Abnormalities of dystrophin, the sarcoglycans, and laminin $\alpha 2$ in the muscular dystrophies

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Abstract

Abnormalities of dystrophin, the sarcoglycans, and laminin $\alpha 2$ are responsible for a subset of the muscular dystrophies. In this study we aim to characterise the nature and frequency of abnormalities of these proteins in an Australian population and to formulate an investigative algorithm to aid in approaching the diagnosis of the muscular dystrophies. To reduce ascertainment bias, biopsies with dystrophic (n=131) and non-dystrophic myopathic (n=71) changes were studied with antibodies to dystrophin, α , β , and γ sarcoglycan, β dystroglycan, and laminin $\alpha 2$, and results were correlated with clinical phenotype. Abnormalities of dystrophin, the sarcoglycans, or laminin $\alpha 2$ were present in 61/131 (47%) dystrophic biopsies and in 0/71 myopathic biopsies, suggesting that immunocytochemical study of dystrophin, the sarcoglycans, and laminin $\alpha 2$ may, in general, be restricted to patients with dystrophic biopsies. Two patients with mutations identified in γ sarcoglycan had abnormal dystrophin (by immunocytochemistry and immunoblot), showing that abnormalities of dystrophin may be a secondary phenomenon. Therefore, biopsies should not be excluded from sarcoglycan analysis on the basis of abnormal dystrophin alone. The diagnostic yield was highest in those with severe, rapidly progressive limb-girdle weakness (92%). Laminin $\alpha 2$ deficiency was identified in 5/131 (4%) patients; 2/5 patients presented after infancy, indicating that abnormalities of laminin $\alpha 2$ are not limited to the congenital muscular dystrophy phenotype. Overall patterns of immunocytochemistry and immunoblotting provided a guide to mutation analysis and, on the basis of this study, we have formulated a diagnostic algorithm to guide the investigation of patients with muscular dystrophy.

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those with later onset limb-girdle weakness, the X linked dystrophinopathies (Duchenne and Becker muscular dystrophy), and the limb-girdle muscular dystrophies (LGMD). The congenital muscular dystrophies (CMD) are traditionally further classified by the presence or absence of central nervous system involvement. The clinical course of those with limb-girdle weakness ranges from the severe phenotype typical of Duchenne muscular dystrophy (DMD), with onset in the first decade and rapid progression to loss of ambulation in the second decade, to milder forms with later onset and slower progression, such as in Becker muscular dystrophy (BMD).¹

The cloning of the DMD/BMD gene at Xp21 and identification of its protein product, dystrophin, led to the isolation of a complex of associated proteins, the sarcolemmal “dystrophin associated proteins” (sarcoglycans, dystroglycans, and syntrophins) and laminin $\alpha 2$ in the extracellular matrix (fig 1).^{2–3} Mutations in the genes encoding α , β , γ , and δ sarcoglycan have recently been described in autosomal recessive limb-girdle muscular dystrophy.^{4–6} Mutations in the LAMA2 gene encoding the laminin $\alpha 2$ chain of merosin have been identified in a subset of “pure” congenital muscular dystrophy without clinical central nervous system involvement, but with characteristic changes in the white matter on cerebral MRI.^{7–9} There has, to date, been no disease identified resulting from primary abnormalities of the dystroglycan or syntrophin complexes, although there is a recent report of a patient with muscular dystrophy and isolated β dystroglycan deficiency.¹⁰ The classification of the muscular dystrophies has recently been restructured according to the primary gene defect.¹¹

Initial studies to determine the frequency of sarcoglycan and laminin $\alpha 2$ abnormalities and the associated phenotypes have been limited by patient selection bias, either at the clinical or histopathological level. For example, the initial search for mutations in components of the sarcoglycan complex was restricted to patients with a severe LGMD phenotype; however, subsequent reports confirm considerable inter- and intrafamilial heterogeneity.^{12–13} Similarly, the search for patients with laminin $\alpha 2$ deficiency focused on patients with “pure” CMD, and although patients with later onset disease resulting from laminin $\alpha 2$ deficiency have been identified, there have been no systematic studies to provide an estimate of frequency.^{9–14–17}

The most comprehensive study to date is that of Duggan *et al*,¹⁸ in which myopathic biopsies from 556 patients (Italian and American) were studied. Patients were excluded if

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The muscular dystrophies are a clinically and genetically heterogeneous group of disorders unified by the presence of “dystrophic” changes on muscle biopsy, defined as increased fibre size variability, increased connective tissue, and the presence of degenerating and regenerating fibres. They are broadly divided on the basis of age of onset and pattern of weakness into those with onset of weakness at birth (congenital muscular dystrophy) and

Figure 2 Results: overview.

