Abnormalities of dystrophin, the sarcoglycans, and laminin α2 in the muscular dystrophies

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Abstract
Abnormalities of dystrophin, the sarcoglycans, and laminin α2 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 α2, and results were correlated with clinical phenotype. Abnormalities of dystrophin, the sarcoglycans, or laminin α2 were present in 61/131 (47%) dystrophic biopsies and in 0/71 myopathic biopsies, suggesting that immunocytochemical study of dystrophin, the sarcoglycans, and laminin α2 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 normal dystrophin alone. The diagnostic yield was highest in those with severe, rapidly progressive limb-girdle weakness (92%). Laminin α2 deficiency was identified in 5/131 (4%) patients; 2/5 patients presented after infancy, indicating that abnormalities of laminin α2 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.

Keywords: muscular dystrophy; dystrophin; sarcoglycan; laminin α2

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 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). 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 α2 in the extracellular matrix (fig 1). Mutations in the genes encoding α, β, γ, and δ sarcoglycan have recently been described in autosomal recessive limb-girdle muscular dystrophy. Mutations in the LAMA2 gene encoding the laminin α2 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. 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. The classification of the muscular dystrophies has recently been restructured according to the primary gene defect.

Initial studies to determine the frequency of sarcoglycan and laminin α2 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. Similarly, the search for patients with laminin α2 deficiency focused on patients with “pure” CMD, and although patients with later onset disease resulting from laminin α2 deficiency have been identified, there have been no systematic studies to provide an estimate of frequency.

The most comprehensive study to date is that of Duggan et al, in which myopathic biopsies from 556 patients (Italian and American) were studied. Patients were excluded if
dystrophin was abnormal (on the basis of immunocytochemistry and immunoblot), and laminin α2 was not studied. Ten percent (55/556) had abnormal immunocytochemical staining with antibodies to a sarcoglycan and, of these, 29/50 (58%) had detectable mutations of the sarcoglycan genes (α-SG (17), β-SG (8), γ-SG(4)). Vainzof et al. and Stec et al. estimated that among sporadic male patients with severe LGMD, 8-12% and 11.8% respectively carry autosomal rather than X chromosome mutations. Hayashi et al. found 5/208 (2%) Japanese muscular dystrophy patients had deficiency of α sarcoglycan with normal dystrophin. The reported frequency of laminin α2 deficiency among patients with “pure” congenital muscular dystrophy varies; American and Japanese studies found 1/12 (4.2%) and 2/34 (5.9%) patients respectively were laminin α2 deficient, whereas in the French and British experience, 13/20 (65%) and 11/24 (46%) respectively were laminin α2 negative.

In this study, we aim to characterise the nature and frequency of abnormalities of dystrophin, the dystrophin associated proteins, and laminin α2 in an Australian population. To address some of the limitations of previous studies, particularly related to ascertainment bias, we studied all available dystrophic muscle biopsies, as well as those with non-dystrophic myopathic changes. We have not limited study of the sarcoglycans to those biopsies with normal dystrophin. In studying patients with a wide range of biopsy findings and clinical phenotypes, we aim to formulate an investigative algorithm to aid in approaching the pathological diagnosis of abnormalities of dystrophin and associated proteins, and to determine the indications for immunocytochemical studies, immunoblotting, and gene mutation analysis.

**Patients and methods**

**PATIENTS**

Patients were ascertained retrospectively and prospectively through archived muscle biopsy material (1979-1996) at the University of Sydney, Sydney Children’s Hospital, and Royal Alexandra Hospital for Children, major reference laboratories in the state of New South Wales for muscle disease. There were no limitations with respect to the age of the patient at biopsy. Biopsies were divided into two groups: (1) “dystrophic” biopsies (as defined above) and (2) biopsies with non-dystrophic myopathic changes. Biopsies were excluded if there was an alternative clinical diagnosis (for example, myofascial dystrophy or facioscapulohumeral dystrophy), or the muscle pathology was strongly suggestive of an alternative diagnosis (for example, neuropathy, necrotising or mitochondrial myopathy, or specific ultrastructural changes such as central cores). Clinical data were collected from medical records, supplemented by clinical assessment where possible.

