



HAL
open science

UMD-DYSF, a novel locus specific database for the compilation and interactive analysis of mutations in the dysferlin gene

Gaëlle Blandin, Christophe Bérout, Veronique Labelle, Karine Nguyen, Nicolas Wein, Dalil Hamroun, Brad Williams, Nilah Monnier, Laura E. Rufibach, Jon Andoni Urtizbera, et al.

► **To cite this version:**

Gaëlle Blandin, Christophe Bérout, Veronique Labelle, Karine Nguyen, Nicolas Wein, et al.. UMD-DYSF, a novel locus specific database for the compilation and interactive analysis of mutations in the dysferlin gene. *Human Mutation*, 2012, 33 (3), pp.E2317-E2331. 10.1002/humu.22015 . hal-01610025

HAL Id: hal-01610025

<https://hal.science/hal-01610025v1>

Submitted on 18 Dec 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

UMD-DYSF, A Novel Locus Specific Database for the Compilation and Interactive Analysis of Mutations in the Dysferlin Gene

Gaëlle Blandin^{1,2}, Christophe Beroud³, Veronique Labelle⁴, Karine Nguyen⁴, Nicolas Wein^{1,2}, Dalil Hamroun³, Brad Williams⁵, Nilah Monnier⁵, Laura E. Rufibach⁵, Jon Andoni Urtizberea⁶, Pierre Cau^{1,2,4}, Marc Bartoli^{1,2}, Nicolas Lévy^{1,2,4}, and Martin Krahn^{1,2,4*}.

1 Aix-Marseille Univ, UMR 910, Faculté de Médecine Timone, 13385, Marseille, France; 2 Inserm, UMR 910, 13385, Marseille, France; 3 Université Montpellier 1, CHU de Montpellier and Inserm U827, 34000, Montpellier, France; 4 APHM, Hôpital d'Enfants de la Timone, Département de Génétique Médicale et de Biologie Cellulaire, 13385, Marseille, France; 5 Jain Foundation, Bellevue, WA, 98005, USA; 6 APHP, Hôpital Marin, 64700, Hendaye, France

ABSTRACT: Mutations in the dysferlin gene (*DYSF*) lead to a complete or partial absence of the dysferlin protein in skeletal muscles and are at the origin of dysferlinopathies, a heterogeneous group of rare autosomal recessive inherited neuromuscular disorders. As a step towards a better understanding of the *DYSF* mutational spectrum, and towards possible inclusion of patients in future therapeutic clinical trials, we set up the Universal Mutation Database for Dysferlin (UMD-DYSF), a Locus-Specific Database developed with the UMD® software. The main objective of UMD-DYSF is to provide an updated compilation of mutational data and relevant interactive tools for the analysis of *DYSF* sequence variants, for diagnostic and research purposes. In particular, specific algorithms can facilitate the interpretation of newly identified intronic, missense- or isosemantic-exonic sequence variants, a problem encountered recurrently during genetic diagnosis in dysferlinopathies. UMD-DYSF v1.0 is freely accessible at www.umd.be/DYSF/. It contains a total of 742 mutational entries corresponding to 266 different disease-causing mutations identified in 558 patients worldwide diagnosed with dysferlinopathy. This article presents for the first time a comprehensive analysis of the dysferlin mutational spectrum based on all compiled *DYSF* disease-causing mutations reported in the literature to date, and using the main bioinformatics tools offered in UMD-DYSF. ©2011 Wiley-Liss, Inc.

KEY WORDS: LSDB, UMD, dysferlin, *DYSF*, LGMD, muscle

INTRODUCTION

In 1998, the groups of Robert H. Brown Jr. (Liu, et al., 1998) and Kate Bushby (Bashir, et al., 1998) identified the genetic cause of the autosomal recessive muscle-wasting diseases Miyoshi myopathy (MIM# 254130), and Limb Girdle Muscular Dystrophy type 2B (LGMD2B; MIM# 253601) as resulting from mutations in a novel gene on chromosome 2p13. The encoded protein was named dysferlin (*DYSF*; MIM# 603009), relating to its involvement in muscular dystrophy, and homology with the *C. elegans* fer-1 protein. Using membrane repair assays on muscle fibers from dysferlin-deficient mouse models, the groups of Paul McNeil/Kevin Campbell (Bansal, et al., 2003) and Robert H. Brown Jr. (Lennon, et al., 2003) subsequently demonstrated a central role for dysferlin in sarcolemmal repair after membrane injury. This established Miyoshi myopathy and LGMD2B as the first entities of a new subgroup of muscular dystrophies, due to defective membrane-repair.

From a clinical point of view, numerous reports corroborated the implication of mutated dysferlin in muscular dystrophy, and in particular in a high proportion of LGMD. In the past ten years, regular mutational analysis has allowed for better characterization of the phenotypic manifestations associated with deleterious mutations in the *DYSF* gene. The main clinical presentations are the distal-onset muscular dystrophy called Miyoshi myopathy and the proximal-onset form LGMD2B, both characterized by progressive muscle weakness, usually appearing in the second decade, and highly elevated serum creatine kinase (CK) levels. Progressively, the description of different phenotypes caused by *DYSF* mutations (Illa, et al., 2001; Klinge, et al., 2008; Nguyen, et al., 2005; Nguyen, et al., 2007; Okahashi, et al., 2008; Paradas, et al., 2009; Seror, et al., 2008; Spuler, et al., 2008; Ueyama, et al., 2002; Wenzel, et al., 2007), in addition to the “typical” LGMD and Miyoshi phenotypes, unraveled a wide spectrum of phenotypes, ranging from clinically asymptomatic, isolated hyperCKemia to severe and early onset presentations (Bushby, 2000; Laval and Bushby, 2004; Urtizberea, et al., 2008). This wide range of clinical presentations is collectively referred to as dysferlinopathies.

DYSF was initially shown to be expressed in the skeletal and cardiac muscle tissues (Bashir, et al., 1998; Liu, et al., 1998), in monocytes (Ho, et al., 2002), as well as in a variety of tissues, including liver, lung, kidney, pancreas, brain, and placenta (Bashir, et al., 1998; Liu, et al., 1998). More recent studies have isolated 14 isoforms that are differentially expressed among tissues. These isoforms originate from the differential use of promoters and alternative exons which have been identified, respectively *DYSF* (AF075575) (Foxton, et al., 2004) and *DYSF_v1* (Pramono, et al., 2006) promoters, and alternative exons 5a and 40a (Pramono, et al., 2009). Isoform 8, which contains the 55 canonical exons transcribed via the *DYSF* promoter, constitutes the major *DYSF* transcript (73%) among all reported isoforms expressed in skeletal muscle, but is not expressed in monocytes where isoform 13 (NM_001130980; containing exon 5a) represents the main dysferlin messenger (44%) (Pramono, et al., 2009). The other isoforms are less expressed in skeletal muscle and blood. In addition to the canonical messenger composed of 55 exons, splice variants lacking exon 17 are expressed at early stages of myogenic cell differentiation and also constitute predominant dysferlin transcripts in mature peripheral nerve tissue (Salani, et al., 2004). To date, the functional role of the different messengers remains unknown.

Due to the large size of the *DYSF* gene, which spans a genomic locus of approximately 233k bp, mutation screening is challenging on a routine clinical basis. Mutational analysis of *DYSF* is further complicated by the large mutational spectrum (detailed in this article), and a high proportion of “private” mutations, which leaves molecular geneticists with the recurrent difficulty of interpreting novel *DYSF* sequence variants, in particular putative splicing and missense variants.