IMMUNOBLOTTING

Western blots of biopsied skeletal muscle were prepared as described²⁵ to quantitate dystrophin and laminin $\alpha 2$. The following modifications were made to the published protocol: cryosections ($10 \times 4 \mu\text{m}$) from biopsied skeletal muscle were collected in pre-chilled eppendorf tubes. Samples were vortexed in $100 \mu\text{l}$ of loading buffer (0.125 mol/l Tris- PO_4 , pH 6.8, 5% sodium dodecyl sulphate (SDS), saturated bromophenol blue, 20% glycerol, 0.1 mol/l DTT, 0.02 mol/l EDTA, 3 mg/ml aprotinin, 2.5 mg/ml leupeptin, 1 mg/ml pepstatin, and 40 mg/ml PMSF), heated for five minutes, and stored at -20°C ; 7.5% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gels were used for dystrophin and laminin $\alpha 2$ assay. Twenty-five micrograms of total protein were loaded in each lane. Protein concentrations of muscle lysates were estimated using a colorimetric assay for staining by amido black²⁶ and equal loading was confirmed by staining of the gel with Coomassie blue. After transfer to the nitrocellulose, the blots were incubated with primary antibody for two hours. Primary antibodies were detected using the ECL chemiluminescence western blotting kit (Amersham, Bucks, UK), followed by exposure to Kodak XAR film for periods of 30 seconds to 15 minutes. Dystrophin and laminin $\alpha 2$ assays were designated normal, decreased in size/amount, or negative.

Results

We studied a total of 202 muscle biopsies, 131 with dystrophic changes and 71 with non-dystrophic, non-specific myopathic changes (fig 2). The average age at biopsy of the patients was 13.7 years (range 0-73 years) with a median age of 5 years. To control for artefact in the immunocytochemical studies, we concurrently studied 30 biopsies in which there were no ultrastructural changes identified by light or electron microscopy, and all stains were normal.

LABORATORY DATA

Abnormalities of dystrophin, the sarcoglycans, or laminin $\alpha 2$ were present in 61/131 (47%) dystrophic biopsies and in 0/71 non-dystrophic myopathic biopsies. Primary dystrophinopathy accounted for 49/61 (80%); patients with abnormal dystrophin on immunocytochemical staining were considered to have a primary dystrophinopathy if they had (1) detectable deletion of the dystrophin gene, (2) clear pedigree evidence of X linked inheritance, (3) a mother with raised CK, or (4) complete absence or altered size of dystrophin on immunoblot. The sarcoglycans were secondarily abnormal, with patchy (discontinuous) staining in 87% of patients with a primary dystrophinopathy. In 7/61 (11%) patients, staining with antibodies to dystrophin or the sarcoglycans or both was abnormal or patchy, but did not satisfy the above criteria for a primary dystrophinopathy (table 1). Three of these patients had abnormalities suggestive of a primary sarcoglycanopathy (patients 1-3) with marked reduction or absence of all components of the sarcoglycan complex (fig 3). Dystrophin staining was markedly abnormal in 2/3 patients (patients 1 and 2) by immunocytochemistry, and of normal size but reduced in quantity on immunoblot. In both cases, mutations in γ sarcoglycan have been identified (C Bonnemant, personal communication). Patient 1 is heterozygous for a four base pair deletion in the splice donor site of intron 2 of the γ sarcoglycan gene and has a large scale deletion of the other allele. Patient 2 is homozygous for the same intronic splice site mutation of the γ sarcoglycan gene. No mutation has yet been identified in patient 3. In the remaining cases (patients 4-7) there was patchy

