congenital muscular dystrophy. We have chosen to include all those patients with laminin α2 abnormality at the protein level, rather than only those that are molecularly defined. However, we have summarised the clinical features in those with *LAMA2* mutations identified. The number of cases reported with mutations in *LAMA2* is small and limited by clinical and immunocytochemical ascertainment bias. Mutation analysis to date has largely focused on those patients with a "typical CMD" phenotype and absence of the protein immunocytochemically. Patients fulfilling the clinical criteria for Fukuyama congenital muscular dystrophy (FCMD), muscle-eye-brain disease (MEB), or Walker-Warburg syndrome (WWS) were excluded from the analysis.[2] However, we will have included some patients who will ultimately be shown to have secondary laminin α2 deficiency, as we cannot accurately predict primary laminin α2 abnormality from the immunocytochemical abnormalities and clinical phenotype alone. Cardiovascular involvement was defined by abnormality on electrocardiogram and/or echocardiogram.

A total of 248 patients with immunohistochemical abnormalities of laminin α2 have been reported to September 2000. Patient ascertainment varies markedly. Most of the initial case series report patients with a clinical and histological diagnosis of classical congenital muscular dystrophy.[5 9 10 48 49] More recent case reports have focused on "atypical cases".[12 13 17 18 50 51] Although there is one comprehensive study with wider ascertainment,[52] the focus remains on patients with a typical congenital muscular dystrophy phenotype. As a result of this variation in ascertainment and variable reporting of clinical findings, it is not possible to determine accurately the frequency of an individual clinical feature. Therefore, we have expressed the frequency of each feature as a percentage of the number of cases where that clinical feature is mentioned. As some clinical features are likely to be under-reported, for example, normal intellect, the frequency is also expressed as a percentage of the total number of cases (n=248). The true frequency of each clinical feature is likely to lie within this frequency range (table 2).

IMMUNOCYTOCHEMICAL FINDINGS

The laminin α2 gene encodes a protein of 390 kDa, and under reducing conditions in vitro laminin α2 chain migrates as an N-terminal

fragment of ~300 kDa and a C-terminal fragment of 80 kDa.[21] A number of different antibodies have been used to detect these two fragments. The Chemicon antibody (MAB1922) was the first available commercially. This antibody is directed against the C-terminal part of the G globular domain of the human laminin α2 chain and detects the 80 kDa fragment of merosin. The commercially available antibody from Alexis (MAB4H8-2) is reported to react predominantly with the 300 kDa N-terminal fragment,[53] and the Novocastra antibody (NCL-merosin) is raised against the whole laminin α2 chain; however, the precise epitope is unknown. More than one antibody to laminin α2 was used in 53/248 (21%) cases and, of these, 21/53 (40%) showed differential staining. In the majority of patients with milder disease, that is, presentation after 6 months of age and/or achievement of independent ambulation, staining was more markedly abnormal with an antibody that detects the 300 kDa fragment than with the Chemicon (MAB1922) antibody to the 80 kDa fragment.[21 24 25 28] However, the opposite staining pattern may also be found in patients with a milder phenotype.[16 51 54]

MUTATION ANALYSIS

In 62/248 cases (25%), the identification of mutations within *LAMA2* confirms that the laminin α2 deficiency is primary rather than secondary.[4 14 22 24 27 31 50 52 61] In 20/62 (32%) there was one or more atypical clinical features (table 2); however, current mutation data are skewed towards those with a "typical CMD" phenotype, as in most reported series these patients were preferentially selected for mutation analysis. It is important to differentiate between primary and secondary abnormalities of laminin α2 staining in unusual or atypical cases. Secondary laminin α2 deficiency also occurs in patients with Fukuyama CMD,[55] muscle-eye-brain disease,[56] and several as yet unclassified forms of CMD.[35 38 51]

CLINICAL FEATURES

The clinical features of the 248 patients are summarised in table 2. A total of 189/248 (76%) fit the definition of classical CMD without clinical CNS involvement; however, 59/248 (24%) were atypical and are described below.

Onset/presentation

A total of 26/248 cases (10%) presented after 6 months of age,[12–14 16 18 20 21 24 25 28] and 3/248 were asymptomatic at the time of reporting (aged 12-30 years).[15 16] These asymptomatic cases were investigated because of raised CK and had partial deficiency of laminin α2 on immunohistochemical staining. MRI was performed in 2/3 asymptomatic patients (at 11 years in one patient, others not reported) and was normal in both, raising the possibility that they do not have primary laminin α2 deficiency. Mutation analysis was not reported in any of the asymptomatic patients.