Until now, the Leiden Open (source) Variation Database established in 1998 for dysferlin (LOVD Dysferlin), has been the unique Locus Specific Database serving as a public repository for human dysferlin variants (www.lovd.nl/DYSF). Our laboratory, as many others, widely uses this valuable resource. On November, 18, 2011, LOVD Dysferlin consisted of 424 unique sequence variants, including published or directly submitted (unpublished) variants, being either disease-causing mutations (ca. 300 variants) or polymorphisms (ca. 100 variants). While LOVD is an efficient and convenient tool for gene-centered collection, curation and display of DNA variation, data analysis options are limited. The Universal Mutation Database (UMD®) Locus Specific Databases (Beroud, et al., 2000; Beroud, et al., 2005) have been developed specifically to allow for the collection of mutational data and provide numerous bioinformatics tools for the interactive analysis of mutational data, including the analysis of novel sequence variants. Even more, the UMD® software is very flexible for the development of novel tools, based on questions arising in the research field.

In the present article, we describe the online version of UMD-DYSF, freely accessible at www.umd.be/DYSF/. In complement to LOVD Dysferlin, UMD-DYSF not only references all previously published disease-causing

mutations identified in the *DYSF* gene but also includes interactive bioinformatics tools for the analysis of *DYSF* sequence variants. In particular, UMD-DYSF offers a computational procedure for the analysis of possible deleterious mutations affecting splicing signals in the dysferlin gene, using the Human Splicing Finder (HSF) algorithm (Desmet, et al., 2009) and integrates the UMD-Predictor tool for the analysis of missense variants (Frederic, et al., 2009). Furthermore, interactive functions allow for analysis of the full UMD-DYSF dataset, single mutational events or customized subsets of mutations referenced in the database. We previously used an offline version of UMD-DYSF to successfully analyse the mutational spectrum of a large cohort of patients analysed for *DYSF* mutations in our diagnostic laboratory (Krahn, et al., 2009a). To further illustrate the use of UMD-DYSF, we here report the results of statistical analyses of the *DYSF* mutational spectrum based for the first time on all compiled *DYSF* disease-causing mutations reported in the literature to date.

THE UMD-DYSF DATABASE

Database description

The UMD-DYSF database was developed using a software package of specific routines, which allows optimized multicriteria search and sorting of data (Beroud, et al., 2000; Beroud, et al., 2005). Mutational data entries are standardized to facilitate mutational analysis, as previously described (Collod-Beroud, et al., 2003; Frederic, et al., 2008). Each entry corresponds to one mutation associated with one affected individual, either index patient or affected relative. At the moment, UMD-DYSF includes *DYSF* mutations described or predicted in the literature as deleterious, exclusively. However, in future versions, UMD-DYSF will include unpublished data for disease-causing variants (see the DATABASE UPDATE section). UMD-DYSF is currently not aimed at collecting polymorphism data from patients because current diagnostic screening methods are not homogenized between laboratories, and results of polymorphism patient data are therefore biased. For users interested in known polymorphism data, the UMD-DYSF website links to the UCSC genome browser page for *DYSF* (Dreszer, et al., 2011) (<http://genome.ucsc.edu>) and each UMD-DYSF mutation description page links to the collection of sequence variations available in LOVD Dysferlin for the corresponding nucleotide position. As dysferlinopathies are an autosomal recessive disease, users should be warned that “polymorphism data” is sued from large-scale “normal” control studies can be “contaminated” with truly pathogenic *DYSF* sequence variants found at a heterozygous state in healthy carriers. These variants should thus be confronted to pathogenicity prediction tools—such as those available in UMD-DYSF—to further evaluate the possibility of a deleterious effect.

The following mutational events can be entered into the database: point mutations, insertions, deletions, and insertions-deletions (intronic and/or exonic); as well as mono- or multi-exonic large-sized deletions or duplications. Several levels of information are provided for each mutation, including the affected exon and codon number, wild-type and mutant codon sequence, type of mutational event, mutation nomenclature, wild-type and mutant amino-acid, affected domain, etc. Whenever available, we also included in the database clinical information; however, in most publications, only the main phenotype data (i.e. LGMD2B or Miyoshi myopathy), but no detailed information, are described.

Mutational events are automatically described using the official nomenclature of the Human Genome Variation Society (den Dunnen and Antonarakis, 2000), and relating to the human *DYSF* cDNA sequence of reference (isoform 8, GenBank #NM_003494.2) which corresponds to the major *DYSF* transcript among all reported isoforms expressed in skeletal muscle (Pramono, et al., 2009). *DYSF* isoform 8 (6243bp) is transcribed under the *DYSF* promoter and contains the 55 canonical exons, with exons 5a and 40a exclusion and exon 17 inclusion. The dysferlin protein sequence was annotated for C2 domains, ferlin family domains, DysF domains and TM domain based on predictions from Pfam 25.0 (Finn, et al., 2010) and SMART 6 (Letunic, et al., 2009) and for highly conserved residues expected to be involved in calcium coordination as described by Therrien and colleagues (Therrien, et al., 2006). Users of UMD-DYSF can verify whether exonic mutations affect annotated structural domains or highly conserved residues.

Interactive analysis of *DYSF* mutational data included in the database was done using previously described sorting- and research-functions (Beroud, et al., 2000; Beroud, et al., 2005). In addition, the present version of the UMD-package includes novel routines to assist the design of new therapeutic tools. Analysis tools and functions accessible on the UMD-DYSF website are described in Table 1, and a brief user guide can be downloaded from the website.

Table 1. Complete list of tools and functions available on the UMD-DYSF website (www.umd.be/DYSF/)

Function or tool name	Function or tool description
I found a mutation	Displays a table of the various mutational events registered in UMD-DYSF for a given position.
I want to analyze the impact of a missense variant	Uses the UMD-Predictor® algorithm to predict the pathogenicity of all possible non-synonymous or synonymous mutations from the <i>DYSF</i> gene.
I want to analyze an intronic variant	Uses the Human Splicing Finder tool to evaluate the consequences of substitutions on splicing.
I want to search the database	Allows the selection of a specific subset of the database. Results are displayed as a list on the screen.
Predicted impact of all previously reported missense variations	Uses the UMD-Predictor® tool to predict the pathogenicity of all UMD-DYSF missense variants localized in the coding sequence.
Global analysis	Gives a summary of mutation types.
Position	Studies the distribution of mutations at the nucleotide level to identify preferential mutation sites.
Potential stop codons	Displays all codons from a specific exon that can be mutated into a stop codon by a single substitution.
Mutation map	Displays the distribution of the various mutations along the gene and the protein.
Deletion map	Displays the distribution of the various deletions along the gene and the protein.
Stop codon map	Displays the exon phasing and the position and number of reported nonsense mutations.
Geographic distribution	Displays geographic origin of patients.
Binary comparison	Displays the distribution of the various mutations along the gene for two chosen subsets of the database.
Stat exons	Studies the distribution of mutations in the different exons. It enables detection of a statistically significant difference between observed and expected mutations
Distribution by exon	Displays the partition of each type of mutation in each exon
Structure	Studies the distribution of allelic mutations both in the various structural domains of the protein and in the highly conserved residues expected to be implicated in calcium coordination

Database entries

The UMD-DYSF v1.0 (April 12, 2011) contains a total of 742 entries corresponding to mutational data from 558 patients diagnosed with primary dysferlinopathy and previously reported in the literature as disease-causing mutations. The total number of patients amounts to 401 index cases (557 mutational entries) and 157 relatives. Among all UMD-DYSF entries, 192 entries from 129 patients correspond to mutations identified in our laboratory (Khadilkar, et al., 2008; Krahn, et al., 2009a; Krahn, et al., 2009b; Krahn, et al., 2010; Nguyen, et al., 2005; Nguyen, et al., 2007; Seror, et al., 2008) while the others correspond to mutational data reported in 55 additional publications (see www.umd.be/DYSF/ for a comprehensive list of references). All mutational data can be visualized through the “Search” function described in Table 1 and downloaded from the UMD-DYSF website.

