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MUTATION IN BRIEF

Analysis of the *DYSF* Mutational Spectrum in a Large Cohort of Patients

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Dysferlinopathies belong to the heterogeneous group of autosomal recessive muscular dystrophies. Mutations in the gene encoding dysferlin (*DYSF*) lead to distinct phenotypes, mainly Limb Girdle Muscular Dystrophy type 2B (LGMD2B) and Miyoshi myopathy (MM). Here, we analysed the mutational data from the largest cohort described to date, a cohort of 134 patients, included based on clinical suspicion of primary dysferlinopathy and/or dysferlin protein deficiency identified on muscle biopsy samples. Data were compiled from 38 patients previously screened for mutations in our laboratory (Nguyen, et al., 2005; Nguyen, et al., 2007), and 96 supplementary patients screened for *DYSF* mutations using genomic DHPLC analysis, and subsequent sequencing of detected variants, in a routine diagnostic setting. In 89 (66%) out of 134 patients, molecular analysis identified two disease-

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causing mutations, confirming the diagnosis of primary Dysferlinopathy on a genetic basis. Furthermore, one mutation was identified in 30 patients, without identification of a second deleterious allele. We are currently developing complementary analysis for patients in whom only one or no disease-causing allele could be identified using the genomic screening procedure. Altogether, 64 novel mutations have been identified in this cohort, which corresponds to approximately 25% of all *DYSF* mutations reported to date. The mutational spectrum of this cohort significantly shows a higher proportion of nonsense mutations, but a lower proportion of deleterious missense changes as compared to previous series. © 2008 Wiley-Liss, Inc.

KEY WORDS: Dysferlin, DYSF, Dysferlinopathy, Limb Girdle Muscular Dystrophy, LGMD2B, Miyoshi myopathy, Mutation analysis.

INTRODUCTION

Mutations in the gene encoding dysferlin (*DYSF*; MIM# 603009, 2p13, GenBank NM_003494.2) cause Limb Girdle Muscular Dystrophy type 2B (LGMD2B; MIM# 253601; Bashir, et al., 1998) and Miyoshi myopathy (MM; MIM# 254130)(Liu, et al., 1998). Additionally, mutations in *DYSF* cause a wide spectrum of phenotypes, ranging from isolated HyperCPKemia to severe disability (Nguyen et al., 2007). Therefore, the generic term of "primary dysferlinopathies" usually defines this heterogeneous group of autosomal recessive muscular dystrophies, caused by mutations in *DYSF*.

Due to the clinical heterogeneity, initial dysferlin protein analysis on muscle biopsy samples is essential to orientate diagnosis. However, diagnosis should be confirmed by molecular analysis of the *DYSF* gene (Bushby, 1999).

DYSF is a large-sized gene (>230 kbp, 55 exons, 6243 coding base pairs) (Aoki et al., 2001; Bashir et al., 1998; Liu et al., 1998). Also, more than 300 different sequence variants, including deleterious mutations and non pathogenic polymorphism, have been reported to date (Leiden Muscular Dystrophy pages © www.dmd.nl). These variants are spread along the entire coding sequence, without any apparent mutational "Hotspot" (exception made of founder mutations). Therefore, methods for mutation screening are particularly useful for efficient molecular analysis on a routine basis.

We previously used SSCP (Single Strand Confirmation Polymorphism analysis) or DHPLC (Denaturing High Pressure Liquid Chromatography) for mutational screening in a series of patients (Nguyen et al., 2005; Nguyen et al., 2007).

In order to better define the mutational spectrum of *DYSF*, we here included 96 additional patients and report the results of mutational screening in the largest cohort reported to date. Altogether, we analysed the molecular data of 134 patients included after diagnostic suspicion of primary dysferlinopathy.

PATIENTS, MATERIALS AND METHODS

Patients: Inclusion criteria and definition of the cohort used for mutational data analysis

Inclusion criteria for index patients were: (i) clinical phenotype consistent with LGMD2 or distal myopathy, and (ii) loss or strong reduction of dysferlin expression evidenced by Western-blotting and/or immunohistochemistry on muscle biopsy. When no biopsy was available (patients indicated in Table 1), patients were included after clinical diagnostic suspicion of LGMD2B or MM. Clinical examination was carried out by neurologists from the French Network on LGMD, mainly from the Institut de Myologie, Hôpital de la Pitié Salpêtrière, AP-HP, Paris, and the Service des maladies neuromusculaires, Hôpital Timone, AP-HM, Marseille, France.

