Relative frequency of congenital muscular dystrophy subtypes: Analysis of the UK diagnostic service 2001–2008

E.M. Clement a, L. Feng a, R. Mein b, C.A. Sewry a, S.A. Robb a, A.Y. Manzur a, E. Mercuri a,c, C. Godfrey a, T. Cullup b, S. Abbs b, F. Muntoni a,*

a Dubowitz Neuromuscular Centre, Institute of Child Health and Great Ormond Street Hospital, London WC1N 1EH, United Kingdom
b DNA Laboratory, GSTS Pathology, Genetics Centre, Guy’s Hospital, London, United Kingdom
c Department of Child Neurology, Catholic University, Rome, Italy

Received 29 November 2011; accepted 26 January 2012

Abstract

The Dubowitz Neuromuscular Centre is the UK National Commissioning Group referral centre for congenital muscular dystrophy (CMD). This retrospective review reports the diagnostic outcome of 214 UK patients referred to the centre for assessment of ‘possible CMD’ between 2001 and 2008 with a view to commenting on the variety of disorders seen and the relative frequency of CMD subtypes in this patient population. A genetic diagnosis was reached in 53 of 116 patients fulfilling a strict criteria for the diagnosis of CMD. Within this group the most common diagnoses were collagen VI related disorders (19%), dystroglycanopathy (12%) and merosin deficient congenital muscular dystrophy (10%). Among the patients referred as ‘possible CMD’ that did not meet our inclusion criteria, congenital myopathies and congenital myasthenic syndromes were the most common diagnoses. In this large study on CMD the diagnostic outcomes compared favourably with other CMD population studies, indicating the importance of an integrated clinical and pathological assessment of this group of patients.

© 2012 Elsevier B.V. All rights reserved.

Keywords: Congenital muscular dystrophy; Epidemiology; Differential diagnosis

1. Introduction

The Dubowitz Neuromuscular Centre was commissioned in 2001 by the UK Department of Health National Commissioning Group (NCG) to provide a national comprehensive service for the assessment, investigation and management of patients affected by congenital muscular dystrophy (CMD). In 2005, the service expanded to include the congenital myopathies. The NCG service can be accessed by the referring clinical team at various levels, including clinical review of patient, assessment of muscle biopsy or molecular genetic analysis and is freely available to UK patients.

CMDs are a heterogeneous group of disorders. They most commonly present with muscle weakness and hypotonia in the first months of life; contractures are common and serum creatine kinase elevated in several but not all subtypes. Other clinical features include muscle wasting or hypertrophy and abnormalities of eye, skin and brain. Respiratory and cardiac involvement is also common.

There are at least 13 genetically distinct forms of CMD reported, for summary of known variants see Table 1 supplementary information. In addition there is a further group of as yet undefined patients in whom all known forms can be excluded. A proportion of these cases is affected by CMD subtypes that are clinically and pathologically indistinguishable from genetically defined forms, while others, despite their clinical and/or pathological similarities to CMD, clearly belong to a different group of conditions. Typical examples of the latter are myotonic
dystrophy, some congenital myopathy variants and congenital myasthenic syndromes [1–3].

Skeletal muscle biopsy is an important part of the diagnostic workup of children with suspected CMD. Muscle biopsy findings in affected individuals range from mild myopathic changes to overtly dystrophic, depending on the muscle biopsied and the age at biopsy. The extent of dystrophic changes necessary to qualify a muscle biopsy to be considered “dystrophic” is also subject to debate. For the purpose of this report, dystrophic change is defined as muscle with fibrosis and necrosis. If necrosis is not observed then muscle fibre regeneration must be present [4].