Progression

A total of 95/127 (75%) were not walking by the age of 2 years. A total of 32/127 (25%) were ambulant at the time of reporting and at least 11/127 (9%) walked by the age of 2 years.[12–14 16 18 20 21 28 34] Weakness was slowly progressive in 13/25 cases,[14 16 23 28 34 50] 12/25 had a static or improving course, and there were no reports of rapid progression. It is likely that those with a non-progressive course are under-reported, so the proportion of cases with progressive weakness is likely to be closer to 13/248 (5%). A total of 11/248 died from respiratory failure at ages ranging from 4 months to 12 years (mean and median 60 months, mode 23 months).

Creatine kinase

CK >1000 U/l was recorded on at least one occasion in 100/124 (81%) cases for whom these results were reported (normal <180-200 U/l). However, the highest CK recorded was <1000 U/l in 24/124 (19%) cases.[5 12 14 16 21–29] In eight cases where CK was >1000 U/l, subsequent CK (age range 11 months-21 years) was <1000 U/l.[23 32 52 57] In one patient, CK was <1000 U/l at 2 years and rose to >1000 U/l at 12 years.[13]

Cerebral MRI

Characteristic white matter hypodensity was present in 156/175 (87%) cases. In 12 of these there was also evidence of cerebral atrophy (for example, mild frontal cortical atrophy, mild ventricular dilatation, hypoplastic pons or cerebellum) in a structurally normal brain.[23 27 28 31] Neuronal migration defect (for example, focal cortical dysplasia, polymicrogyria, or cortical anomaly) was present in 9/175 (5%),[30–33 58] and MRI was normal in 10/175 (6%) of patients (18 months-13 years).[15 16 27 34–36] Four out of 10 patients with normal MRI had "typical" primary laminin α2 deficiency with absence of laminin α2 on immunocytochemistry, a classical CMD phenotype, and identified *LAMA2* gene mutations.[27] The age at biopsy of these four patients ranged from 3-13 months; however, the specific age at cerebral imaging was not reported. Of the remaining six patients with normal MRI in whom mutations have not been reported, all had partial laminin α2 deficiency, and 2/6 were not linked to the *LAMA2* locus, consistent with secondary laminin α2 deficiency.

Only 1/9 patients with neuronal migration defect on MRI have been reported to have a *LAMA2* mutation.[31] All patients with neuronal migration defect had a severe CMD phenotype, with absence of laminin α2 immunocytochemical staining.

Clinical CNS involvement

Mental retardation was present in 7-12% (that is, between 17/248 of all cases and 17/138 in which intellect was specifically reported).[5 12 16 32 34 35] Normal intellect is probably under-reported, although some of the cases were too young for mental retardation to be apparent. The retardation was mild in 8/17 cases and moderate to severe in 9/17 cases.

There was no apparent correlation between mental retardation and severity of weakness. Seizures were present in three of these cases with mental retardation (one mild, one moderate, and one severe), not present in nine cases, and not reported in five cases. MRI showed "typical" white matter changes in all cases with mild impairment, and of those with moderate to severe impairment "typical" changes were present in 1/9, neuronal migration defects in 3/9, MRI was normal in 2/9, and not reported in 3/9. Immunohistochemical staining varied from partial deficiency to complete absence. *LAMA2* mutations have been reported in 5/17 cases with mental retardation, and in only one patient with severe mental retardation.[31]

Seizures were reported in 19/97 (20%); however, the absence of seizures is likely to be under-reported and therefore the frequency of seizures is likely to be closer to 19/248 (8%). Seizures were both partial and complex, with no consistent pattern,[5 9 12 15 21 27 28 31–33] and may be found in patients with primary laminin α2 deficiency.[27 31]

There were no patients reported with structural abnormalities of the eyes.

Electrocardiogram and/or echocardiogram were reported in 20 cases and were abnormal in 7/20 (35%).[34 43–46] More than half (4/7) of these patients were asymptomatic and therefore would not have been detected if routine investigation was not performed. There was a range of cardiac abnormalities including right bundle branch block with normal echocardiogram, dilated cardiomyopathy, and "borderline changes in cardiac function". Mutations were not reported in these cases.