Table 1 Immunocytochemical results: abnormal dystrophin and associated proteins (excluding confirmed primary dystrophinopathy)

Patient No	Patient's current age (sex)	Pattern of weakness/clinical course	Immunofluorescence									Western blot	Diagnosis
			Dystrophin			Sarcoglycans				Laminin α2			
			Dys 1	Dys 2	Dys 3	α-SG	β-SG	γ-SG	β-DG				
1 (CS)	12 (F)	Limb-girdle (S)	++	↓	++	++	--+	-	N	N	↓ amount, N size	γ-SG (mutation detected)	
2 (MD)	9 (F)	Limb-girdle (S)	++	+	++	--+	--+	-	++	N	—	γ-SG (mutation detected)	
3 (EA)	14 (F)	Limb-girdle (S)	N	N	↓	+	-	++	N	N	N	Probably primary sarcoglycanopathy	
4 (SC)	33 (M)	Limb-girdle (M)	↓	N	↓↓	↓	↓	N	N	N	↓ amount, N size	Probably dystrophinopathy	
5 (MG)	21 (M)	Limb-girdle (M)	N	↓	-	↓	N	N	N	N	↓ amount, N size	Probably dystrophinopathy	
6 (ME)	9 (M)	Limb-girdle (S)	-	+	+	++	+	++	N	N	↓ amount, N size	Probably dystrophinopathy/ ?sarcoglycanopathy	
7 (MD)	19 (F)	Limb-girdle (S)	++	N	N	N	N	N	N	N	↓ amount, N size	?Dystrophinopathy/ ?sarcoglycanopathy	

N = normal, - = negative, + and ++ = patchy (discontinuous) staining, ↓ = decreased staining, ↓ amount = decreased amount, N size = normal size, SG = sarcoglycan, β -DG = β -dystroglycan, CMD = congenital muscular dystrophy, (S) = severe limb-girdle weakness with rapid progression, (M) = milder limb-girdle weakness with slower progression.

Figure 3 Immunocytochemistry of biopsied skeletal muscle from patient 1 (B, D, F), a 12 year old girl with primary γ sarcoglycanopathy and severe Duchenne-like muscular dystrophy, compared with a normal control (A, C, E). Sections of the biopsy were stained with antibodies to α sarcoglycan (A, B), β sarcoglycan (C, D), and γ sarcoglycan (E, F). γ sarcoglycan staining was completely absent and α and β sarcoglycan staining was markedly reduced and patchy. (Images examined under fluorescein microscopy at $20\times$ magnification; scale bar represents $100\text{ }\mu\text{m}$.)

or discontinuous staining in dystrophin or sarcoglycans or both. In the absence of a family history suggesting X linked inheritance or detection of the primary gene mutation we were unable to classify these patients definitively.

Laminin $\alpha 2$ was abnormal in 5/131 (4%) dystrophic biopsies. In 3/5 patients (patients 8, 10, 11), laminin $\alpha 2$ was negative on immunoblot consistent with primary laminin $\alpha 2$ deficiency, and two patients (9, 12) had partial deficiency of laminin $\alpha 2$ (table 2). Patient 10, with absence of laminin $\alpha 2$, also had abnormal staining with dystrophin and α sarcoglycan. Laminin $\alpha 2$ staining was normal in all other patients with abnormal dystrophin or sarcoglycan staining.

CLINICAL DATA

Clinical data were available on 110/131 patients with dystrophic muscle biopsies (summarised in fig 4). Progressive limb-girdle weakness was present in 83/110 (75%) patients with dystrophic biopsies; of these, 52/83 (63%) had a severe phenotype (with onset of weakness in the first decade and rapid progression to loss of ambulation before 16 years), and in 31/83 (37%) the phenotype was milder with later onset and slower rate of progression. In 48/52 (92%) patients with the severe phenotype, there were abnormalities of dystrophin or sarcoglycans or both; 44/52 (85%) patients had confirmed primary dystrophinopathy (DMD), 3/52 had likely sarcoglycanopathy, 1/52 had likely dystrophinopathy, in 1/52 we were unable

Table 2 Immunocytochemical and clinical results: patients with abnormal laminin α2