Merosin (laminin2) deficient congenital muscular dystrophy type 1A (MDC1A) was the first CMD pathologically defined due to a characteristic deficiency of the laminin α2 chain of merosin on skeletal muscle biopsy; this also became the first CMD variant for which the genetic defect, mutations in laminin α2 (LAMA2), was found. The availability of a straightforward pathological diagnosis and genetic testing, led to the rapid proliferation of manuscripts reporting the clinical features and relative frequency of this condition in several populations [5–7]. The collagen VI related disorders, Ullrich congenital muscular dystrophy (UCMD) and its milder allelic variant Bethlem myopathy (BM), represent another significant proportion of CMD cases. Although many cases of BM present after the first 2 years of life, with some remaining asymptomatic in adulthood, early or congenital onset cases are well documented and for this reason have been included here. The biochemical and molecular diagnosis of collagen VI related disorders is more complicated due to the subtle reduction of protein expression in many UCMD patients and the lack of clear pathological markers on muscle biopsy for most BM cases. However improved detection techniques such as double immunofluorescence of collagen VI and proteins such as perlecan and collagen IV on muscle biopsy, or immunohistochemical studies on cultured fibroblasts followed by the genetic screening of the three collagen 6A chains, have led to the appreciation that collagen VI defects are one of the most common CMD subtypes [8–10]. Muscle imaging also plays an important role in the diagnosis of the collagen VI related conditions [11]. A third subgroup of patients are grouped together in the so called ‘dystroglycanopathy’ variant, identified by defective glycosylation of alpha dystroglycan in skeletal muscle, and appears to be a relatively frequent CMD subtype [12]. The spectrum of clinical severity observed in the dystroglycanopathies is extremely variable, ranging from early lethal conditions with associated structural brain and eye malformations such as walker warburg syndrome (WWS), to much milder variants only affecting muscle and overlapping in clinical severity with limb girdle muscular dystrophies (for a review see [12]). Intermediate variants exist, and include muscle eye brain disease (MEB), Fukuyama CMD (FCMD), MDC1D and MDC1C. Separate conditions are the deficiency of α7 integrin [13] and CMD caused by mutations in LMNA, the gene more commonly involved in Emery Dreifuss muscular dystrophy (EDMD2) [14]. Rigid spine muscular dystrophy (RSMD1) is a CMD caused by mutations in SEPN1. Mutations in the same gene also cause other pathological phenotypes including congenital fibre type disproportion, multiminicore disease and desmin-related myopathy with mallory body-like inclusions. The phenotypes and pathology in this group of conditions show considerable overlap, however the fact that pathological changes in axial muscle are invariably dystrophic supports this condition being a dystrophic rather than myopathic variant [15].

Few studies have looked at the incidence of CMD but from the reports available it has been estimated at between $4.7 \times 10^{-5}$ live births in the North of Italy with a point prevalence of $2.5 \times 10^{-5}$ in Western Sweden [16,17]. The prevalence of different subtypes of CMD varies in different populations, often due to founder mutations. MDC1A has historically been thought of as the most common form of CMD with estimates suggesting that it accounts for between 30% and 50% of cases [1,18]. In Japan however, Fukuyama CMD is the most common form of CMD reported but has rarely been seen outside this population [19,20].

In this study we review the diagnostic outcome of 214 UK patients clinically assessed in our department between 2001 and 2008 with a view to describing the frequency of the various disorders encountered in our patient population.

2. Materials and methods

The UK referrals to the NCG Dubowitz Neuromuscular Centre between April 2001 and January 2008 as ‘possible CMD’ were retrospectively reviewed. Ethical approval was obtained from the Hammersmith Hospital and Queen Charlotte Ethics Committee. Only patients who had been clinically assessed by us at the centre and from whom a muscle biopsy was available for the diagnostic studies were included in this report. Patients where biopsy sample was unavailable or insufficient for analysis, or cases who had not been assessed in person at the centre, but in whom a diagnostic muscle or DNA sample had been forwarded for diagnosis have been excluded. Where more than one member of a family was seen, only one member of the family has been included for the purpose of analysis (the patient with the most information available).

Clinical notes, genetic tests and muscle biopsy reports were reviewed for all patients fulfilling the criteria. Diagnostic and biopsy information was gathered firstly for all referrals, which however consisted of a considerable number of patients not affected by CMD and then subsequently for patients who fulfilled the tighter inclusion criteria for CMD. This included: (1) Dystrophic, myopathic or minimal change muscle biopsy, with evidence of regeneration. Patients were excluded from this subgroup analysis if biopsy showed additional structural change (e.g. rods) suggesting an alternative diagnosis. Patients with cores were included, as long as RYR1 mutations had been excluded,
as cores have been reported in patients with RSMD1 and also more rarely also EDMD2 and UCMD (Sewry and Muntoni, personal observation). (2) Presentation before 2 years of age with hypotonia, weakness, contractures, delayed motor milestones, raised CK or characteristic eye or brain abnormality. (3) Non-CMD diagnoses (or patients in whom alternative diagnosis is strongly suspected) excluded.