Discussion

Initial studies of patients with laminin α2 abnormalities were limited by ascertainment bias, both clinical and histopathological. In our own series, we aimed to minimise ascertainment bias and to characterise the spectrum of phenotypes associated with abnormalities of laminin α2 and their relative frequency. All patients with abnormalities of laminin α2 had dystrophic changes on muscle biopsy, markedly raised CK, and 4/5 had characteristic abnormalities of white matter on cerebral MRI scan; however, there was a range of clinical phenotypes. Only one patient, case 1, has the typical phenotype of classical congenital muscular dystrophy with laminin α2 deficiency. Although case 2 does strictly satisfy the diagnostic criteria for classical CMD,[2] she has mental retardation and seizures. These occur with increased frequency in CMD owing to laminin α2 deficiency, but in this case may be secondary to neonatal intracranial haemorrhage. Therefore although there is a "typical" phenotype of complete laminin α2 deficiency, with severe classical CMD, there is variability in onset and severity of weakness, and in the degree of CNS involvement. Many of these variable cases have proven primary laminin α2 deficiency, but some may have secondary laminin α2 deficiency.

The majority of cases (88%) presented in the first 6 months of life. At least 25% of patients were walking at the time of reporting, in contrast to initial reports where very few of the patients achieved independent ambulation.[5 9 12 52] Some patients have later onset with a static or slowly progressive LGMD phenotype,[12 14 21] although, importantly, there were no reports of rapidly progressive weakness. Three patients were asymptomatic at the time of reporting. The primary abnormality in these patients is unclear; there was partial laminin α2 deficiency on immunohistochemical staining, cerebral MRI was normal, and mutations have not been reported suggesting that the laminin α2 abnormality may be secondary. The long term prognosis appears to be more favourable in the group of patients with later onset phenotype and laminin α2 deficiency than in the primary dystrophinopathies and limb-girdle muscular dystrophies resulting from sarcoglycan deficiency.

The highest creatine kinase (CK) recorded was less than 1000 U/l in almost 20% of cases, but in those with reported mutations, CK was less than 1000 U/l in only 3/26 cases (measured in early childhood). Eight percent of patients with CK >1000 U/l had subsequent CK levels recorded of <1000 U/l (ranging between 11/12-21 years), so the CK level can vary, usually decreasing with age, and age should be taken into account when interpreting CK levels.

Intellect is usually normal in classical congenital muscular dystrophy (ENMC diagnostic criteria).[2] Mental retardation was present in 7-12% of cases of laminin α2 deficiency; however, many of these patients with mental retardation do not have mutations reported to date in the laminin α2 gene. These patients (for example, case 5) may represent part of the spectrum of patients with FCMD or MEB, or they may have a primary abnormality of another, as yet unidentified protein. In some cases (for example, case 2), mental retardation may be secondary to another cause, such as hypoxia or intracranial haemorrhage. Seizures were reported in 20% of patients, but absence of seizures is likely to be under-reported, so the proportion of patients with seizures is between 8% and 20%. There was no consistent pattern of seizures, but they tended to present in early childhood. Seizures were reported in 5/62 (8%) patients with *LAMA2* mutations, confirming that seizures may be present in a significant proportion of patients with primary laminin α2 mutations, some in association with normal intellect and structurally normal brain on MRI.

There were no patients reported in these series with structural abnormalities of the eyes and laminin α2 deficiency, as these are assigned an alternative diagnosis (for example, FCMD, WWS) and were therefore excluded. There may be some overlap, and a subset of the patients with structural abnormalities of the brain, mental retardation, and seizures but without structural eye abnormalities may represent the mild end of the MEB spectrum, with secondary laminin α2 deficiency.

Characteristic white matter hypodensity on MRI was present in the majority of cases (90%) and in 87% (33/38) of patients with confirmed

primary *LAMA2* mutations. Although abnormalities of neuronal migration were present in 5% of patients with laminin α2 abnormalities, only one of these cases had reported *LAMA2* mutations. This may be related to ascertainment bias in the patients selected for mutation analysis or it may be that the laminin α2 abnormality is secondary in these patients. Interestingly, in 4% of cases MRI was completely normal, and in 4/10 of these patients *LAMA2* mutations have been identified. The age at which MRI was performed was not specifically reported, which is important as the MRI changes may be difficult to assess in the early stages of disease when brain myelination is incomplete.