Bioinformatics tools for the interpretation of sequence variants

A recurrent problem in genetic diagnosis is the interpretation of sequence variants, including the difficulty in predicting the impact of a genomic variation on the pre-mRNA maturation and the mRNA translation mechanisms, and in predicting any deleterious effect on the mRNA and protein stability. The Human Gene Mutation Database (professional release 2010.4) which collects all known gene lesions responsible for human inherited diseases

(Stenson, et al., 2009), reports a total of 108046 mutational entries, 54% of which are missense mutations, as well as mutations affecting RNA splicing. Interpretation of the effect of *DYSF* missense variants and identification of *DYSF* splice variants is facilitated by a number of bioinformatics tools integrated into UMD-DYSF and available online.

The HSF tool is based on UMD algorithms and predicts consequences of mutations affecting existing splice signals (donor and acceptor sites, branchpoints and *cis*-acting elements such as exonic splicing enhancers and silencers) or possibly creating novel ectopic splicing sequences. These algorithms are integrated into UMD-DYSF to allow for the analysis of sequence variants. Detailed analysis of UMD-DYSF abnormal splicing variants is described below.

To further discriminate between neutral and pathogenic sequence variations, UMD-DYSF also integrates the recently developed UMD-Predictor tool (Frederic, et al., 2009). UMD-Predictor combines data such as localization within the protein, conservation and biochemical properties of the mutant and wild-type residues, as well as results from HSF analysis to calculate a pathogenicity score ranging from 0 to 100 for each missense variant (score >65 indicates a probable or highly likely pathogenicity). Its efficiency for predicting pathogenic missense mutations was demonstrated by a sensitivity of 95.4% and a positive predictive value of 99.5% (Frederic, et al., 2009). The UMD-Predictor score was computed for all UMD-DYSF missense variant entries and can be consulted on the UMD-DYSF website using the “Predicted Impact of all Previously Reported Missense Variations” function. Although all variants predicted or described in the literature to be deleterious were entered into UMD-DYSF, 5% were predicted as probable or likely polymorphisms using UMD-Predictor (pathogenicity score <65). These variants could correspond to true polymorphisms in patients for whom the accurate deleterious mutation has been missed during genetic testing (incomplete mutation detection rates of pre-screening techniques such as Single Strand Conformation Polymorphism analysis or Denaturing High Pressure Liquid Chromatography; mutations not detected using routine sequencing approaches such as large genomic rearrangements and “deep” intronic mutations; etc.). More likely, these variants spot cases for which the UMD-Predictor algorithm lacked predictive elements to accurately interpret the pathogenic effect of the sequence variant. More generally, for variants of unclear pathogenicity, definitive conclusion on their possible deleterious effect will only be achievable with integration of novel functional data into the UMD-Predictor algorithm. In particular, bioinformatics predictions can greatly benefit from sequencing data of mutated *DYSF* RNAs and proteins, and from novel functional elements that would shed light on molecular roles and functions of dysferlin, domain organisation and critical residues of the protein.

Bioinformatics routines to assist the design of therapeutic strategies

Two interesting tools available on the UMD-DYSF website (Table 1) have been designed to help develop certain types of therapeutic approaches for dysferlinopathies. In particular, several nonsense mutations could be targets for possible therapeutic approaches based on aminoglycoside read through of stop codons (Wang, et al., 2010). The “Potential Stop Codon” function gives the list of codons that can lead to a premature termination codon (PTC) when mutated by a single substitution; along with the number of such mutations reported in UMD-DYSF. This function also provides statistical calculation about the environment of observed PTC compared to potential PTC for which no mutation has ever been reported. The distribution of nonsense mutations reported in the *DYSF* gene is described below. In addition, the “Stop Codon Map” function is a UMD newly implemented tool that displays the exon phasing and the position and number of reported nonsense mutations. This function has been designed to facilitate envisaging exon skipping strategies (Aartsma-Rus, et al., 2010; Levy, et al., 2010; Wein, et al., 2010).

Analysis of the *DYSF* mutational spectrum

General statistics

Mutational data from large cohorts of patients repeatedly revealed a large mutational spectrum for the *DYSF* gene, with a high proportion of missense changes, or frameshifting insertions and/or deletions (for example, (Aoki, et al., 2001; Cagliani, et al., 2003; De Luna, et al., 2007; Guglieri, et al., 2008; Klinge, et al., 2010; Krahn, et al., 2009a; Mahjneh, et al., 1996; Nguyen, et al., 2005; Tagawa, et al., 2003; Takahashi, et al., 2003)). Accordingly, most of the UMD-DYSF entries correspond to “private” or rare *DYSF* disease-causing mutations. In the 401 reported index patients, 266 disease-causing variants were identified along the *DYSF* coding sequence. Within the

index cases population, 379 heterozygous variants and 178 homozygous variants were identified and constitute a set of 735 alleles.

Founder mutations and recurrent mutations

Among *DYSF* disease-causing mutations, several different founder mutations have been suggested or demonstrated in patients of various geographic/ethnic origins (Argov, et al., 2000; Cagliani, et al., 2003; Leshinsky-Silver, et al., 2007; Santos, et al., 2010; Vernengo, et al., 2011; Vilchez, et al., 2005; Weiler, et al., 1999) (Table 2). In addition, interrogation of the database shows that 51 mutations have been recurrently identified in at least three non-related index patients (see updated list on the UMD-DYSF website). These recurrent mutations are distributed along the coding sequence and canonic splice sites without any apparent mutational « hotspot » (Figure 1).

Table 2. List of *DYSF* founder mutations

Mutation nomenclature on cDNA (RNA, protein)	Geographic/ethnic origin of the population	Evaluated carrier frequency	Reference
c.1180_1180+7delAGTGCGTG (r.1054_1284del, p.Glu353_Leu429del)	Portuguese	Unknown	Vernengo et al., 2011
c.2372C>G (p.Pro791Arg)	Native canadian	Unknown	Weiler et al., 1999
c.2779delG (p.Ala927LeufsX21)	Caucasian jewish	4%	Leshinsky-Silver et al., 2007
c.2875C>T (p.Arg959Trp)	Italian	Unknown	Cagliani et al., 2003
c.4872_4876delinsCCCC (p.Glu1624AspfsX10)	Libyan jewish	10%	Argov et al., 2000
c.5492G>A (exon skipping)	Portuguese	Unknown	Santos et al., 2010
c.5713C>T (p.Arg1905X)	Spanish (region of Sueca)	2%	Vilchez et al., 2005

Mutations are described using the official nomenclature of the Human Genome Variation Society, and relating to the human *DYSF* cDNA sequence of reference (isoform 8, GenBank #NM_003494.2).