DYSF mutational data analysis was carried out on a cohort of 134 patients, for which mutational screening has been performed in our laboratory including 38 patients previously reported by our laboratory (Nguyen, et al., 2005; Nguyen, et al., 2007), and 96 newly included patients. Among the 134 included patients (Table 1), 113 were initial index cases, including 95 sporadic cases, and 18 familial index cases. A total of 21 affected relatives was also

included. The familial cases subdivide as follows: 15 families with one other affected member, and 3 families with two other affected members.

Protein analysis

Immunohistochemistry and multiplex Western-blotting on muscle biopsies were carried out as previously described (Anderson and Davison, 1999), using antibodies to dysferlin (NCL-Hamlet antibody, Novocastra Newcastle upon Tyne, UK).

Genomic mutation screening and mutational data analysis

After informed consent, genomic DNA was extracted from peripheral blood obtained from all patients. The 55 exons and flanking intronic boundaries of *DYSF* were PCR-amplified, then analysed using DHPLC and subsequent direct sequencing of abnormally eluted fragments, as previously described (Nguyen et al., 2005).

Sequence chromatograms were analysed using the Sequencher® software (Gene Codes Corporation, Ann Arbor, MI, USA) and compared to the human *DYSF* sequence (NM_003494.2). Mutational data are described using the nomenclature of the Human Genome Variation Society (www.hgvs.org/mutnomen). Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines. The initiation codon is codon 1.

Mutational data were compiled and analysed using the bioinformatics algorithms available in the UMD software package (Beroud et al., 2000; Beroud et al., 2005).

Sequence variants identified in this study were determined as deleterious : (i) if pathogenicity had been previously reported in the Leiden Muscular Dystrophy pages database © (www.dmd.nl), based on reports in the literature or direct submission; or (ii) for novel sequence variants, if the type of mutation is *per se* deleterious (nonsense-, frameshifting-, splice-donor-site- or splice-acceptor-site mutations), or for variants of missense-type, a pathogenicity score of ≥65 determined using a novel algorithm, "UMD predictor" (Frederic et al., submitted) and absence of the variant in at least 200 control chromosomes. Selected intronic variants were further analysed using the Splicing Sequences Finder prediction algorithm (www.umd.be/SSF/).

All novel disease-causing mutations identified in this study were submitted to the Leiden Muscular Dystrophy pages database © (www.dmd.nl) (december 2007).

RESULTS

DYSF mutational data analysis was carried out on a cohort of 134 patients, included for genomic mutational screening following diagnostic suspicion of primary dysferlinopathy.

Identification of *DYSF* disease-causing mutations and genetic diagnosis of primary dysferlinopathy in the patients (Table 1)

Altogether, 212 deleterious mutations were identified in the 134 patients included in this study. Two mutations clearly considered as disease-causing were identified in 89 patients (66%), including three patients in whom more than two mutations initially considered as possibly disease-causing were identified (patients F1-18-1-2, F1-47-1-2, and F1-65-1-2). The mutational status of the included patients subdivides as follows.

In 49 index patients, and 8 corresponding relatives, two disease-causing mutations were identified at a compound heterozygous state.

24 index patients, and 8 corresponding relatives, carry homozygous mutations in a context of known familial consanguinity.

In addition, 4 index patients from families with no known consanguinity were found to be homozygous.

In 30 patients (26 index patients and 4 corresponding relatives), only one mutation undoubtedly considered as disease-causing was identified. No disease-causing mutation was found in 10 index patients and 1 corresponding relative.

In the different groups of patients, we identified sequence variants that have been previously reported, but without conclusive information on pathogenicity, and not previously reported sequence variants (listed in Table 1

and Table 2; bioinformatics analysis of pathogenicity detailed in Table 3 for exonic variants, in Table 4 for intronic variants).