The molecular basis for variations in clinical phenotype has not been fully determined. There are insufficient numbers of patients of varying phenotypes with defined mutations to allow genotype/phenotype correlation. The majority of published cases have been defined immunocytochemically, and only 21% have been studied with more than one laminin α2 antibody. There is considerable variability in immunocytochemical staining pattern depending on the antibody used, and in almost half there was differential staining, with normal staining with one antibody in some cases. Most patients with a milder phenotype, in whom more than one antibody was used, had relatively preserved staining with the antibody to the 80 kDa fragment when compared with staining using other antibodies.[17 25] However, in our case 3, who has a mild phenotype, the opposite staining pattern was seen. There was absence of staining of the 80 kDa fragment and relative preservation of staining of the 300 kDa fragment.[54]

In addition to absence of laminin α2, case 4 has absence of dystrophin on immunocytochemistry and immunoblot. Cerebral MRI changes are typical of those found in primary laminin α2 deficiency and she has non-progressive weakness with normal intellect. Although laminin α2 staining is usually maintained in the primary dystrophinopathies, Tachi *et al*[59] reported a female patient with a typical CMD phenotype with proven primary dystrophinopathy, who also had secondary deficiencies of laminin α2, dystroglycan, and syntrophin on immunocytochemistry. Dystrophin gene analysis has not been performed in our patient. The combination of dystrophin and laminin α2 abnormalities is unusual, and may indicate that the laminin α2 deficiency in this patient is secondary to another, as yet unidentified protein, or to disruption of the interaction between the dystrophin associated proteins.

Bushby *et al*[60] reported seven patients in whom immunohistochemistry was normal (with antibodies to the 80 kDa and 300 kDa fragments of laminin α2); however, an absence or near absence of laminin α2 was noted on immunoblotting. These patients (including two sib pairs) had predominantly late onset limb-girdle weakness, with normal MRI in 3/7 patients and CK at least 10 times normal. All biopsies showed "non-specific dystrophic features" and haplotype analysis was consistent with absence of linkage to the 6q locus. This is an unusual finding, and the authors postulate it may be related to the processing of the sample.

Mutations confirming primary abnormality of laminin α2 have only been reported in 25% of cases, so the relative incidence of secondary deficiency of laminin α2 is as yet unclear. Pegoraro *et al*[27] suggested that primary *LAMA2* mutations underlie the majority of cases with complete deficiency of laminin α2. There are, however, cases with complete deficiency of laminin α2 in whom mutations have not been identified.[27 61] Current mutation data are skewed towards this group of patients with absence of the protein, but there are a number of patients reported with *LAMA2* mutations and partial deficiency of the protein.

In summary, although there is a "typical" phenotype of laminin α2 deficiency, with severe classical CMD, there is variability in onset and severity of weakness, and variability in CNS manifestations. In our population, with comprehensive ascertainment of all patients with a dystrophic muscle biopsy, the atypical phenotypes are more common than the "typical" phenotype. Isolated absence of laminin α2 is frequently associated with primary laminin α2 deficiency, and Pegoraro *et al*[27] found that a high proportion of such cases have *LAMA2* mutations. However, if there is only partial deficiency or atypical clinical phenotype then mutation analysis is important to confirm the primary abnormality. We suggest that all dystrophic muscle biopsies, regardless of clinical phenotype, should be studied with antibodies to more than one region of laminin α2. The yield in biopsies with non-specific myopathic changes appears low compared to patients with dystrophic changes. The diagnostic yield may be increased by using antibodies against different regions of laminin α2, and further studies may elucidate some correlation between immunocytochemical changes and the severity of the clinical phenotype.

We are grateful to the Muscular Dystrophy Association of NSW for their generous financial support and Sarah Kim and Michelle Mills for their contribution to the immunocytochemistry and immunoblotting.

1 Dubowitz V. The muscular dystrophies. In: Dubowitz V, ed. *Muscle disorders in childhood*. London: Saunders, 1995:34-133.
2 Dubowitz V. Congenital muscular dystrophy. In: Emery AEH, ed. *Diagnostic criteria for neuromuscular disorders*. The Netherlands: Baarn, 1994:32-4.
3 Dubowitz V. New developments in congenital muscular dystrophy. *Neuromusc Disord* 1996;**6**:228.
4 Helbling-Leclerc A, Zhang X, Topaloglu H, Cruaud C, Tesson F, Weissenbach J, Tome FMS, Schwartz K, Fardeau M, Tryggvason K, Guicheney P. Mutations in the laminin alpha 2-chain gene (LAMA2) cause merosin-deficient congenital muscular dystrophy. *Nat Genet* 1995;**11**:216-18.
5 Philpot J, Sewry C, Pennock J, Dubowitz V. Clinical phenotype in congenital muscular dystrophy: correlation with expression of merosin in skeletal muscle. *Neuromusc Disord* 1995;**5**:301-5.
6 Hayashi YK, Koga R, Tsukahara T, Ishii H, Matsuishi T, Yamashita Y, Nonaka I, Arahata K. Deficiency of laminin alpha 2-chain mRNA in muscle in a patient with merosin-negative congenital muscular dystrophy. *Muscle Nerve* 1995;**18**:1027-30.
7 North KN, Specht L, Sethi RK, Shapiro F, Beggs AH. Congenital muscular dystrophy associated with merosin deficiency. *J Child Neurol* 1996;**11**:291-5.
8 Philpot J, Topaloglu H, Pennock J, Dubowitz V. Familial concordance of brain magnetic resonance imaging changes in congenital muscular dystrophy. *Neuromusc Disord* 1995;**5**:227-31.