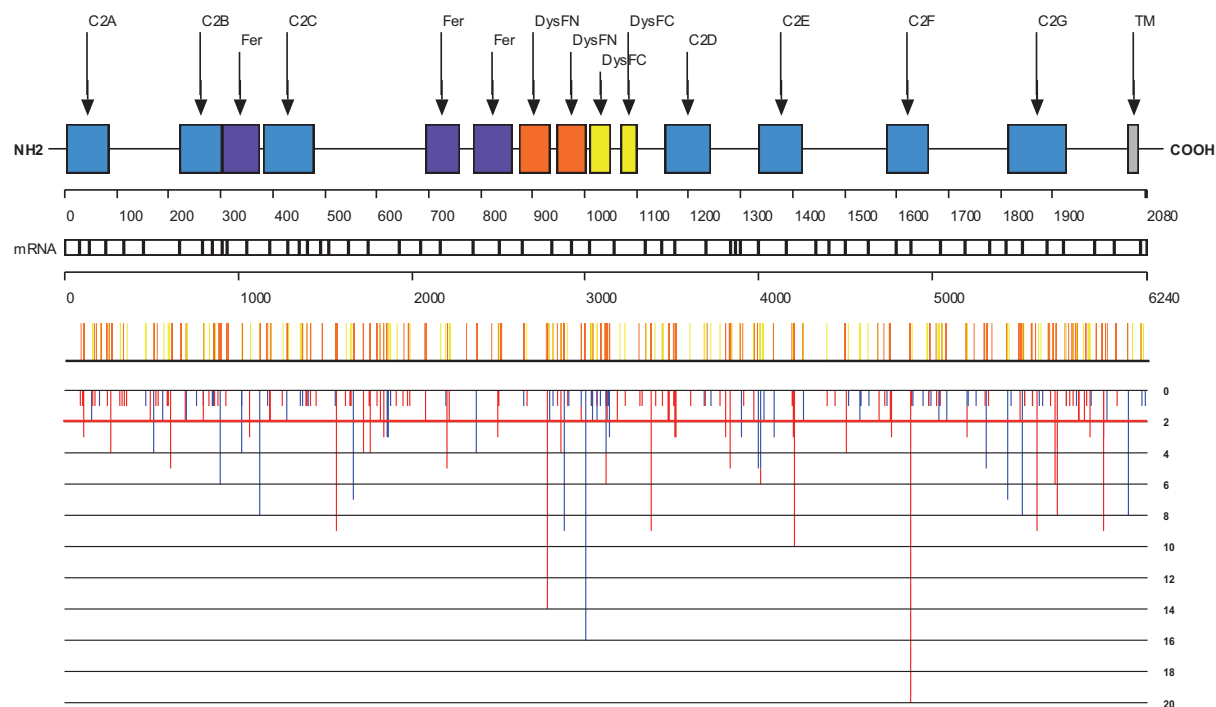


Figure 1. Distribution of exonic disease-causing mutations reported in the dysferlin sequence. Above a scale at the amino acid level, the colored boxes represent the various structural or functional domains annotated for the protein. Above a scale at the nucleotide level, the various white boxes represent the exons of the gene. The middle panel displays the distribution of all exonic mutations identified in patients first diagnosed with LGMD2B (yellow vertical lines) or Miyoshi myopathy (orange vertical lines). The bottom panel displays the number of the various exonic mutational entries found in the index cases population and classified as missense and in-frame insertion or deletion mutations (blue vertical lines) or nonsense and frameshifting mutations (red vertical lines). Mutations below the red horizontal line represent recurrent mutations identified in at least three non-related index patients.

Type of mutational events

Among the 266 different reported mutational events, the following type of mutations were identified: 175 single base substitutions (65.8%), 54 deletions (20.3%), 26 duplications (9.8%), 6 insertions (2.3%) and 5 insertion/deletions (1.9%). Among the total deletion and insertion events, 51.8% of deletions and 68.7% of insertions occurred within a repeated sequence. A total of 220 (82.7%) distinct mutations affect exonic sequences and the remaining 46 (17.3%) mutations involve change of intronic nucleotides. Altogether, among all disease-causing mutations in UMD-DYSF, exonic mutations segregate into missense mutations (33.1%), nonsense mutations (18.0%), frameshifting mutations (27.8%) and in-frame exonic insertions or deletions (3.8%) (Table 3A). The partition of the different mutation types found within the UMD-DYSF allele set is summarized in Table 3B. Moreover, UMD-DYSF reports six large rearrangements found in eight index patients involving deletion or duplication of one or several exons (Table 4). Because such large mutational events are not systematically searched for in genetic testing, this figure is expected to be an underestimate of the real large rearrangements frequency (Krahn, et al., 2009b).

Table 3. Types of disease-causing mutations recorded in UMD-DYSF

Type of mutations	A. Number of different mutations	B. Number of alleles from index patients*	C. Number of homozygous alleles from LGMD2B index patients*	D. Number of homozygous alleles from Miyoshi index patients*
Exonic point mutations	136 (51.1%)	365 (49.7%)	70 (46.1%)	62 (40.3%)
Missenses	88 (33.1%)	236 (32.1%)	56 (36.8%)	30 (19.5%)
Nonsenses	48 (18.0%)	129 (17.6%)	14 (9.2%)	32 (20.8%)
Exonic deletions and insertions	84 (31.6%)	261 (35.5%)	54 (35.5%)	74 (48.1%)
Deletions	49 (18.4%)	149 (20.3%)	28 (18.4%)	42 (27.3%)
<i>Out of frame deletions</i>	45 (16.9%)	139 (18.9%)	24 (15.8%)	40 (26.0%)
<i>In frame deletions</i>	4 (1.5%)	10 (1.4%)	4 (2.6%)	2 (1.3%)
Insertions	30 (11.3%)	74 (10.1%)	14 (9.2%)	16 (10.4%)
<i>Out of frame insertions</i>	27 (10.2%)	71 (9.7%)	14 (9.2%)	16 (10.4%)
<i>In frame insertions</i>	3 (1.1%)	3 (0.4%)	0 (0.0%)	0 (0.0%)
Indels	5 (1.9%)	38 (5.2%)	12 (7.9%)	16 (10.4%)
<i>Out of frame indels</i>	2 (0.8%)	33 (4.5%)	6 (6.6%)	0 (0%)
<i>In frame indels</i>	3 (1.1%)	5 (0.7%)	2 (1.3%)	16 (10.4%)
Intronic mutations	46 (17.3%)	109 (14.8%)	28 (18.4%)	18 (11.7%)
TOTAL	266 (100%)	735 (100%)	152 (100%)	154 (100%)

All percentages are calculated with respect to the value in the TOTAL line. * For each patient, heterozygous disease-causing mutations are counted once and homozygous disease-causing mutations are counted twice.

Table 4. List of large rearrangements identified in the *DYSF* gene

Mutation nomenclature	Duplicated or deleted exons	Number of occurrence in probands
c.89-643_4411-2493del	2 to 40	1
c.343-?_457+?del 5		1
c.2512-?_3174+?del	25 to 29	3
c.3904-?_4333+?dup	37 to 39	1
c.5768-?_5946+?del 52		1
c.6205-?*+?del 55		1

Large deletions can be displayed using the “Deletion Map” function on the UMD-DYSF website. Mutations are described using the official nomenclature of the Human Genome Variation Society, and relating to the human *DYSF* cDNA sequence of reference (isoform 8, GenBank #NM_003494.2).