Diagnostic standards for diagnosis were as for Dubowitz and Sewry (CMD and congenital myopathy) [4], Godfrey et al. (dystroglycanopathy) [12], Geremmayeh et al. (MDC1A) [21], Nadeau et al. (collagen VI) [22] and Kinali et al. (congenital myasthenia) [23]. All biopsy samples from patients referred with suspected CMD underwent a panel of routine investigation including histological, histochemical and immunohistochemical analysis except for those where sample size limited testing. For further information on standard departmental testing procedure see www.ich.ucl.ac.uk/gosh/clinicalservices/neuromuscular_services.

Genetic testing was directed by biopsy and clinical findings and often supported by muscle magnetic resonance imaging (MRI) [24]. Molecular genetic analysis of CMD and congenital myopathy genes was provided by Guys and St Thomas’ Trust, London (part of CMD NCG service), the NCG Referral Centre for LGMD at the Institute of Human Genetics at Newcastle University and National Commissioning Group funded Oxford Congenital Myasthenic Syndrome Service. Key references for the listed diseases and their diagnostic workup can be found at: www.musclegenetable.org/.

3. Results

3.1. Part 1: total cohort analysis ‘all referrals’

Muscle biopsy samples were received from 415 patients referred with ‘possible CMD’ during the study period. Of these, 218 patients were clinically assessed at our centre. Four siblings were excluded, leaving 214 patients included in subsequent analysis, with an average age at biopsy assessment of 6.2 years (range 1 day–37.2 years).

The referrals represent a very mixed group in terms of ethnicity reflecting the London and wider UK population. Patients were referred from throughout the UK with 74/214 referred from the Greater London area.

All 214 patients were assessed to see if they conformed to stricter CMD inclusion criteria including; (1) Dystrophic, myopathic or minimal change muscle biopsy, with evidence of regeneration. (2) Presentation before 2 years of age with hypotonia, weakness, contractures, delayed motor milestones, raised CK or characteristic eye or brain abnormality. (3) Non-CMD diagnoses excluded.

One hundred and sixteen-patients fulfilled all three criteria. Average age at biopsy assessment was 6.4 years (range 8 days–28.4 years).

A molecular diagnosis was reached in 53 of 116 (46%) patients and another five patients (4%) could be unequivocally assigned to a pathological subgroup (4%), see Table 1. The most common CMD diagnosis made was collagen VI related disorders (21%; genetically confirmed in 22/116 and pathologically confirmed in a further two patients) followed by dystroglycanopathy (15%; genetically confirmed in 14/116 and pathologically confirmed in a further three patients). The relative frequency of the different CMDs is shown in Fig. 1.

Of 57/116 patients in the CMD subgroup with clearly dystrophic biopsies, a molecular diagnosis was made in 43 (75%) and a further three patients could be unequivocally assigned to a pathological subgroup (data not shown).

Ninety-eight of the 214 patients referred for assessment of ‘possible CMD’ did not meet our strict CMD inclusion criteria. In this group a genetic diagnosis was made in 30/98 and a pathological diagnosis was made in a further 17

---

Table 1

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>CMD subgroup</th>
<th>Number</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic (n = 53)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen VI related</td>
<td></td>
<td>22</td>
<td>Collagen VI</td>
</tr>
<tr>
<td>(BM = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(UCMD = 17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dystroglycanopathy</td>
<td></td>
<td>14</td>
<td>POMT1 = 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>POMT2 = 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>POMGNT1 = 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FKRP = 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LARGE = 1</td>
</tr>
<tr>
<td>EDMD2</td>
<td></td>
<td>4</td>
<td>LMNA = 4</td>
</tr>
<tr>
<td>MDC1A</td>
<td></td>
<td>12</td>
<td>LAMA2 = 12</td>
</tr>
<tr>
<td>RSMD1</td>
<td></td>
<td>1</td>
<td>SEPNI = 1</td>
</tr>
<tr>
<td>Pathological (n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen VI related</td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Dystroglycanopathy</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Unknown (n = 58)</td>
<td></td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>116</td>
<td></td>
</tr>
</tbody>
</table>

BM, Bethlem myopathy; UCMD, Ullrich congenital muscular dystrophy; EDMD2, autosomal dominant Emery–Dreifuss muscular dystrophy; MDC1A, merosin deficient CMD; RSMD1, rigid spine muscular dystrophy.