9 Vainzof M, Marie SK, Reed UC, Schwartzman JS, Pavanello RM, Passos-Bueno MR, Zatz M. Deficiency of merosin (laminin M or alpha 2) in congenital muscular dystrophy associated with cerebral white matter alterations. *Neuropediatrics* 1995;**26**:293-7.
10 Fardeau M, Tome FM, Helbling-Leclerc A, Evangelista T, Ottolini A, Chevallay M, Barois A, Estournet B, Harpey JP, Faure S, Guicheney P, Hillaire D. Congenital muscular dystrophy with merosin deficiency: clinical, histopathological, immunocytochemical and genetic analysis. *Rev Neurol (Paris)* 1996;**152**:11-19.
11 Reed UC, Marie SK, Vainzof M, Salum PB, Levy JA, Zatz M, Diament A. Congenital muscular dystrophy with cerebral white matter hypodensity. Correlation of clinical features and merosin deficiency. *Brain Dev* 1996;**18**:53-8.
12 Herrmann R, Straub V, Meyer K, Kahn T, Wagner M, Voit T. Congenital muscular dystrophy with laminin alpha 2 chain deficiency: identification of a new intermediate phenotype and correlation of clinical findings to muscle immunohistochemistry. *Eur J Pediatr* 1996;**155**:968-76.
13 Mora M, Moroni I, Uziel G, Di Blasi C, Barresi R, Farina L, Morandi L. Mild clinical phenotype in a 12-year-old boy with partial merosin deficiency and central and peripheral nervous system abnormalities. *Neuromusc Disord* 1996;**6**: 377-81.
14 Naom I, D'Alessandro M, Topaloglu H, Sewry C, Ferlini A, Helbling Leclerc A, Guicheney P, Weissenbach J, Schwartz K, Bushby K, Philpot J, Dubowitz V, Muntoni F. Refinement of the laminin alpha 2 locus to human chromosome 6q2 in severe and mild merosin deficient congenital muscular dystrophy. *J Med Genet* 1997;**34**:99-104.
15 Farina L, Morandi L, Milanesi I, Ciceri E, Mora M, Moroni I, Pantaleoni C, Savoiardo M. Congenital muscular dystrophy with merosin deficiency: MRI findings in five patients. *Neuroradiology* 1998;**40**:807-11.
16 Morandi L, Di Blasi C, Farina L, Sorokin L, Uziel G, Azan G, Pini A, Toscano A, Lanfossi M, Galbiati S, Cornelio F, Mora M. Clinical correlations in 16 patients with total or partial laminin alpha2 deficiency characterized using antibodies against 2 fragments of the protein. *Arch Neurol* 1999;**56**:209-15.
17 Tan E, Topaloglu H, Sewry C, Zorlu Y, Naom I, Erdem S, D'Alessandro M, Muntoni F, Dubowitz V. Late onset muscular dystrophy with cerebral white matter changes due to partial merosin deficiency. *Neuromusc Disord* 1997;7:85-9.
18 Naom I, D'Alessandro M, Sewry CA, Philpot J, Manzur AY, Dubowitz V, Muntoni F. Laminin alpha 2-chain gene mutations in two siblings presenting with limb-girdle muscular dystrophy. *Neuromusc Disord* 1998;**8**:495-501.
19 Dubowitz V. 50th ENMC International Workshop: Congenital Muscular Dystrophy, 28 February to 2 March 1997, Naarden, The Netherlands. *Neuromusc Disord* 1997; 7:539-47.
20 Di Blasi C, Mora M, Pareyson D, Farina L, Sghirlanzoni A, Vignier N, Blasevich F, Cornelio F, Guicheney P, Morandi M. Partial laminin alpha 2 deficiency in a patient with a myopathy resembling inclusion body myositis. *Ann Neurol* 2000;**47**:811-16.
21 Cohn RD, Herrmann R, Sorokin L, Wewer UM, Voit T. Laminin alpha2 chain-deficient congenital muscular dystrophy: variable epitope expression in severe and mild cases. *Neurology* 1998;**51**:94-100.
22 Nissinen M, Helbling Leclerc A, Xu Z, Evangelista T, Topaloglu H, Cruaud C, Weissenbach J, Fardeau M, Tome FMS, Schwartz K, Tryggvason K, Guicheney P. Substitution of a conserved cysteine-996 in a cysteine-rich motif of the laminin alpha2-chain in congenital muscular with partial deficiency of the protein. *Am J Hum Genet* 1996;**58**: 1177-84.