Exonic variants

The 220 exonic mutations are distributed along the entire coding sequence, affecting regions of the protein both within or outside of predicted functional domains, and without any defined mutational hotspot (Figure 1). A total of 122 exonic mutations are predicted to disrupt the open reading frame and/or to lead to a premature stop codon. These mutations can be classified into insertions or deletions events (74 frameshifting mutations) and nonsense mutations (48 mutations) (Table 3A) and are found evenly distributed along the coding sequence (Figure 1). Overall, the events that presumably lead to the translation of a truncated and unstable dysferlin protein represent 50.6% of the proband allele population (Table 3B). We examined the distribution of missense and in-frame exonic

insertion or deletion mutations and compared their proportion either within or outside annotated domains (Figure 1). We show that mutations recorded in UMD-DYSF affect 3.3% of all amino acids residing outside annotated domains and 5.0% of all amino acids residing within domains. In particular, we confirm the susceptibility of the repeated DysF domain to mutations (Patel, et al., 2008) as the UMD-DYSF mutations affect 7.9% of the amino acids within this domain. The “Structure” function summarizes the distribution of small rearrangements in structural domains and in possible calcium binding residues of the dysferlin protein. Within the group of proband alleles, 453 (81.3%) correspond to *DYSF* variants mutated within regions encoding a predicted structural or functional domain. Overall, C2 domains are the most frequently affected (266 mutational entries), followed by DysF and ferlin domains (126 and 60 mutational entries, respectively) whereas one single index patient was identified with a deleterious mutation (12 bp insertion/deletion) in the region coding for the carboxy-terminal transmembrane domain (Guglieri, et al., 2008). Interestingly, mutations in predicted calcium binding residues of C2 domains were reported for only three patients, within C2B, C2C and C2F domains (De Luna, et al., 2007; Nguyen, et al., 2005; Walter, et al., 2003).

Splice variants

Among the 266 different mutational events reported in UMD-DYSF, 46 splice variants consist of both intronic or exonic mutations associated with a predicted or experimentally described abnormal splicing of the *DYSF* gene (Table 5). Intronic variants include 31 mutations directly affecting 5' splice donor-sites, 14 mutations affecting 3' splice acceptor-sites and one deleterious mutation within a branchpoint signal. In addition, two exonic mutations have been shown to produce aberrantly spliced transcripts by either abolishing the canonical donor splice site (c.5429G>A) (Santos, et al., 2010) or by creating a novel ectopic acceptor splice site (c.1555G>A) (De Luna, et al., 2007). Altogether, splice variants constitute 14.8% of the allele population in UMD-DYSF index patients (Table 3B). Using dedicated functions included in UMD and HSF, a pathogenic effect on the splice donor or acceptor sites, or in the branchpoint (c.3443-33A>G), was correctly predicted in all cases, exception made for one mutation, c.5525+3A>G. This mutation was shown to promote exon 49 skipping (De Luna, et al., 2007). HSF analysis predicts an effect on the splice donor site, but below the threshold of pathogenicity. However, possible effects on exonic splicing enhancer and silencer sites are also predicted, and may cause the experimentally proven exon 49 skipping in this case.

Mutation status

Altogether, 280 patients carry at least one homozygous mutation. Among them, two patients carry two or three homozygous mutations (F1-47-1-2 and F1-18-1-2) and three patients carry one homozygous mutation and one heterozygous mutation (UK2-29-1-0, UK2-47-1-0 and UK2-49-1-0). A total of 176 patients carry at least two compound heterozygous mutations, including two patients carrying three heterozygous mutations (F1-65-1-2 and UK2-35-1-0). The identification of more than two distinct possibly disease-causing mutations in a patient may be related to the existence of hypomorphic sequence variants, or complex alleles. For 102 patients, only one heterozygous disease-causing mutation was identified. Among these are two symptomatic dysferlin mutation carriers described by Illa and colleagues (Illa, et al., 2007). Overall, both disease-causing alleles were identified in 323 index patients (80.5%), whereas only one disease-causing allele was identified in the other 78 index cases (19.5%), thus underlining incomplete sensitivity of the currently used mutation detection techniques. However, these figures do not reflect the overall detection rate of dysferlin mutation screening procedures since in patients with a clinical diagnosis of dysferlinopathy, it is estimated that for approximately 10% of them, mutational analyses did not confirm them as carriers of any disease-causing mutation in the dysferlin gene and these patients are thus not recorded in UMD-DYSF (the inclusion criteria being the identification of at least one deleterious mutation).

Table 5. List of reported splice mutations within the *DYSF* gene

Localisation	Mutation nomenclature	Effect at the RNA	Original description
IVS3	c.236+1G>T	r.143_236del (E3S, FS)	Liewluck et al. 2009
IVS5	c.457+1insG	r.spl?	Nguyen et al. 2005
IVS5	c.457+2T>G	r.343_457del (E5S, FS)	Cagliani et al. 2005
IVS6	c.663+1G>C	r.spl?	Saito et al. 2002
IVS6	c.664-9_667del13	r.spl?	Klinge et al. 2010
IVS8	c.855+1delG	r.spl?	Nguyen et al. 2005
IVS10	c.937+1G>A	r.spl?	Saito et al. 2002
IVS11	c.1053+5G>A	r.spl?	Klinge et al. 2008
IVS12	c.1180+2T>C	r.spl?	Cuglieri et al. 2008
IVS12	c.1181-2A>C	r.1181_1212del (FS)	Cagliani et al. 2005
IVS13	c.1284+2T>C	r.spl?	Tagawa et al. 2003
IVS13	c.1285-2A>G	r.spl?	Spuler et al. 2008
IVS14	c.1353+1G>A	[r.1353+1_1354-1ins; r.1353+1g>a] (I14R, FS)	de Luna et al. 2007
IVS14	c.1354-1G>A	r.spl?	Klinge et al. 2010
IVS16	c.1480+1delG	r.1398_1480del (E16S, FS)	Therrien et al. 2006
IVS16	c.1481-1G>A	r.spl?	Rosales et al. 2010
Exon17	c.1555G>A	r.1523_1556del (FS)	de Luna et al. 2007
IVS22	c.2163-1G>T	r.spl?	Klinge et al. 2010
IVS24	c.2511+1G>A	r.spl?	Nguyen et al. 2005
IVS25	c.2643+1G>A	r.spl?	Matsuda et al. 2001
IVS25	c.2643+2T>C	r.spl?	Klinge et al. 2010
IVS25	c.2643+2T>G	r.2512_2643del (E25S, IF)	Therrien et al. 2006
IVS25	c.2644-2A>G	r.spl?	Matsuda et al. 2001
IVS26	c.2810+1G>A	r.spl?	Nguyen et al. 2005
IVS26	c.2810+1G>C	r.spl?	Cuglieri et al. 2008
IVS28	c.3031+2T>C	r.spl?	Nguyen et al. 2005
IVS30	c.3348+1delGTAT	r.spl?	Nguyen et al. 2005
IVS30	c.3349-2A>G	r.spl?	Klinge et al. 2010
IVS31	c.3443-33A>G	r.3443_3520del (E32S, IF)	Sinnreich et al. 2006
IVS33	c.3702+1G>A	r.spl?	Nguyen et al. 2005
IVS33	c.3703-1G>A	r.spl?	Nguyen et al. 2005
IVS34	c.3843+1G>A	r.spl?	Nguyen et al. 2005
IVS34	c.3843+2T>A	r.spl?	Rosales et al. 2010
IVS37	c.4005+1G>A	r.spl?	Nguyen et al. 2005
IVS38	c.4167+1G>C	r.spl?	Nguyen et al. 2005