Fig. 1. Graph showing distribution of diagnoses (n = 58) in the ‘CMD subgroup’. MDC1A, merosin deficient CMD; EDMD2, autosomal dominant Emery–Dreifuss muscular dystrophy; RSMD1, rigid spine muscular dystrophy.
patients, see Tables 2a and 2b. Congenital myopathy (24/98 either genetically or pathologically confirmed), congenital myasthenia (8/98) and limb girdle muscular dystrophy (6/98) were the most common diagnoses.

There were a further 23/214 patients in whom a clinical diagnosis is strongly suspected but a definitive genetic or pathological diagnosis could not be reached.

4. Discussion

We present here the diagnostic outcome in a large cohort of UK patients referred to the CMD NCG centre for clinical review and muscle biopsy analysis between 2001 and 2008.

Of those patients fulfilling the diagnostic criteria for CMD, a diagnosis of CMD was made in 50% (58/116) and was genetically confirmed in 53 of 116 (46%). Within this group, collagen VI related myopathy was the most common diagnosis with 24/116 (21%), followed by dystroglycanopathies with 17/116 (15%). The most common dystroglycanopathy genes found to be mutated were POMGNT1 and FKRP, with four affected cases each. A diagnosis of MDC1A was made in 12/116 (10%), EDMD2 in 4/116 (3%) and RSMD1 in 1/116 (1%). Two further genetically proven cases of RSMD1 were excluded from this ‘CMD’ subgroup analysis as they presented after 2 years of age. We found no cases of integrin a7 deficiency in our population. A final diagnosis could not be determined in 58 cases (50%). It should be noted that we documented a further five cases of collagen VI related disorders confirmed from analysis of skin biopsy alone in patients not assessed at our centre and therefore not included in this report, reinforcing this as the most prevalent CMD subtype in our referral population.

Our results contrast those of a recent Australian study by Peat et al. where a specific histopathological diagnosis could be allocated in 49% of 101 CMD cases and a definitive genetic diagnosis could be made in 24% [25]. It should be noted that patients with biopsies containing cores were excluded in their study therefore direct comparison with our data should be done with care. Even taking this into account, our definitive (genetic) diagnosis rate is almost twice as high (46% v 24%) when comparing our data to the Australian CMD cohort. The reasons for the differences in the diagnostic pick up rate are most likely a reflection of the fact that all of the patients in our study were clinically assessed in the specialised diagnostic service. The pathology department has a longstanding tradition of interpretation of protein abnormalities in muscular dystrophies and routinely uses a very extensive panel of antibodies to characterise not only the primary protein deficiency but also secondary protein changes which can be very helpful. Furthermore many patients also had additional supportive investigations including analysis of collagen VI in fibroblasts and muscle MRI imaging to help direct genetic investigations. We have previously reported
that muscle MRI is a particularly useful diagnostic adjunct in investigating muscular dystrophies with a high specificity and sensitivity for different subtypes of CMD [24]. We have found MRI to be especially useful in the case of collagen VI related disorders where we have found it to be a more reliable indicator of collagen VI pathology than fibroblast analysis. Indeed abnormal production of collagen VI in fibroblast cultures is a sensitive indicator but not specific for collagen VI abnormality (Muntoni, unpublished data).

In the Australian study, dystroglycanopathy was found to be the most common group representing 25% of cases, with collagen VI abnormalities found in 12% [25]. Studies from Japan reveal FCMD to be the most prevalent form of CMD in the Japanese population (49.2% of CMD cases in one series) but this is due to a FKTN founder mutation. The second most common form of CMD encountered in the Japanese population is collagen VI deficiency with an estimated frequency of 7.2% in their CMD cohort. Interestingly, a 2009 paper from the North of England [26] found MDC1A to be the most prevalent CMD disorder (0.6/100,000) and much more common than dystroglycanopathies (0.03/100,000), UCMD (0.13/100,000) and RSMD1 (0.13/100,000). However, mutations in FKRP are more frequently seen in that LGMD cohort (0.43/100,000). In addition, BM has an estimated prevalence of 0.77/100,000 in their series but as there is no information about the age of presentation of these patients, comparison between the collagen VI related disorders in that study and ours is of limited value.