**ANTIBODIES**

The following antibodies were used in this study: monoclonal dystrophin (NCL-DYS1 [1:1], NCL-DYS2[1:2], NCL-DYS3[1:1], Novocastra) for immunocytochemistry, polyclonal antibody to the rod domain of dystrophin (DYS6-10[1:1000]) provided by C Bonneman and L Kunkel for immunoblotting, monoclonal α sarcoglycan (NCL-50DAG[1:50] Novocastra), affinity purified β and γ sarcoglycan (BSG[1:50], γ-SG[1:1000], β-DG[1:100]) provided by C Bonneman and L Kunkel), and laminin α2 (merosin) antibodies (MAB1922[1:2000] Chemicon).

**IMMUNOCYTOCHEMISTRY**

All muscle biopsies were frozen in isopentane, pre-chilled with liquid nitrogen. Indirect immunofluorescence staining was applied to cryostat sections of 8 µm. Sections were incubated with primary antibodies in phosphate buffered saline (PBS) with 10% fetal calf serum for two hours at room temperature and washed with PBS. Secondary antibodies, FITC conjugated or Cy3 conjugated (Jackson ImmunoResearch Laboratories Inc, Westgrove, PA), were applied and incubated for 30 minutes at room temperature followed by three washes with PBS. Sections were mounted and sealed with Immumount (Shandon, Pittsburgh, PA) and examined and photographed with a Leica DMRBE fluorescence microscope. Staining was graded as negative (-), patchy (+ + +), decreased but continuous (-), or normal (N). Photographs were taken with Ektachrome P1600 film under identical conditions with the same exposure time and developed at 800 ASA.
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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 α2 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 Bonnemann, 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)

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Patient's current age (sex)</th>
<th>Pattern of weakness/clinical course</th>
<th>Immunofluorescence</th>
<th>Western blot</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dystrophin</td>
<td>Sarco-glycans</td>
<td>Laminin α2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dyst 1  Dyst 2  Dyst 3</td>
<td>α-SG  β-SG  γ-SG  β-DG</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1(CS)</td>
<td>12 (F)</td>
<td>Limb-girdle (S)</td>
<td>++    ±    ±</td>
<td>++  --  --  N  N</td>
<td></td>
</tr>
<tr>
<td>2(MD)</td>
<td>9 (F)</td>
<td>Limb-girdle (S)</td>
<td>++    ±    ±</td>
<td>++  --  --  N  N</td>
<td></td>
</tr>
<tr>
<td>3(EA)</td>
<td>14 (F)</td>
<td>Limb-girdle (S)</td>
<td>N    N    N</td>
<td>{}     {}    {}</td>
<td></td>
</tr>
<tr>
<td>4(SC)</td>
<td>33 (M)</td>
<td>Limb-girdle (M)</td>
<td>{}    {}    {}</td>
<td>{}     {}    {}</td>
<td></td>
</tr>
<tr>
<td>5(MG)</td>
<td>21(M)</td>
<td>Limb-girdle (M)</td>
<td>N    N    N</td>
<td>{}     {}    {}</td>
<td></td>
</tr>
<tr>
<td>6(ME)</td>
<td>9 (M)</td>
<td>Limb-girdle (S)</td>
<td>+    +    +</td>
<td>++  ++  ++  N  N</td>
<td></td>
</tr>
<tr>
<td>7(MD)</td>
<td>19 (F)</td>
<td>Limb-girdle (S)</td>
<td>++    N    N</td>
<td>N    N    N    N</td>
<td></td>
</tr>
</tbody>
</table>

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.

IMMUNOBLOTTING

Western blots of biopsied skeletal muscle were prepared as described to quantitate dystrophin and laminin α2. The following modifications were made to the published protocol: cryosections (10 × 4 μm) from biopsied skeletal muscle were collected in pre-chilled eppendorf tubes. Samples were vortexed in 100 μl of loading buffer (0.125 mol/l Tris-PO4, 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 α2 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 black 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 α2 assays were designated normal, decreased in size/amount, or negative.