Localisation	Mutation nomenclature	Effect at the RNA	Original description
IVS40	c.4411-5C>G	r.spl?	Klinge et al. 2008
IVS45	c.5057+4_delCGT	r.?_5057del (FS)	Cagliani et al. 2003
IVS45	c.5057+5G>A	r.spl	McNally et al. 2000
IVS45	c.5057+4_5057+5ins23	r.spl?	Anderson et al. 2000
IVS46	c.5200+1G>A	r.spl?	Cagliani et al. 2005
IVS47	c.5341-1G>A	r.spl?	Klinge et al. 2010
Exon48	c.5429G>A	r.5341_5429del * (E48S, FS)	Santos et al. 2010
IVS48	c.5430-2A>G	r.spl?	Kesari et al. 2008
IVS49	c.5525+3A>G	r.5430_5525del (E49S, IF)	De Luna et al. 2007
IVS49	c.5526-1G>A	r.spl?	Rosales et al. 2010
IVS50	c.5668-7G>A	[r.5668-5_5668-1ins;r.5668-7g>a] (FS)	Cagliani et al. 2005
IVS51	c.5767+1G>A	r.spl?	Nguyen et al. 2005
IVS52	c.5946+1G>A	r.spl?	Liu et al. 1998

Mutations affect canonical intronic splice signals (5' and 3' splice sites, branchpoints) or exonic nucleotides. Effect on RNA splicing was either predicted (r.spl?) or experimentally described. Disruption of canonical splice signals or creation of novel splice signals can promote exon skipping (ES), intron retention (IR), or other sequence insertion/deletion in the mRNA. Mutations are predicted to either maintain the reading frame (IF) or introduce a frameshift (FS) leading to the translation of a truncated product and possibly to nonsense-mediated mRNA decay. * Predominant transcript. Mutations are described using the official nomenclature of the Human Genome Variation Society, and relating to the human *DYSF* cDNA sequence of reference (isoform 8, GenBank #NM_003494.2).

Comparison of mutational profiles of the LGMD2B and Miyoshi myopathy phenotypes

Dysferlinopathies are characterized by the two main clinical phenotypes, LGMD2B and Miyoshi myopathy, and additional clinical variants, thus presenting a broad range of symptoms and onset. In all cases the genotype-phenotype relationship has always remained difficult to define. In UMD-DYSF, 88% of patients present with either a LGMD2B or Miyoshi myopathy phenotype, as described in the original publications. We have compared the distribution of the mutations along the *DYSF* gene (Figure 1) and the type of mutations between the two main clinical groups (Table 3C and D, with patients with one homozygous mutation) and no significant difference was observed between them (Chi² test, p>0.01). Therefore, available mutational data do not point out any genotype-phenotype correlation for dysferlin mutations with regard to the two main clinical presentations, LGMD2B or Miyoshi myopathy. It can be speculated that the observed clinical heterogeneity in dysferlinopathies may rather be related to the implication of genetic or environmental modifiers.

DATABASE UPDATE

The UMD-DYSF v 1.0 database and subsequent updated versions are available at www.umd.be/DYSF/. Curation of the UMD-DYSF database by a dedicated curator will allow continuous updating. Clinicians and researchers are encouraged to submit unpublished variants by contacting the curator of the database. Notification of omissions and errors in the current version, as well as specific phenotypic data, would be gratefully received by the curator. The software package is available on a collaborative basis and will be expanded as the database grows, with the implementation of new specific functions according to the requirements of its users. In referring to UMD-DYSF, we kindly ask all users of the database to cite this article.

ACKNOWLEDGMENTS

We sincerely thank Kate Bushby, Brigitta von Rekowski and Hans Lochmüller for helpful advice on the UMD-DYSF website, Andrew Phillips for his help with the HGMD statistics, and Bruno Eymard, Jean Pouget, Shahram Attarian and Emmanuelle Campana-Salort for helpful discussions.

REFERENCES

- Aartsma-Rus A, Singh KH, Fokkema IF, Ginjaar IB, van Ommen GJ, den Dunnen JT, van der Maarel SM. 2010. Therapeutic exon skipping for dysferlinopathies? *Eur J Hum Genet* 18(8):889-94.
- Aoki M, Liu J, Richard I, Bashir R, Britton S, Keers SM, Oeltjen J, Brown HE, Marchand S, Bourg N, Belay C, McKenna-Yasek D, Arahata K, Bohlega S, Cupler E, Illa I, Majneh I, Barohn RJ, Urtizberea JA, Fardeau M, Amato A, Angelini C, Bushby K, Beckmann JS, Brown RH, Jr. 2001. Genomic organization of the dysferlin gene and novel mutations in Miyoshi myopathy. *Neurology* 57(2):271-8.
- Argov Z, Sadeh M, Mazor K, Soffer D, Kahana E, Eisenberg I, Mitrani-Rosenbaum S, Richard I, Beckmann J, Keers S, Bashir R, Bushby K, Rosenmann H. 2000. Muscular dystrophy due to dysferlin deficiency in Libyan Jews. *Clinical and genetic features. Brain* 123 (Pt 6):1229-37.
- Bansal D, Miyake K, Vogel SS, Groh S, Chen CC, Williamson R, McNeil PL, Campbell KP. 2003. Defective membrane repair in dysferlin-deficient muscular dystrophy. *Nature* 423(6936):168-72.
- Bashir R, Britton S, Strachan T, Keers S, Vafiadaki E, Lako M, Richard I, Marchand S, Bourg N, Argov Z, Sadeh M, Mahjneh I, Marconi G, Passos-Bueno MR, Moreira Ede S, Zatz M, Beckmann JS, Bushby K. 1998. A gene related to Caenorhabditis elegans spermatogenesis factor fer-1 is mutated in limb-girdle muscular dystrophy type 2B. *Nat Genet* 20(1):37-42.
- Beroud C, Collod-Beroud G, Boileau C, Soussi T, Junien C. 2000. UMD (Universal mutation database): a generic software to build and analyze locus-specific databases. *Hum Mutat* 15(1):86-94.
- Beroud C, Hamroun D, Collod-Beroud G, Boileau C, Soussi T, Claustres M. 2005. UMD (Universal Mutation Database): 2005 update. *Hum Mutat* 26(3):184-91.
- Bushby KM. 2000. Dysferlin and muscular dystrophy. *Acta Neurol Belg* 100(3):142-5.
- Cagliani R, Fortunato F, Giorda R, Rodolico C, Bonaglia MC, Sironi M, D'Angelo MG, Pirella A, Locatelli F, Toscano A, Bresolin N, Comi GP. 2003. Molecular analysis of LGMD-2B and MM patients: identification of novel DYSF mutations and possible founder effect in the Italian population. *Neuromuscul Disord* 13(10):788-95.
- Collod-Beroud G, Le Bourdelles S, Ades L, Ala-Kokko L, Booms P, Boxer M, Child A, Comeglio P, De Paepe A, Hyland JC, Holman K, Kaitila I, Loeys B, Matyas G, Nuytinck L, Peltonen L, Rantamaki T, Robinson P, Steinmann B, Junien C, Beroud C, Boileau C. 2003. Update of the UMD-FBN1 mutation database and creation of an FBN1 polymorphism database. *Hum Mutat* 22(3):199-208.
- De Luna N, Freixas A, Gallano P, Caselles L, Rojas-Garcia R, Paradas C, Nogales G, Dominguez-Perles R, Gonzalez-Quereda L, Vilchez JJ, Marquez C, Bautista J, Guerrero A, Salazar JA, Pou A, Illa I, Gallardo E. 2007. Dysferlin expression in monocytes: a source of mRNA for mutation analysis. *Neuromuscul Disord* 17(1):69-76.
- den Dunnen JT, Antonarakis SE. 2000. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. *Hum Mutat* 15(1):7-12.
- Desmet FO, Hamroun D, Lalonde M, Collod-Beroud G, Claustres M, Beroud C. 2009. Human Splicing Finder: an online bioinformatics tool to predict splicing signals. *Nucleic Acids Res* 37(9):e67.
- Dreszer TR, Karolchik D, Zweig AS, Hinrichs AS, Raney BJ, Kuhn RM, Meyer LR, Wong M, Sloan CA, Rosenbloom KR, Roe G, Rhead B, Pohl A, Malladi VS, Li CH, Learned K, Kirkup V, Hsu F, Harte RA, Guruvadoo L, Goldman M, Giardine BM, Fujita PA, Diekhans M, Cline MS, Clawson H, Barber GP, Haussler D, James Kent W. 2011. The UCSC Genome Browser database: extensions and updates 2011. *Nucleic Acids Res*.
- Finn RD, Mistry J, Tate J, Coggill P, Heger A, Pörlington JE, Gavin OL, Gunasekaran P, Ceric G, Forslund K, Holm L, Sonnhammer EL, Eddy SR, Bateman A. 2010. The Pfam protein families database. *Nucleic Acids Res* 38(Database issue):D211-22.