Analysis of our data suggests that MDC1A, in various series of patients reported to be the most common form of CMD, is not the most frequent diagnosis in our population, as both collagen VI related disorders and dystroglycanopathy are more prevalent. This may be a reflection of improved diagnostic assays and greater awareness of the clinical and pathological presentation of other CMD forms; we cannot exclude regional variation in the prevalence of CMD subtypes.

It is also of interest to note the large number of non CMD diagnoses (47/214, including two patients with RSMD1 presenting after 2 years of age) made in the patients referred with ‘possible CMD’, reflecting the importance of an integrated approach to the diagnosis of these rare and heterogeneous conditions, which includes clinical examination, pathological diagnosis and muscle imaging directing the genetic testing.

These results also confirm our previous observations of conditions resembling CMD, which include several congenital myopathies and congenital myasthenias which may show significant clinical and some pathological overlap with CMD [3]. Indeed, this is the reason why the NCG service in London has expanded to include assessment and diagnostic testing for congenital myopathy cases.

Despite our integrated approach, we were unable to reach a diagnosis in half (58/116) of the CMD subgroup. These undiagnosed patients are a varied group. Whilst the diagnosis cannot be confirmed, approximately 20% have a collagen VI-like phenotype. This suggests either that current collagen VI diagnostic methods are not sensitive enough or that similar disorders exist but have yet to be genetically and pathologically refined. A further two patients probably have a ‘syndromic’ cause of their problems with others having suspected Marinosco-Sjögren syndrome and motor neuronopathy.

The remainder of these cases are likely to comprise a number who have an as yet undefined form of CMD or congenital myopathy caused by mutations in a novel gene and also some patients with a defined form of CMD in whom we are unable to confirm this pathologically or genetically. Although the sensitivity of genetic testing has steadily improved, large heterozygous deletions or duplications are likely to be missed using present techniques. In some patients variants of unknown significance will have been detected that cannot be proven to be pathogenic using current methods. Some of the undiagnosed patients will eventually ‘reveal’ their diagnosis as their symptomatology and histopathology evolves. For example, recent studies highlight the mild initial pathological features in UCMD [27], and the selectivity of muscle involvement which characterises many of these conditions [24]. In others, a diagnosis may be made as a result of new technology, in particular the emergence of the hybridisation arrays for assessing copy number variations in DNA of affected individuals.

With regard to the clinical presentation of the CMDs; although MDC1A is usually seen as a classical presentation of severe early weakness, hypotonia and later characteristic features on brain MRI, the collagen VI related disorders and dystroglycanopathies are frequently more challenging to diagnose due to their often milder phenotypes at presentation. In both groups and also in EDMD2 and RSMD1, the classification lines between CMD, myopathy and LGMD are frequently blurred.

The geographically diverse nature of our referral population unfortunately precludes any formal estimation of prevalence or incidence for the different CMD subtypes, none the less, the information gathered here we feel is an accurate reflection of clinical activity in the CMD NCG service. It is of interest to note that over 50% of our patients were referred from outside greater London highlighting the nationwide uptake of services.

In summary, the data presented in this study represents the largest series of UK CMD referral data and diagnostic outcome reported. It reveals collagen VI related disorders to be the most common form of CMD in our patient group, accounting for 21% of referrals that fulfil the CMD inclusion criteria. Patients with dystroglycanopathy follow closely behind accounting for 15% with MDC1A seen in 10%. There remain a large proportion of cases (50%) that fulfil the clinical and pathological criteria for CMD, in whom no diagnosis can be made. This suggests that further CMD subtypes and genes are yet to be discovered.
Acknowledgements

The authors wish to thank the Muscular Dystrophy Campaign (MDC) for the centre and Research Grants (RA3/734 and PO0916). E.C. is a MDC clinical research fellow. The support of the National Commissioning Group to the Dubowitz Neuromuscular Centre is also gratefully acknowledged. F.M. is supported by the Great Ormond Street Children’s Charity.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2012.01.010.

References

[10] Pepe G, Bertini E, Bonaldo P, et al.. Bethlem myopathy (BETHLEM) and Ullrich scierotonic muscular dystrophy 100th ENMC interna-