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Clinical and genetic characterization of manifesting carriers of *DMD* mutations

Payam Soltanzadeh [a], Michael J. Friez [e], Diane Dunn [a], Andrew von Niederhausern [a], Olga L. Gurvich [a,1], Kathryn J. Swoboda [b,c], Jacinda B. Sampson [b], Alan Pestronk [f], Anne M. Connolly [f], Julaine M. Florence [f], Richard S. Finkel [g], Carsten G. Bönnemann [g], Livija Medne [g], Jerry R. Mendell [h], Katherine D. Mathews [i], Brenda L. Wong [j], Michael D. Sussman [k], Jonathan Zonana [l], Karen Kovak [l], Sidney M. Gospe Jr. [m], Eduard Gappmaier [d], Laura E. Taylor [h], Michael T. Howard [a], Robert B. Weiss [a], Kevin M. Flanigan [h,*]

[a] *Department of Human Genetics, University of Utah, Salt Lake City, UT, USA*
[b] *Department of Neurology, University of Utah, Salt Lake City, UT, USA*
[c] *Department of Pediatrics, University of Utah, Salt Lake City, UT, USA*
[d] *Department of Physical Therapy, University of Utah, Salt Lake City, UT, USA*
[e] *Molecular Diagnostic Laboratory, Greenwood Genetic Center, Greenwood, SC, USA*
[f] *Department of Neurology, Washington University, St. Louis, MO, USA*
[g] *Department of Neurology, Children's Hospital of Philadelphia, Philadelphia, PA, USA*
[h] *Center for Gene Therapy, Nationwide Children's Hospital, Department of Pediatrics, Ohio State University, Columbus, OH, USA*
[i] *Department of Pediatrics, University of Iowa, Iowa City, IA, USA*
[j] *Departments of Neurology and Pediatrics, Cincinnati Children's Hospital, Cincinnati, OH, USA*
[k] *Department of Orthopedic Surgery, Shriners Hospital for Children, Portland, OR, USA*
[l] *Department of Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR, USA*
[m] *Departments of Neurology and Pediatrics, University of Washington, Division of Pediatric Neurology, Seattle Children's Hospital, Seattle, WA, USA*

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ABSTRACT

Manifesting carriers of *DMD* gene mutations may present diagnostic challenges, particularly in the absence of a family history of dystrophinopathy. We review the clinical and genetic features in 15 manifesting carriers identified among 860 subjects within the United Dystrophinopathy Project, a large clinical dystrophinopathy cohort whose members undergo comprehensive *DMD* mutation analysis. We defined manifesting carriers as females with significant weakness, excluding those with only myalgias/cramps. DNA extracted from peripheral blood was used to study X-chromosome inactivation patterns. Among these manifesting carriers, age at symptom onset ranged from 2 to 47 years. Seven had no family history and eight had male relatives with Duchenne muscular dystrophy (DMD). Clinical severity among the manifesting carriers varied from a DMD-like progression to a very mild Becker muscular dystrophy-like phenotype. Eight had exonic deletions or duplications and six had point mutations. One patient had two mutations (an exonic deletion and a splice site mutation), consistent with a heterozygous compound state. The X-chromosome inactivation pattern was skewed toward non-random in four out of seven informative deletions or duplications but was random in all cases with nonsense mutations. We present the results of *DMD* mutation analysis in this manifesting carrier cohort, including the first example of a presumably compound heterozygous *DMD* mutation. Our results demonstrate that improved molecular diagnostic methods facilitate the identification of *DMD* mutations in manifesting carriers, and confirm the heterogeneity of mutational mechanisms as well as the wide spectrum of phenotypes.

1. Introduction

Mutations in the *DMD* gene, which encodes dystrophin, result in Duchenne muscular dystrophy (DMD), the milder Becker muscular dystrophy (BMD), or X-linked dilated cardiomyopathy (XLDC). Most heterozygous female carriers of *DMD* mutations are asymptomatic; however, between 2.5% and 7.8% of these carriers are manifesting carriers (MCs) who develop symptoms ranging from mild muscle weakness to a rapidly progressive DMD-like muscular dystrophy [1–3]. Dystrophin labeling of muscle biopsies from MCs shows a mosaic pattern, with some fibers having continuous membrane immunostaining, other fibers uniformly unstained, and some fibers with discontinuous or partial dystrophin staining; these abnormalities are often seen in a non-uniform distribution, with groups of normal and abnormal fibers [4].

* Corresponding author. Address: Center for Gene Therapy, Nationwide Children's Hospital, 700 Children's Drive, WA4023, Columbus, OH 43205, USA. Tel.: +1 614 355 2947; fax: +1 614 722 3273.
E-mail address: kevin.flanigan@nationwidechildrens.org (K.M. Flanigan).
[1] Present address: Cork Cancer Research Centre, BioSciences Institute, University College Cork, Cork, Ireland.

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The diagnosis of a dystrophinopathy carrier state may be considered on clinical grounds alone in the setting of a clear X-linked family history of muscular dystrophy. However, mutational analysis of the *DMD* gene is typically required for diagnosis, particularly in the absence of a family history. Use of modern molecular diagnostic methods facilitates the identification of MCs without a positive family history of dystrophinopathy (“isolated” MCs), who may account for around 10% of women with hyperCKemia and a myopathic muscle biopsy [5], and some of whom may have in the past been diagnosed as having autosomal recessive limb-girdle muscular dystrophy or unknown myopathy [6].

Here we describe a series of genetically confirmed MCs identified from subjects enrolled in the United Dystrophinopathy Project (UDP). Our data expand the mutational spectrum associated with manifesting carriers (including the first example of a presumably compound heterozygous *DMD* mutation in an isolated MC), demonstrate that skewed XCI is present in 38% of cases, and highlight the need for a high index of clinical suspicion among neuromuscular clinicians.