Patient No	Current age (sex)	Laminin a2	Immunofluorescence							Western blot	Clinical course	Cerebral MRI
			Dystrophin		Sarcoglycans							
			Dys 1	Dys 2	Dys 3	α-SG	β-SG	γ-SG				
8 (JD)	6 (F)	—	N	N	N	N	N	N	Negative	CMD	Typical white matter changes	
9 (SM)	3 (F)	↓	N	N	N	N	N	N	↓ amount, N size	CMD, DD ?secondary to ICH	Typical white matter changes	
10 (B-MD)	3 (F)	++	N	++	N	+--+	N	N	Negative	Non-progressive weakness	Typical white matter changes	
11 (BD)	6 (M)	—	N	N	N	N	N	N	Negative	Non-progressive weakness	Typical white matter changes	
12 (LR)	5 (F)	↓	N	ND	ND	N	N	N	↓ amount, N size	CMD, DD, seizures	Patchy white matter changes	

N = normal, - = negative, + and ++ = patchy (discontinuous) staining, ↓ = decreased staining, ND = not done, ↓ amount = decreased amount, N size = normal size, SG = sarcoglycan, β-DG = β-dystroglycan, CMD = congenital muscular dystrophy, DD = developmental delay, ICH = intracranial haemorrhage.

to predict the primary abnormality, and in 3/52 patients all stains were normal. In contrast, only 8/31 (26%) of patients with the milder phenotype had abnormalities of dystrophin or sarcoglycans or both, five of these with definite primary dystrophinopathy (BMD) and two with likely primary dystrophinopathy.

Clinical data of the patients with abnormal sarcoglycans or dystrophin or both (excluding those with confirmed primary dystrophinopathy) are outlined in table 3. The two patients with confirmed primary γ sarcoglycan deficiency (patients 1 and 2) and patient 3 with probable primary sarcoglycanopathy had similar clinical presentations with a severe phenotype. All are female, white Australian, born to unrelated parents, presented between 6 and 9 years of age with progressive limb-girdle weakness, and remain ambulant over short distances only at 12, 9, and 14 years, respectively. CK was raised more than 25 times the upper limit of normal. Intellect, cardiac (ECG and echocardiogram), and respiratory function are normal

to date, and cerebral MRI (performed in patient 1) was normal.

Nineteen of 110 (17%) patients with a dystrophic biopsy (for whom clinical data were available) fulfilled the diagnostic criteria for congenital muscular dystrophy, with presentation of hypotonia, weakness, or contractures within the first 6 months of life, and 1/19 had structural central nervous system involvement.^{9 27} Laminin α2 deficiency was present in 3/19 (16%) patients with CMD (patients 8, 9, 12). Patients 8 and 9 had structurally normal brains on cranial MRI with characteristic white matter changes. Patient 9 has seizures and severe intellectual impairment; however, we believe this was on the basis of intracranial haemorrhage in the neonatal period. A mutation has been identified in one copy of the LAMA2 gene in this patient (P Guicheney, personal communication). Patient 12 has severe intellectual impairment, without eye abnormalities, and on cranial MRI there were patchy white matter changes and irregular

Figure 4 Clinical data: dystrophic biopsies. CNS=central nervous system.

Table 3 Clinical results: abnormal dystrophin and associated proteins (excluding primary dystrophinopathy)

Patient No	Current age (sex)	Pattern of weakness	Clinical course	Age at loss of ambulation	Highest serum CK ($\times$ normal upper limit) (age in y)	Calf hypertrophy	Cardiac involvement	Intellect	Other
1 (CS)	12 (F)	Limb-girdle	Rapid progression	Still ambulant	45 $\times$ (12)	+	–	Normal	Short stature, scoliosis
2 (MD)	9 (F)	Limb-girdle	Rapid progression	Still ambulant	42 $\times$ (8)	–	–	Normal	Asymmetrical weakness
3 (EA)	14 (F)	Limb-girdle	Rapid progression	Still ambulant	25 $\times$ (10)	+	–	Normal	
4 (SC)	33 (M)	Limb-girdle	Slow deterioration	Still ambulant	15 $\times$ (30)	–	–	Normal	Acromegaly
5 (MG)	21 (M)	Limb-girdle	Slow deterioration	Still ambulant	29 $\times$ (17)	–	–	Normal	
6 (ME)	9 (M)	Limb-girdle	Rapid progression	Still ambulant	162 $\times$ (9)	+	–	Normal	
7 (MD)	19 (F)	Limb-girdle	Rapid progression	13 years	140 $\times$ (7)	+	–	Mild learning difficulties	