- Foxton R M, Laval SH, Bushby K M. 2004. Characterisation of the dysferlin skeletal muscle promoter. *Eur J Hum Genet* 12(2):127-31.
- Frederic MY, Hamroun D, Faivre L, Boileau C, Jondeau G, Claustres M, Beroud C, Collod-Beroud G. 2008. A new locus-specific database (LSDB) for mutations in the TGFBR2 gene: UMD-TGFBR2. *Hum Mutat* 29(1):33-8.
- Frederic MY, Lalande M, Boileau C, Hamroun D, Claustres M, Beroud C, Collod-Beroud G. 2009. UMD-predictor, a new prediction tool for nucleotide substitution pathogenicity -- application to four genes: FBN1, FBN2, TGFBR1, and TGFBR2. *Hum Mutat* 30(6):952-9.
- Guglieri M, Magri F, D'Angelo MG, Prella A, Morandi L, Rodolico C, Cagliani R, Mora M, Fortunato F, Bordoni A, Del Bo R, Ghezzi S, Pagliarani S, Lucchiarini S, Salani S, Zecca C, Lamperti C, Ronchi D, Aguenouz M, Ciscato P, Di Blasi C, Ruggieri A, Moroni I, Turconi A, Toscano A, Moggi M, Bresolin N, Comi GP. 2008. Clinical, molecular, and protein correlations in a large sample of genetically diagnosed Italian limb girdle muscular dystrophy patients. *Hum Mutat* 29(2):258-66.
- Ho M, Gallardo E, McKenna-Yasek D, De Luna N, Illa I, Brown Jr RH. 2002. A novel, blood-based diagnostic assay for limb girdle muscular dystrophy 2B and Miyoshi myopathy. *Ann Neurol* 51(1):129-33.
- Illa I, De Luna N, Dominguez-Perles R, Rojas-Garcia R, Paradas C, Palmer J, Marquez C, Gallano P, Gallardo E. 2007. Symptomatic dysferlin gene mutation carriers: characterization of two cases. *Neurology* 68(16):1284-9.
- Illa I, Serrano-Munuera C, Gallardo E, Lasa A, Rojas-Garcia R, Palmer J, Gallano P, Baiget M, Matsuda C, Brown RH. 2001. Distal anterior compartment myopathy: a dysferlin mutation causing a new muscular dystrophy phenotype. *Ann Neurol* 49(1):130-4.
- Khadilkar SV, Singh RK, Agarwal P, Kohn M, Levy N. 2008. Twenty-two year follow-up of an Indian family with dysferlinopathy-clinical, immunocytochemical, western blotting and genetic features. *Neurol India* 56(3):388-90.
- Klinge L, Aboumoussa A, Eagle M, Hudson J, Sarkozy A, Vita G, Charlton R, Roberts M, Straub V, Barresi R, Lochmuller H, Bushby K. 2010. New aspects on patients affected by dysferlin deficient muscular dystrophy. *J Neurol Neurosurg Psychiatry* 81(9):946-53.
- Klinge L, Dean AF, Kress W, Dixon P, Charlton R, Muller JS, Anderson LV, Straub V, Barresi R, Lochmuller H, Bushby K. 2008. Late onset in dysferlinopathy widens the clinical spectrum. *Neuromuscul Disord* 18(4):288-90.
- Krahn M, Beroud C, Labelle V, Nguyen K, Bernard R, Bassez G, Figarella-Branger D, Fernandez C, Bouvenot J, Richard I, Ollagnon-Roman E, Bevilacqua JA, Salvo E, Attarian S, Chapon F, Pellissier JF, Pouget J, Hammouda el H, Laforet P, Urtizberea JA, Eymard B, Leturcq F, Levy N. 2009a. A analysis of the DYSF mutational spectrum in a large cohort of patients. *Hum Mutat* 30(2):E345-75.
- Krahn M, Borges A, Navarro C, Schuit R, Stojkovic T, Torrente Y, Wein N, Pecheux C, Levy N. 2009b. Identification of different genomic deletions and one duplication in the dysferlin gene using multiplex ligation-dependent probe amplification and genomic quantitative PCR. *Genet Test Mol Biomarkers* 13(4):439-42.
- Krahn M, Wein N, Bartoli M, Lostal W, Courrier S, Bourg-Alibert N, Nguyen K, Vial C, Streichenberger N, Labelle V, DePetris D, Pecheux C, Leturcq F, Chau P, Richard I, Levy N. 2010. A naturally occurring human minidysferlin protein repairs sarcolemmal lesions in a mouse model of dysferlinopathy. *Sci Transl Med* 2(50):50ra69.
- Laval SH, Bushby K M. 2004. Limb-girdle muscular dystrophies--from genetics to molecular pathology. *Neuropathol Appl Neurobiol* 30(2):91-105.
- Lennon NJ, Kho A, Bacskai BJ, Perlmutter SL, Hyman BT, Brown RH, Jr. 2003. Dysferlin interacts with annexins A1 and A2 and mediates sarcolemmal wound-healing. *J Biol Chem* 278(50):50466-73.
- Leshinsky-Silver E, Argov Z, Rozenboim L, Cohen S, Tzofit Z, Cohen Y, Wirguin Y, Dabby R, Lev D, Saadeh M. 2007. Dysferlinopathy in the Jews of the Caucasus: a frequent mutation in the dysferlin gene. *Neuromuscul Disord* 17(11-12):950-4.
- Letunic I, Doerks T, Bork P. 2009. SMART 6: recent updates and new developments. *Nucleic Acids Res* 37(Database issue):D229-32.
- Levy N, Wein N, Barthelemy F, Moulou V, Garcia L, Kohn M, Bartoli M. 2010. Therapeutic exon 'switching' for dysferlinopathies? *Eur J Hum Genet* 18(9):969-70; author reply 971.