23 Trevisan CP, Martinello F, Ferruzza E, Fanin M, Chevallay M, Tome FMS. Brain alterations in the classical form of congenital muscular dystrophy. Clinical and neuroimaging follow-up of 12 cases and correlation with the expression of merosin in muscle. *Childs Nerv Sys* 1996;**12**:604-10.
24 Allamand V, Sunada Y, Salih MA, Straub V, Ozo CO, Al-Turaiki MH, Akbar M, Kolo T, Colognato H, Zhang X, Sorokin LM, Yurchenco PD, Tryggvason K, Campbell KP. Mild congenital muscular dystrophy in two patients with an internally deleted laminin alpha2-chain. *Hum Mol Genet* 1997;**6**:747-52.
25 Sewry CA, Naom I, D'Alessandro M, Sorokin L, Bruno S, Wilson LA, Dubowitz V, Muntoni F. Variable clinical phenotype in merosin-deficient congenital muscular dystrophy associated with differential immunolabelling of two fragments of the laminin alpha 2 chain. *Neuromusc Disord* 1997;7:169-75.
26 Ter Laak HJ, Leyten QH, Gabreels FJ, Kuppen H, Renier WO, Sengers RC. Laminin-alpha2 (merosin), beta-dystroglycan, alpha-sarcoglycan (adhalin), and dystrophin expression in congenital muscular dystrophies: an immunohistochemical study. *Clin Neurol Neurosurg* 1998; **100**:5-10.
27 Pegoraro E, Marks H, Garcia CA, Crawford T, Mancias P, Connolly AM, Fanin M, Martinello F, Trevisan CP, Angelini C, Stella A, Scavina M, Munk RL, Servidei S, Bonnemann CC, Bertorini T, Ascadi G, Thompson CE, Gagnon D, Hoganson G, Carver V, Zimmerman RA, Hoffman EP. Laminin alpha2 muscular dystrophy: genotype/phenotype studies of 22 patients. *Neurology* 1998;**51**:101-10.
28 Martinello F, Angelini C, Trevisan CP. Congenital muscular dystrophy with partial merosin deficiency and late onset epilepsy. *Eur Neurol* 1998;**40**:37-45.
29 Castro-Gago M. Occidental-type cerebromuscular dystrophy versus congenital muscular dystrophy with merosin deficiency. *Childs Nerv Sys* 1998;**14**:531.
30 Mackay MT, Kornberg AJ, Shield L, Phelan E, Kean MJ, Coleman LT, Dennett X. Congenital muscular dystrophy, white-matter abnormalities, and neuronal migration disorders: the expanding concept. *J Child Neurol* 1998;**13**: 481-7.
31 Mercuri E, Gruter-Andrew J, Philpot J, Sewry C, Counsell S, Henderson S, Jensen A, Naom I, Bydder G, Dubowitz V, Muntoni F. Cognitive abilities in children with congenital muscular dystrophy: correlation with brain MRI and merosin status. *Neuromusc Disord* 1999;**9**:383-7.
32 Pini A, Merlini L, Tome FM, Chevallay M, Gobbi G. Merosin-negative congenital muscular dystrophy, occipital epilepsy with periodic spasms and focal cortical dysplasia. Report of three Italian cases in two families. *Brain Dev* 1996;**18**:316-22.
33 Tsao CY, Mendell JR, Rusin J, Luquette M. Congenital muscular dystrophy with complete laminin-alpha2-deficiency, cortical dysplasia, and cerebral white-matter changes in children. *J Child Neurol* 1998;**13**:253-6.
34 Prelle A, Comi GP, Rigoletto C, Turconi A, Felisari G, Ciscato P, Fortunato F, Messina S, Bresolin N, Mora M, Moggio M, Scarlato G. An atypical case of partial merosin deficiency congenital muscular dystrophy. *J Neurol* 1997;**244**: 391-5.
35 Topaloglu H, Talim B, Vignier N, Helbling-Leclerc AH, Yetuk M, Afsin IE, Caglar M, Kale G, Guicheney P. Merosin-deficient congenital muscular dystrophy with severe mental retardation and normal cranial MRI: a report of two siblings. *Neuromusc Disord* 1998;**8**:169-74.