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.
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 α2 was abnormal in 5/131 (4%) dystrophic biopsies. In 3/5 patients (patients 8, 10, 11), laminin α2 was negative on immunoblot consistent with primary laminin α2 deficiency, and two patients (9, 12) had partial deficiency of laminin α2 (table 2). Patient 10, with absence of laminin α2, also had abnormal staining with dystrophin and α sarcoglycan. Laminin α2 staining was normal in all other patients with abnormal dystrophin or sarcoglycan staining.

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
Abnormalities of dystrophin, the sarcoglycans, and laminin a2

Table 2 Immunocytochemical and clinical results: patients with abnormal laminin a2

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Current age (sex)</th>
<th>Laminin a2</th>
<th>Dystrophin</th>
<th>Sar coglycans</th>
<th>Western blot</th>
<th>Clinical course</th>
<th>Cerebral MRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 (JD)</td>
<td>6 (F)</td>
<td>—</td>
<td>N N N N N N</td>
<td>N N</td>
<td>Negative</td>
<td>CMD</td>
<td>Typical white matter changes</td>
</tr>
<tr>
<td>9 (SM)</td>
<td>3 (F)</td>
<td>↓</td>
<td>N N N N N N</td>
<td>++</td>
<td>Negative</td>
<td>CMD, DD secondary to ICH</td>
<td>Typical white matter changes</td>
</tr>
<tr>
<td>10 (R-MD)</td>
<td>3 (F)</td>
<td>++</td>
<td>N N N N N N</td>
<td>++</td>
<td>Negative</td>
<td>CMD, DD, seizures</td>
<td>Typical white matter changes</td>
</tr>
<tr>
<td>11 (BD)</td>
<td>6 (M)</td>
<td>—</td>
<td>N N N N N N</td>
<td>Non-progressive weakness</td>
<td>Typical white matter changes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 (LR)</td>
<td>5 (F)</td>
<td>↓</td>
<td>N N N N N N</td>
<td>Positive</td>
<td>CMD, DD, seizures</td>
<td>Patchy white matter changes</td>
<td></td>
</tr>
</tbody>
</table>

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.

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.

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. Intelect, 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.27 Laminin a2 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)

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Current age (sex)</th>
<th>Pattern of weakness</th>
<th>Clinical course</th>
<th>Age at loss of ambulation</th>
<th>Highest serum CK (&lt; normal upper limit) (age in y)</th>
<th>Calf hypertrophy</th>
<th>Cardiac involvement</th>
<th>Intellect</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (CS)</td>
<td>12 (F)</td>
<td>Limb-girdle</td>
<td>Rapid progression</td>
<td>Still ambulant</td>
<td>45x (12)</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
<td>Short stature, scoliosis</td>
</tr>
<tr>
<td>2 (MD)</td>
<td>9 (F)</td>
<td>Limb-girdle</td>
<td>Rapid progression</td>
<td>Still ambulant</td>
<td>42x (8)</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
<td>Asymmetrical weakness</td>
</tr>
<tr>
<td>3 (EA)</td>
<td>14 (F)</td>
<td>Limb-girdle</td>
<td>Rapid progression</td>
<td>Still ambulant</td>
<td>25x (10)</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
<td>Acromegaly</td>
</tr>
<tr>
<td>4 (SC)</td>
<td>33 (M)</td>
<td>Limb-girdle</td>
<td>Slow deterioration</td>
<td>Still ambulant</td>
<td>15x (30)</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>5 (MG)</td>
<td>21 (M)</td>
<td>Limb-girdle</td>
<td>Slow deterioration</td>
<td>Still ambulant</td>
<td>29x (17)</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>6 (ME)</td>
<td>9 (M)</td>
<td>Limb-girdle</td>
<td>Rapid progression</td>
<td>Still ambulant</td>
<td>162x (9)</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>7 (MD)</td>
<td>19 (F)</td>
<td>Limb-girdle</td>
<td>Rapid progression</td>
<td>13 years</td>
<td>140x (7)</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
<td></td>
</tr>
</tbody>
</table>

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.

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 α2. 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
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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. 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.

The frequency of laminin a2 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%). Two of five patients with abnormal laminin a2 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 a2 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. Bonnemann, 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 a2, particularly to determine the indications for immunocytochemical studies, immunoblotting, and gene mutation analysis (fig 5).

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