- Liu J, Aoki M, Illa I, Wu C, Fardeau M, Angelini C, Serrano C, Urtizberea JA, Hentati F, Hamida MB, Bohlega S, Culper EJ, Amato AA, Bossie K, Oeltjen J, Bejaoui K, McKenna-Yasek D, Hosler BA, Schurr E, Arahata K, de Jong PJ, Brown RH, Jr. 1998. Dysferlin, a novel skeletal muscle gene, is mutated in Miyoshi myopathy and limb girdle muscular dystrophy. *Nat Genet* 20(1):31-6.
- Mahjneh I, Bushby K, Pizzi A, Bashir R, Marconi G. 1996. Limb-girdle muscular dystrophy: a follow-up study of 79 patients. *Acta Neurol Scand* 94(3):177-89.
- Nguyen K, Bassez G, Bernard R, Krahn M, Labelle V, Figarella-Branger D, Pouget J, Hammouda el H, Beroud C, Urtizberea A, Eymard B, Leturcq F, Levy N. 2005. Dysferlin mutations in LGMD2B, Miyoshi myopathy, and atypical dysferlinopathies. *Hum Mutat* 26(2):165.
- Nguyen K, Bassez G, Krahn M, Bernard R, Laforet P, Labelle V, Urtizberea JA, Figarella-Branger D, Romero N, Attarian S, Leturcq F, Pouget J, Levy N, Eymard B. 2007. Phenotypic study in 40 patients with dysferlin gene mutations: high frequency of atypical phenotypes. *Arch Neurol* 64(8):1176-82.
- Okahashi S, Ogawa G, Suzuki M, Ogata K, Nishino I, Kawai M. 2008. Asymptomatic sporadic dysferlinopathy presenting with elevation of serum creatine kinase. Typical distribution of muscle involvement shown by MRI but not by CT. *Intern Med* 47(4):305-7.
- Paradas C, Gonzalez-Quereda L, De Luna N, Gallardo E, Garcia-Consuegra I, Gomez H, Cabello A, Illa I, Gallano P. 2009. A new phenotype of dysferlinopathy with congenital onset. *Neuromuscul Disord* 19(1):21-5.
- Patel P, Harris R, Geddes SM, Strehle EM, Watson JD, Bashir R, Bushby K, Driscoll PC, Keep NH. 2008. Solution structure of the inner DysF domain of myoferlin and implications for limb girdle muscular dystrophy type 2b. *J Mol Biol* 379(5):981-90.
- Pramono ZA, Lai PS, Tan CL, Takeda S, Yee WC. 2006. Identification and characterization of a novel human dysferlin transcript: *dysferlin_v1*. *Hum Genet* 120(3):410-9.
- Pramono ZA, Tan CL, Seah IA, See JS, Kam SY, Lai PS, Yee WC. 2009. Identification and characterization of human dysferlin transcript variants: implications for dysferlin mutational screening and isoforms. *Hum Genet* 125(4):413-20.
- Salani S, Lucchiari S, Fortunato F, Crimi M, Corti S, Locatelli F, Bossolasco P, Bresolin N, Comi GP. 2004. Developmental and tissue-specific regulation of a novel dysferlin isoform. *Muscle Nerve* 30(3):366-74.
- Santos R, Oliveira J, Vieira E, Coelho T, Carneiro AL, Evangelista T, Dias C, Fortuna A, Geraldo A, Negrao L, Guimaraes A, Bronze-da-Rocha E. 2010. Private dysferlin exon skipping mutation (c.5492G>A) with a founder effect reveals further alternative splicing involving exons 49-51. *J Hum Genet* 55(8):546-9.
- Seror P, Krahn M, Laforet P, Leturcq F, Misonobe T. 2008. Complete fatty degeneration of lumbar erector spinae muscles caused by a primary dysferlinopathy. *Muscle Nerve* 37(3):410-4.
- Spuler S, Carl M, Zabojszcza J, Straub V, Bushby K, Moore SA, Bähring S, Wenzel K, Vinkemeier U, Rocken C. 2008. Dysferlin-deficient muscular dystrophy features amyloidosis. *Ann Neurol* 63(3):323-8.
- Stenson PD, Ball EV, Howells K, Phillips AD, Mort M, Cooper DN. 2009. The Human Gene Mutation Database: providing a comprehensive central mutation database for molecular diagnostics and personalized genomics. *Hum Genomics* 4(2):69-72.
- Tagawa K, Ogawa M, Kawabe K, Yamanaka G, Matsumura T, Goto K, Nonaka I, Nishino I, Hayashi YK. 2003. Protein and gene analyses of dysferlinopathy in a large group of Japanese muscular dystrophy patients. *J Neurol Sci* 211(1-2):23-8.
- Takahashi T, Aoki M, Tateyama M, Kondo E, Mizuno T, Onodera Y, Takano R, Kawai H, Kamakura K, Mochizuki H, Shizuka-Ikeda M, Nakagawa M, Yoshida Y, Akanuma J, Hoshino K, Saito H, Nishizawa M, Kato S, Saito K, Miyachi T, Yamashita H, Kawai M, Matsumura T, Kuzuhara S, Ibi T, Sahashi K, Nakai H, Kohnosu T, Nonaka I, Arahata K, Brown RH, Jr., Itoyama Y. 2003. Dysferlin mutations in Japanese Miyoshi myopathy: relationship to phenotype. *Neurology* 60(11):1799-804.
- Therrien C, Dodig D, Karpati G, Sinnreich M. 2006. Mutation impact on dysferlin inferred from database analysis and computer-based structural predictions. *J Neurol Sci* 250(1-2):71-8.
- Ueyama H, Kumamoto T, Horinouchi H, Fujimoto S, Aono H, Tsuda T. 2002. Clinical heterogeneity in dysferlinopathy. *Intern Med* 41(7):532-6.
- Urtizberea JA, Bassez G, Leturcq F, Nguyen K, Krahn M, Levy N. 2008. Dysferlinopathies. *Neurol India* 56(3):289-97.

- Vernengo L, Oliveira J, Krahn M, Vieira E, Santos R, Carrasco L, Negro L, Panuncio A, Leturcq F, Labelle V, Bronze-da-Rocha E, Mesa R, Pizzarossa C, Levy N, Rodriguez MM. 2011. Novel ancestral Dysferlin splicing mutation which migrated from the Iberian peninsula to South America. *Neuromuscul Disord*.
- Vilchez JJ, Gallano P, Gallardo E, Lasa A, Rojas-Garcia R, Freixas A, De Luna N, Calafell F, Sevilla T, Mayordomo F, Baiget M, Illa I. 2005. Identification of a novel founder mutation in the DYSF gene causing clinical variability in the Spanish population. *Arch Neurol* 62(8):1256-9.
- Walter MC, Braun C, Vorgerd M, Poppe M, Thirion C, Schmidt C, Schreiber H, Knirsch UI, Brummer D, Muller-Felber W, Pongratz D, Muller-Hocker J, Huebner A, Lochmuller H. 2003. Variable reduction of caveolin-3 in patients with LGMD2B/MM. *J Neurol* 250(12):1431-8.
- Wang B, Yang Z, Brisson BK, Feng H, Zhang Z, Welch E, Peltz S, Barton ER, Brown RH, Jr., Sweeney HL. 2010. Membrane blebbing as an assessment of functional rescue of dysferlin-deficient human myotubes via nonsense suppression. *J Appl Physiol*.
- Weiler T, Bashir R, Anderson LV, Davison K, Moss JA, Britton S, Nylen E, Keers S, Vafiadaki E, Greenberg CR, Bushby CR, Wrogemann K. 1999. Identical mutation in patients with limb girdle muscular dystrophy type 2B or Miyoshi myopathy suggests a role for modifier gene(s). *Hum Mol Genet* 8(5):871-7.
- Wein N, Avril A, Bartoli M, Beley C, Chaouch S, Laforet P, Behin A, Butler-Browne G, Mouly V, Krahn M, Garcia L, Levy N. 2010. Efficient bypass of mutations in dysferlin deficient patient cells by antisense-induced exon skipping. *Hum Mutat* 31(2):136-42.
- Wenzel K, Geier C, Qadri F, Hubner N, Schulz H, Erdmann B, Gross V, Bauer D, Dechend R, Dietz R, Osterziel KJ, Spuler S, Ozcelik C. 2007. Dysfunction of dysferlin-deficient hearts. *J Mol Med* 85(11):1203-14.