2. Methods

2.1. Patient selection/clinical data ascertainment

We reviewed all records in the United Dystrophinopathy Project (UDP) database to identify only subjects with an identified mutation in the *DMD* gene. The UDP is a seven-center consortium to prospectively study the mutational mechanisms, clinical features and genotype/phenotype relationships in dystrophinopathies. Enrollment requires a clinical history compatible with dystrophinopathy, and at least one of the following criteria: an X-linked family history of muscular dystrophy; a muscle biopsy that shows absent or altered expression of dystrophin by immunohistochemical, immunofluorescent, or immunoblot analysis; or a previous mutation analysis report showing a *DMD* mutation. All females were enrolled in order to obtain mutation analysis because they were symptomatic. Details regarding the phenotypic evaluation are included in Supplemental Table 1.

We defined patients as MCs if they had muscle weakness in at least one muscle group by manual muscle testing, or dilated cardiomyopathy. Our definition of MCs is similar to previously published criteria, although in contrast to previous studies we did not use dynamometry results in isolation to establish inclusion criteria [7,8]. Asymptomatic carriers or carriers with daily muscle cramps/myalgias but no muscle weakness were excluded from our analysis. The severity of phenotype was first categorized according to clinical course. We designated subjects as “DMD-like” if the clinical progression was similar to that of DMD in males. Subjects who began using a wheelchair by age 30 were designated “severe BMD-like” and those still walking independently after 30 years of age were grouped as “mild BMD-like”. If the phenotype was milder than DMD but the patient was too young to be assigned to either mild or severe categories, only the term “BMD-like” was used. In addition to designating the clinical course, we defined the current degree of muscle weakness, independent of patient age, at the most recent examination. We relied on functional descriptions (e.g., wheelchair use) or the description of expert clinicians to label the current degree of weakness as “mild”, “moderate”, or “severe”, as noted in Table 1.

2.2. Mutation analysis

Under an Institutional Review Board approved protocol, DNA extracted from peripheral blood underwent mutational analysis using a combination of either single condition amplification/internal primer (SCAIP) direct sequencing analysis, MLPA analysis, or both, using conditions as previously described [9]. MLPA was performed using Salsa MLPA kit (MRC-Holland, Inc., Amsterdam). For selected patients, archived muscle specimens were obtained, and messenger RNA was extracted for reverse-transcription PCR and sequencing of the *DMD* cDNA using published primers [10]. Nucleotide positions were determined according to the standard *DMD* reference sequence (GenBank Accession No. NM_004006).

2.3. X-chromosome inactivation (XCI) analysis

Methylation of the highly polymorphic HpaII restriction endonuclease site in the androgen-receptor (*AR*) locus correlates with XCI. We used HpaII digestion followed by PCR to determine the methylation status of both the maternal and paternal X chromosomes in lymphocyte-derived DNA [11,12]. In this method, parental alleles are distinguished based on the difference between the numbers of CpGs in the CpG island within the *AR* locus. Alleles that are active will be digested while the inactive alleles are not. The ratio of undigested parental alleles gives the pattern of inactivation. If the assay does not differentiate the maternal and paternal alleles, the result is reported as “uninformative”. As per published criteria, XCI ratios of less than or equal to 80:20 were considered “random” pattern, ratios greater than 80:20 but less than or equal to 90:10 were considered “moderately skewed” pattern and ratios greater than 90:10 were considered “highly skewed” pattern [13].

3. Results

We identified 15 manifesting carrier females among 860 UDP subjects with an identified *DMD* mutation. Clinical and genetic data for each MC subject are shown in Table 1. Eight patients had a relative with the diagnosis of DMD and seven had no family history of dystrophinopathy (isolated MCs). Age at onset of symptoms varied from 2 to 47 years (median: 8; mean: 14.9). Muscle weakness was the most common presenting symptom (reported in 80% of the cases), followed by myalgia and/or muscle cramps (reported in 60%). One subject (#8) had a phenotype as severe as a typical DMD boy, while other patients showed a mild to severe BMD-like phenotype.

Clinically notable asymmetry in muscle weakness was seen in three subjects in whom there were at least two muscle groups with left–right differences of at least 2 grades in mMRC scale: subject #4, in which left versus right differences for knee extensors, shoulder abductors, shoulder external rotators, elbow flexors, elbow extensors, and thumb abductors were $\geqslant 2$ grades in the mMRC scale; subject #11, who showed $\geqslant 2$ mMRC grade differences in her elbow flexors and wrist flexors; and subject #14, with $\geqslant 2$ mMRC grade difference in hip flexion and ankle dorsiflexion. Based on mean intra-individual right–left difference in mMRC scores for each muscle group, muscle weakness in the lower extremity tends to be more symmetric than in the upper extremity (Supplemental Table 1).

Dilated cardiomyopathy was seen in five subjects, two with severe BMD-like phenotypes (subjects #4 and #6) and three with mild BMD-like symptoms (#5, #12, and #14). One severe BMD-like subject (#4) had a rapid decline in cardiac function over one year, with an ejection fraction that dropped from 56% at the age of 28 to 41% at 29 years old (with a fractional shortening of 13%). One mild BMD-like subject (#12) had a postpartum cardiomyopathy that improved with treatment over 18 months (with a rise in ejection fraction from 45% to 65%). A sixth subject (#11; mild BMD-like) had cardiac symptoms but not a dilated cardiomyopathy: she had syncopal episodes due to a drug-resistant bradytachycardia syndrome and required a cardiac pacemaker implantation at the