Figure 5 Investigative algorithm. The first line investigation for a male with limb-girdle weakness and raised CK is dystrophin gene mutation analysis, as this may immediately confirm the diagnosis of primary dystrophinopathy. Muscle biopsy should be performed if this is negative or to gain further prognostic information.

areas of pachygyria and polymicrogyria. Laminin $\alpha 2$ was present but reduced in quantity on immunocytochemistry and immunoblot, raising the possibility of secondary laminin $\alpha 2$ abnormality. These findings are consistent with Fukuyama congenital muscular dystrophy; the distribution of white matter changes does not correlate with those described as “occipital agyria” by van der Knaap *et al.*²⁸ CK was more markedly raised in the CMD patients with abnormal laminin $\alpha 2$ (range 2009–7121 U/l, normal <200 U/l, average 3750 U/l) than in the laminin $\alpha 2$ positive CMD patients (range 22–1710 U/l, average 324 U/l).

A subset of patients (8/110) presented after 6 months of age with non-progressive proximal or generalised weakness, with no abnormality noted in infancy. Two of these patients (patients 10 and 11) had abnormal laminin $\alpha 2$. Both patients have normal intellect with typical white matter changes on cerebral MRI. They presented at 2 and 4 years respectively with delayed motor milestones, lordotic, waddling gait, and use of a Gowers manoeuvre to rise from the floor. There has been no progression in weakness, and in patient 11 there has been some functional improvement; he is now able to rise from the floor without a Gowers

manoeuvre, his gait is almost normal, but his run remains slow and awkward.

Discussion

Abnormalities of dystrophin, the sarcoglycans, or laminin $\alpha 2$ were present in 61/202 (30%) myopathic muscle biopsies; however, abnormalities were only found in those with dystrophic changes. The diagnostic yield was therefore considerably increased when only dystrophic biopsies were considered (61/131, 47%). When further subdivided on the basis of clinical findings, abnormalities of either dystrophin, the sarcoglycans, or laminin $\alpha 2$ were present in 48/52 (92%) patients with severe limb-girdle weakness, compared with 8/31 (26%) patients with a milder phenotype, 3/19 (16%) with congenital muscular dystrophy, and 2/7 (29%) with non-progressive weakness. Our data suggest that sarcoglycanopathy in patients with myopathic biopsies is considerably less common in the Australian population (1.5%, 3/202) than in the American and Italian populations (10%).¹⁸ However, we cannot directly compare the frequency of dystrophin and sarcoglycan abnormalities in our study with those of Duggan *et al.*,¹⁸ as although patients with a wide range of clinical phenotypes and muscle histology were included in their study, patients with dystrophic biopsies were not analysed separately, and patients with abnormal dystrophin were excluded from further sarcoglycan analysis.

Our results do not support the assumption that the diagnosis of a primary dystrophinopathy can be based exclusively on decreased or patchy staining with dystrophin antibodies and abnormalities on immunoblot. Secondary abnormalities of dystrophin staining were observed in three patients with sarcoglycanopathy (patients 1–3) and one patient with laminin $\alpha 2$ deficiency (patient 10), a finding previously noted by Vainzof *et al.*¹² In those patients with proven γ sarcoglycanopathy, dystrophin was abnormal on immunocytochemistry and immunoblot; staining was patchy and discontinuous, and the protein was of normal molecular weight but reduced in quantity in patient 1 and absent in patient 2. In contrast, patient 3 had absent staining with β sarcoglycan, patchy staining with α and γ sarcoglycan, and relatively normal dystrophin staining, a pattern suggesting a primary abnormality of another component of the sarcoglycan complex. The pattern of dystrophin abnormalities found in some primary sarcoglycanopathies is similar to that seen in the milder primary dystrophinopathies (BMD). Duggan *et al.*¹⁸ commented that “because biopsy specimens from patients with dystrophin gene abnormalities reveal secondary deficiencies of the sarcoglycans, testing for sarcoglycan proteins is informative only if dystrophin is normal”, and therefore restricted sarcoglycan analysis to those patients with normal dystrophin staining. This may underestimate the incidence of primary sarcoglycanopathy, as the dystrophin abnormalities may be a secondary finding, and may have considerable genetic counselling implications.