36 Muntoni F, Taylor J, Sewry CA, Naom I, Dubowitz V. An early onset muscular dystrophy with diaphragmatic involvement, early respiratory failure and secondary alpha 2 laminin deficiency unlinked to the LAMA2 locus on 6q22. *Eur J Paediatr Neurol* 1998;**1**:19-26.
37 van der Knaap MS, Smit LME, Barth PG, Catsman-Berrevoets CE, Brouwer OF, Begeer JH, de Coo IFM, Valk J. Magnetic resonance imaging in classification of congenital muscular dystrophies with brain abnormalities. *Ann Neurol* 1997;**42**:50-9.
38 Philpot J, Cowan F, Pennock J, Sewry C, Dubowitz V, Bydder G, Muntoni F. Merosin-deficient congenital muscular dystrophy: the spectrum of brain involvement on magnetic resonance imaging. *Neuromusc Disord* 1999;**9**:81-5.
39 Mercuri E, Muntoni F, Berardinelli A, Pennock J, Sewry C, Philpot J, Dubowitz V. Somatosensory and visual evoked potentials in congenital muscular dystrophy: correlation with MRI changes and muscle merosin status. *Neuropediatrics* 1995;**26**:3-7.
40 Mercuri E, Dubowitz L, Berardinelli A, Pennock J, Jongmans M, Henderson S, Muntoni F, Sewry C, Philpot J, Dubowitz V. Minor neurological and perceptuo-motor deficits in children with congenital muscular dystrophy: correlation with brain MRI changes. *Neuropediatrics.* 1995; **26**:156-62.
41 Shorer Z, Philpot J, Muntoni F, Sewry C, Dubowitz V. Demyelinating peripheral neuropathy in merosin-deficient congenital muscular dystrophy. *J Child Neurol* 1995;**10**: 472-5.
42 Mercuri E, Pennock J, Goodwin F, Sewry C, Cowan F, Dubowitz L, Dubowitz V, Muntoni F. Sequential study of central and peripheral nervous system involvement in an infant with merosin deficient congenital muscular dystrophy. *Neuromusc Disord* 1996;**6**:425-9.
43 Cil E, Topaloglu H, Caglar M, Ozme S. Left ventricular structure and function by echocardiography in congenital muscular dystrophy. *Brain Dev* 1994;**16**:301-3.
44 Sewry CA, Naom I, D'Alessandro M, Ferlini A, Philpot J, Mercuri E, Dubowitz V, Muntoni F. The protein defect in congenital muscular dystrophy. *Biochem Soc Trans* 1996;**24**: 281S.
45 Dubowitz V. 41st ENMC International Workshop on Congenital Muscular Dystrophy, 8-10 March 1996, Naarden, The Netherlands. *Neuromusc Disord* 1996;**6**:295-304.
46 Spyrou N, Philpot J, Foale R, Camici PG, Muntoni F. Evidence of left ventricular dysfunction in children with merosin-deficient congenital muscular dystrophy. *Am Heart J* 1998;**136**:474-6.
47 Jones KJ, Kim SS, North KN. Abnormalities of dystrophin, the sarcoglycans, and laminin alpha2 in the muscular dystrophies. *J Med Genet* 1998;**35**:379-86.
48 Tome FM, Evangelista T, Leclerc A, Sunada Y, Manole E, Estournet B, Barois A, Campbell KP, Fardeau M. Congenital muscular dystrophy with merosin deficiency. *C R Acad Sci III* 1994;**317**:351-7.
49 Dubowitz V. Proceedings of the 27th ENMC Sponsored Workshop on Congenital Muscular Dystrophy, 22-24 April 1994, The Netherlands. *Neuromusc Disord* 1995;**3**:253-8.
50 Hayashi YK, Ishihara T, Domen K, Hori H, Arahata K. A benign allelic form of laminin alpha 2 chain deficient muscular dystrophy. *Lancet* 1997;**349**:1147.
51 Dubowitz V. 68th ENMC International Workshop (5th International Workshop) on Congenital Muscular Dystrophy, 9-11 April 1999, Naarden, The Netherlands. *Neuromusc Disord* 1999; **9**:446-54.
52 Pegoraro E, Mancias P, Swerdlow SH, Raikow RB, Garcia C, Marks H, Crawford T, Carver V, Di Cianno B, Hoffman EP. Congenital muscular dystrophy with primary laminin alpha2 (merosin) deficiency presenting as inflammatory myopathy. *Ann Neurol* 1996;**40**:782-91.

