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Congenital muscular dystrophies in the UK population: Clinical and molecular spectrum of a large cohort diagnosed over a 12-year period

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Abstract

Congenital muscular dystrophies (CMDs) are clinically and genetically heterogeneous conditions; some fatal in the first few years of life and with central nervous system involvement, whereas others present a milder course. We provide a comprehensive report of the relative frequency and clinical and genetic spectrum of CMD in the UK. Genetic analysis of CMD genes in the UK is centralised in London and Newcastle. Between 2001 and 2013, a genetically confirmed diagnosis of CMD was obtained for 249 unrelated individuals referred to these services. The most common CMD subtype was laminin-α2 related CMD (also known as MDC1A, 37.4%), followed by dystroglycanopathies (26.5%), Ullrich-CMD (15.7%), *SEPN1* (11.65%) and *LMNA* (8.8%) gene related CMDs. The most common dystroglycanopathy phenotype was muscle–eye–brain-like disease. Fifteen patients carried mutations in the recently discovered *ISPD*, *GMPPB* and *B3GALNT2* genes. Pathogenic allelic mutations in one of the CMD genes were also found in 169 unrelated patients with milder phenotypes, such as limb girdle muscular dystrophy and Bethlem myopathy. In all, we identified 362 mutations, 160 of which were novel. Our results provide one of the most comprehensive reports on genetics and clinical features of CMD subtypes and should help diagnosis and counselling of families with this group of conditions.

Keywords: Congenital muscular dystrophy; Prevalence; Dystroglycanopathies; *ISPD*; *GMPPB*; *B3GALTN2*; *COL6A1-A2-A3*; *LAMA2*; *SEPN1*; *LMNA*

1. Introduction

Congenital muscular dystrophies (CMD) are a highly heterogeneous group of conditions clinically characterised by muscle weakness with onset at birth or shortly after, and variable involvement of eyes, heart and central nervous system [1]. Some forms of CMD can be fatal in the first years of life, whereas others have a milder course and survival into adulthood is possible [2]. Following the initial classification of CMD based on clinical features and country of origin, it was soon recognised that different CMDs show significant clinical overlap and marked genetic heterogeneity. In the past few years, the improved understanding of the molecular mechanisms underlying these diseases has allowed a genetic classification based on the function of the involved protein [3,4]. Although CMD have been associated with mutations in more than 20 genes, the number of clinically and histologically proven CMD cases without a genetic diagnosis is not entirely clear, but it is considered to be significant [5]. Only during the last few years, with the advent of full exome and genome sequencing, several new genes have been described, including *ISPD*, *GMPPB* and *B3GALNT2*, encoding for proteins involved in the glycosylation of alpha-dystroglycan (α-DG) [6–8]. It is also now recognised that allelic mutations in genes responsible for CMD can cause

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milder phenotypes and in particular limb girdle muscular dystrophies (LGMD) [9–11]. An accurate genetic diagnosis in CMD is of paramount importance not only for improved phenotype–genotype correlation, but also to facilitate genetic and prenatal counselling and to inform prognosis and aspects of management, as well as to facilitate clinical trials and possible future treatments specific for an individual genetic subtype or mutation.

Information on the incidence and prevalence of CMD in various populations is limited. Point prevalence of CMD in Northern England was calculated as 0.9×100.000 [12] while a recent study on the Italian population showed slightly lower figures at 0.563×100.000 [13]. Relative frequency of individual types seems to differ among populations often because of founder mutations [14,15]. For example, in the Japanese population the most common type is Fukuyama CMD, due to a founder insertion of a 3-kb retrotransposition element in the 3′ untranslated region of fukutin (*FKTN*) gene [15], while this mutation is virtually absent outside Japan.

In the UK, there is the Highly Specialised Services (HSS) commission for the diagnosis and management of rare neuromuscular diseases, with the Dubowitz Neuromuscular Centre (DNC) providing a comprehensive clinical, histopathological and molecular service for diagnosis of CMD. In the UK genetic analysis of CMD genes is offered at the DNC only, with the exception of the *FKRP* and *LMNA* gene, also tested at the HSS for LGMD in Newcastle in view of the most common and milder phenotypes (LGMD2I and AD-EDMD, respectively).