All three patients with proven or probable sarcoglycanopathy had absent or markedly diminished staining with all components of the sarcoglycan complex. The complete absence of a protein on immunocytochemical staining may be used as a guide for determining the primary abnormality by mutation analysis, as suggested by Vainzof *et al.*¹² In patients 1 and 2 with primary γ sarcoglycanopathy, γ sarcoglycan staining was completely absent on immunocytochemistry and α and β sarcoglycan staining was markedly reduced and patchy. Our numbers of patients with proven primary sarcoglycanopathy are small, and it is difficult to make conclusions about genotype-phenotype correlation. However, both of our patients with γ sarcoglycan (patients 1 and 2) had normal intellect and normal cardiac and respiratory function in accordance with the clinical features described by Sewry *et al.*²⁹

The frequency of laminin $\alpha 2$ abnormality in “pure” congenital muscular dystrophy without structural central nervous system involvement was 11% (2/18), higher than in the American (4.2%) and Japanese (5.9%) populations, but not approaching that seen in European studies (46–65%).^{7, 22–24} Two of five patients with abnormal laminin $\alpha 2$ presented after the first year of life with non-progressive generalised weakness; however, these patients may have had mild weakness from birth which was not noted. White matter changes were present on MRI, with a structurally normal brain. In both patients (10 and 11) there was no correlation between the severity of the phenotype and level of reduction of protein.

Conclusion

Immunocytochemical study of dystrophin, the sarcoglycans, and laminin $\alpha 2$ can, in general, be restricted to patients with dystrophic biopsies. However, there has been one patient with a primary sarcoglycanopathy in which the muscle pathology was myopathic rather than dystrophic, showing that there are always exceptions (C Bonnemant, personal communication). The diagnostic yield is highest in patients with a severe, rapidly progressive phenotype. Abnormalities of dystrophin should not be used to exclude patients from sarcoglycan analysis without confirmation of primary dystrophinopathy, as dystrophin abnormalities may be secondary. Immunocytochemistry and immunoblotting patterns can be used to guide mutation analysis, particularly in the sarcoglycanopathies. At this early stage of defining the characteristics of the sarcoglycanopathies, it is recommended that all available sarcoglycan antibodies are used in screening (α and β sarcoglycan, which are currently commercially available, γ and δ sarcoglycan). On the basis of this study, we have formulated an investigative algorithm to aid in approaching the diagnosis of abnormalities of dystrophin, the sarcoglycans, and laminin $\alpha 2$, particularly to determine the indications for immunocytochemical studies, immunoblotting, and gene mutation analysis (fig 5).