53 Schuler F, Sorokin L. Expression of laminin isoforms in mouse myogenic cells in vitro and in vivo. *J Cell Sci* 1995; **108**:3795-805.
54 He Y, Jones KJ, Vignier N, Morgan G, Chevallay M, Barois A, Estournet-Mathiaud B, Hori H, Mizuta T, Tomé FMS, North KN, Guicheney P. Congenital muscular dystrophy with primary partial laminin 2 chain deficiency: molecular study. *Neurology* (in press).
55 Hayashi YK, Engvall E, Arikawa Hirasawa E, Goto K, Koga R, Nonaka I, Sugita H, Arahata K. Abnormal localization of laminin subunits in muscular dystrophies. *J Neurol Sci* 1993;**119**:53-64.
56 Haltia M, Leivo I, Somer H, Pihko H, Paetau A, Kivela T, Tarkkanen A, Tome FMS, Engvall E, Santavuori P. Muscle-eye-brain disease: a neuropathological study. *Ann Neurol* 1997;**41**:173-80.
57 Yamashita Y, Ohtaka E, Matsuishi T, Osari S, Kobayashi O, Nonaka I. Merosin-negative non-Fukuyama-type congenital muscular dystrophy: a case report. *Brain Dev* 1996;**18**: 131-4.
58 Sunada Y, Edgar TS, Lotz BP, Rust RS, Campbell KP. Merosin-negative congenital muscular dystrophy associated with extensive brain abnormalities. *Neurology* 1995;**45**: 2084-9.
59 Tachi N, Kamimura S, Ohya K, Chiba S, Sasaki K. Congenital muscular dystrophy with partial deficiency of merosin. *J Neurol Sci* 1997;**151**:25-7.
60 Bushby K, Anderson LV, Pollitt C, Naom I, Muntoni F, Bindoff L. Abnormal merosin in adults. A new form of late onset muscular dystrophy not linked to chromosome 6q2. *Brain* 1998;**121**:581-8.
61 Guicheney P, Vignier N, Zhang X, He Y, Cruaud C, Frey V, Helbling-Leclerc A, Richard P, Estournet B, Merlini L, Topaloglu H, Mora M, Harpey JP, Haenggeli CA, Barois A, Hainque B, Schwartz K, Tome FMS, Fardeau M, Tryggvason K. PCR based mutation screening of the laminin alpha2 chain gene (LAMA2): application to prenatal diagnosis and search for founder effects in congenital muscular dystrophy. *J Med Genet* 1998;**35**:211-17.
62 Brett FM, Loring P, Caesar A, Burke M, Brennan RP, King M, Farrell MA. Immunohistochemical evaluation of merosin deficiency in congenital muscular dystrophies. *Arch Pathol Lab Med* 1998;**122**:69-71.
63 Hillaire D, Leclerc A, Faure S, Topaloglu H, Chiannilkulchai N, Guicheney P, Grinas L, Legos P, Philpot J, Evangelista T, Routon MC, Mayer M, Pellissier JF, Estournet B, Barois A, Hentati F, Feingold N, Beckmann JS, Dubowitz V, Tome FMS, Fardeau M. Localization of merosin-negative congenital muscular dystrophy to chromosome 6q2 by homozygosity mapping. *Hum Mol Genet* 1994;**3**: 1657-61.
64 Tachi N, Ohya K, Chiba S, Matsuo M, Patria SY, Matsumura K. Deficiency of syntrophin, dystroglycan, and merosin in a female infant with a congenital muscular dystrophy phenotype lacking cysteine-rich and C-terminal domains of dystrophin. *Neurology* 1997;**49**:579-83.
65 Taratuto AL, Lubieniecki F, Diaz D, Schultz M, Ruggieri V, Saccoliti M, Dubrovsky A. Merosin-deficient congenital muscular dystrophy associated with abnormal cerebral cortical gyration: an autopsy study. *Neuromusc Disord* 1999;**9**: 86-94.
66 Vajsar J, Chitayat D, Becker LE, Ho M, Ben-Zeev B, Jay V. Severe classical congenital muscular dystrophy and merosin expression. *Clin Genet* 1998;**54**:193-8.
67 Talim B, Kale G, Topaloglu H, Akcoren Z, Caglar M, Gogus S, Elkay M. Clinical and histopathological study of merosin-deficient and merosin-positive congenital muscular dystrophy. *Pediatr Dev Pathol* 2000;**3**:168-76.