We have previously reported the relative frequency of CMD among children specifically referred for a clinical opinion to the DNC Centre in London who had been assessed by the multidisciplinary team [16]. A genetic diagnosis was reached in 53 patients (46%); the most common diagnosis in this selected population was collagen VI disorders (19%), followed by dystroglycanopathies (12%) and laminin-α2 related CMD (MDC1A) (10%). However our previous work did not take into account patients with a clinical or pathological diagnosis of CMD who were referred to the DNC for genetic testing only and as such it likely represented a subpopulation of patients in which the local centres required support from a clinical and pathological perspective.

In the present study we have extended our previous observation and report the result of genetic screening of CMD genes in all the UK patients referred to the two HSS diagnostic laboratories between 2001 and 2013, with the main aim of investigating frequency and relative prevalence of CMD subtypes in the UK population. We also summarise observed genotypes and novel mutations identified in the CMD genes.

2. Patients and methods

2.1. Patients

Between 2001 and 2013, 3734 DNA samples were sent to the DNC and the HSS in Newcastle for genetic testing of CMD genes. Among these, 1042 samples were referred to the DNC for sequencing of the 14 CMD genes offered for analysis until 2014 (*LAMA2*, *POMT1*, *POMT2*, *POMGnT1*, *FKRP*, *FKTN*, *LARGE*, *ISPD*, *GMPPB*, *B3GALNT2*, *COL6A1*, *COL6A2*, *COL6A3* and *SEPN1*), while further 2692 samples were sent to the HSS in Newcastle for analysis of the *LMNA* and *FKRP* genes only. These figures also include samples of patients with milder allelic phenotypes, such as LGMD and Bethlem myopathy (BM). All patients were UK resident at time of referral for genetic testing.

Written informed consent for diagnostic genetic testing was obtained from patients and/or parents prior to DNA collection by referring clinicians. Genetic testing was gate kept by clinical members of the two services based on relevant medical history, clinical features and clinical investigations (including serum CK values, EMG, muscle biopsy analysis, brain and/or muscle magnetic resonance imaging) provided in clinical referral forms. Clinical information was collected retrospectively from the clinical referral forms only for patients in whom pathogenic mutation/s were identified. Patients were divided into two clinical subgroups: (a) CMD and (b) milder phenotype. Standard diagnostic criteria were used to define CMD [4,5]. Of note, increased creatine kinase (CK) levels were not considered an inclusion criterion as not all CMD have raised CK levels. Patients who did not fulfil the diagnostic criteria for CMD but who had a clear dystrophic muscle pathology phenotype were included in the milder phenotype subgroup. We excluded possible overlap at the two HSS centres by cross checking patients referred for *FKRP* testing.

The spectrum of clinical severity for dystroglycanopathy patients was described using the classification proposed by Godfrey et al.: MEB/FCMD = Muscle–Eye–Brain/Fukuyama Congenital Muscular Dystrophy Like; CMD-MR = Congenital Muscular Dystrophy with Mental Retardation; CMD-NOMR = Congenital Muscular Dystrophy with No Mental Retardation; CMD-CRB = Congenital Muscular Dystrophy with cerebellar Involvement; LGMD-MR = Limb Girdle Muscular Dystrophy with Mental Retardation; LGMD-NOMR = Limb Girdle Muscular Dystrophy with No Mental Retardation [17].

2.2. Molecular analysis

Molecular genetic analysis of the *LAMA2*, *POMT1*, *POMT2*, *POMGnT1*, *FKRP*, *FKTN*, *LARGE*, *ISPD*, *GMPPB, B3GALNT2*, *COL6A1*, *COL6A2*, *COL6A3* and *SEPN1* genes was performed at the Viapath Molecular Laboratory at Guys and St Thomas' Trust, London, as part of Highly Specialised Service (HSS) for CMD, while the HSS in Newcastle performed analysis of the *FKRP* and *LMNA* genes. Sequencing of gene/s by a candidate gene approach was guided by phenotypic features, brain and muscle MRI and muscle biopsy review. In case of suspicion of dystroglycanopathy, DNAs underwent analysis of the following 9 α-DG genes (*POMT1*, *POMT2*, *POMGnT1*, *FKRP*, *FKTN*, *LARGE*, *ISPD*, *GMPPB*, *B3GALNT2)*.

The entire coding regions and flanking intronic regions of each gene were sequenced from peripheral genomic DNA. Mutation nomenclature was based on the reference sequence, with nucleotide number 1 corresponding to the first base of the translation initiation codon. To investigate whether novel changes