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- 1 Dubowitz V. The muscular dystrophies. In: Dubowitz V, ed. *Muscle disorders in childhood*. London: W B Saunders, 1995:34-133.
- 2 Koenig M, Hoffman EP, Bertelson CJ, et al. Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organisation of the DMD gene in normal and affected individuals. *Cell* 1987;50:509-17.
- 3 Campbell KP. Three muscular dystrophies: loss of cytoskeleton-extracellular matrix linkage. *Cell* 1995;80:675-9.
- 4 Roberds SL, Leturcq F, Allamand V, et al. Missense mutations in the adhalin gene linked to autosomal recessive muscular dystrophy. *Cell* 1994;78:625-33.
- 5 Noguchi S, McNally EM, Ben Othmane K, et al. Mutations in the dystrophin-associated protein gamma-sarcoglycan in chromosome 13 muscular dystrophy (see comments). *Science* 1995;270:819-22.
- 6 Bonnemann CG, Modi R, Noguchi S, et al. Beta-sarcoglycan (A3b) mutations cause autosomal recessive muscular dystrophy with loss of the sarcoglycan complex. *Nat Genet* 1995;11:266-73.
- 7 Tome FM, Evangelista T, Leclerc A, et al. Congenital muscular dystrophy with merosin deficiency. *C R Acad Sci III* 1994;317:351-7.
- 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 Dubowitz V. 41st ENMC International workshop on congenital muscular dystrophy, 8-10 March 1996, Naarden, The Netherlands. *Neuromusc Disord* 1996;6:295-304.
- 10 Salih MAM, Sunada Y, Al-Nasser M, et al. Muscular dystrophy associated with beta-dystroglycan deficiency. *Ann Neurol* 1996;40:925-8.
- 11 Dubowitz V. Neuromuscular disorders: gene location. *Neuromusc Disord* 1996;6:I-X.
- 12 Vainzof M, Passos-Bueno MR, Canovas ES, et al. The sarcoglycan complex in the six autosomal recessive limb-girdle muscular dystrophies. *Hum Mol Genet* 1996;5:1963-9.
- 13 Morandi L, Barresi R, Di Blasi C, et al. Clinical heterogeneity of adhalin deficiency. *Ann Neurol* 1996;39:196-202.
- 14 Naom I, D'Alessandro M, Topaloglu H, et al. Refinement of the laminin alpha 2 locus to human chromosome 6q2 in severe and mild merosin deficient congenital muscular dystrophy. *Neuromusc Disord* 1997;34:99-104A.
- 15 Tan E, Topaloglu H, Sewry C, et al. Late onset muscular dystrophy with cerebral white matter changes due to partial merosin deficiency. *Neuromusc Disord* 1997;7:85-9.
- 16 Mora M, Moroni I, Uziel G, et al. 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.
- 17 Herrmann R, Straub V, Meyer K, et al. 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.
- 18 Duggan DJ, Gorospe JR, Fanin M, Hoffman EP, Angelini C. Mutations in the sarcoglycan genes in patients with myopathy. *N Engl J Med* 1997;336:618-24.
- 19 Vainzof M, Pavanello RM, Pavanello-Filho I, et al. Screening of male patients with autosomal recessive Duchenne dystrophy through dystrophin and DNA studies. *Hum Mol Genet* 1991;39:38-41.
- 20 Stec I, Kress W, Meng G, Muller B, Grimm T. Estimate of severe autosomal recessive limb-girdle muscular dystrophy (LGMD2C, LGMD2D) among sporadic muscular dystrophy males: a study of 415 families. *J Med Genet* 1995;32:930-3.
- 21 Hayashi YK, Mizuno Y, Yoshida M, et al. The frequency of patients with 50 kDa dystrophin associated glycoprotein (50DAG or adhalin) deficiency in a muscular dystrophy patient population in Japan: immunocytochemical analysis of 50DAG, 43DAG, dystrophin, and utrophin. *Neurology* 1995;45:551-4.
- 22 North KN, Beggs AH. Deficiency of a skeletal muscle isoform of alpha actinin (alpha actinin 3) in merosin positive congenital muscular dystrophy. *Neuromusc Disord* 1996;6:229-35.
- 23 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.
- 24 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.
- 25 Hoffman EP, Fischbeck KH, Brown RH, et al. Characterisation of dystrophin in muscle biopsy specimens from patients with Duchenne or Becker muscular dystrophy. *N Engl J Med* 1988;318:1363-8.
- 26 Nakamura K, Tanaka T, Kuwahara A, Takeo K. Microassay for proteins on nitrocellulose filter using protein dye-staining procedure. *Anal Biochem* 1985;148:311-19.
- 27 Dubowitz V. Congenital muscular dystrophy. In: Emery AEH, ed. *Diagnostic criteria for neuromuscular disorders*. Baarn, The Netherlands: European Neuromuscular Centre, 1994:32-4.
- 28 van der Knaap MS, Smit LME, Barth PG, et al. Magnetic resonance imaging in classification of congenital muscular dystrophies with brain abnormalities. *Ann Neurol* 1997;42:50-9.
- 29 Sewry C, Taylot J, Anderson LVB, et al. Abnormalities in alpha, beta, and gamma sarcoglycan in patients with limb-girdle muscular dystrophy. *Neuromusc Disord* 1996;6:467-74.