Additionally, different sequence variants previously reported as non pathogenic polymorphism (Leiden Muscular Dystrophy pages © www.dmd.nl) were identified in the patients (data not shown).

Identification of novel disease-causing mutations and analysis of the *DYSF* mutational spectrum

We identified a total of 22 distinct novel mutations, considered as clearly deleterious regarding the type of mutation (nonsense-, frameshifting-, splice-donor-site- or splice-acceptor-site mutations) (Table 2).

Moreover, using bioinformatics predictive algorithms, we analysed variants identified in this study as corresponding to: (i) previously reported disease-causing missense mutations, novel missense and isosemantic exonic variants (Table 3) and (ii) previously reported and novel intronic variants whose pathogenicity is unknown (Table 4).

We identified 8 novel missense variants considered to be pathogenic after bioinformatics analysis (UMD predictor pathogenicity score ≥65)(Table 3): c.463G>A (p.Gly155Arg, exon 6); c.851T>C (p.Ile284Thr, exon 8); c.1276G>A (p.Gly426Arg, exon 13); c.2192C>G (p.Pro731Arg, exon 23); c.3086C>T (p.Pro1029Leu, exon 29); c.3683T>C (p.Leu1228Pro, exon 33); c.4577A>C (p.Lys1526Thr, exon 42); c.5908C>T (p.Pro1970Ser, exon 52).

A deleterious effect was excluded for 8 novel exonic variants (7 isosemantic and 1 missense)(Table 3) and 8 novel intronic variants (Table 4).

We analysed the distribution of disease-causing mutations identified in index cases included in this study. The deleterious mutations were distributed along the entire coding sequence, without identified clustering of mutations in a particular exon or domain (Figure 1A).The types of mutations were as follows: 28% of frameshifting mutations, 26% of missense mutations, 26% of nonsense mutations, 18% of intronic mutations and 1% of in-frame deletions (Figure 1B).

Clinical data

Detailed clinical data were available only for 38 patients, indicated in Table 1, and have been reported elsewhere (Nguyen et al., 2005; Nguyen et al., 2007). Briefly, these patients subdivided as: 10 cases of MM, 9 cases of LGMD, 13 cases of proximo-distal myopathy (distal limb-girdle myopathy (Ueyama et al., 2002)), 4 cases of pseudo-metabolic myopathy, and 2 cases of isolated hyperCPKemia.

Regarding the 96 patients additionally included, available clinical information is detailed in Table 1. At onset, 51 patients presented with typical features of MM, 34 with LGMD (including respectively 25 and 21 cases with available results of Western blot analysis showing severely reduced/absence of dysferlin), 6 with isolated hyperCPKemia, and one with myalgia. One additional case initially diagnosed as MM presented normal expression of dysferlin on Western blot. No information on the initial phenotype at inclusion was available for 3 patients, thus included only on the basis of absent dysferlin expression.

Altogether, results from dysferlin Western blot analysis were available for 90 patients. In 89 cases (including 5 affected relatives of index patients) a severely reduced quantity or absence of dysferlin was found.

We compared the type of disease-causing mutations between patients classified respectively as affected with a “MM”, and a “LGMD” phenotype (Figure 1B). No significant difference was evidenced between the two subgroups.

DISCUSSION

The aim of this study was to analyse mutational data from a large cohort of patients, to further characterise the *DYSF* mutational spectrum. Altogether, we identified 212 mutations clearly considered as disease-causing in our cohort of 134 patients.

As expected from previous reports (Leiden Muscular Dystrophy pages © www.dmd.nl), showing that most of the *DYSF* disease-causing mutations are private sequence variants, we identified a high proportion of novel

mutations in the newly included patients: we identified 30 not previously reported deleterious mutations (in addition to 34 novel mutations identified in part of this cohort, and already reported (Nguyen et al., 2005; Nguyen et al., 2007)), considered as deleterious either because of the mutation-type, or, in case of missense variants, based on bioinformatics analysis. Among these novel mutations, two were identified each in two non related patients, and may therefore be rare recurrent mutations: c.2217C>A (p.Tyr739X, exon 23) and c.4005+1G>A (IVS 37).

At completion of the present study (February 2008), 352 different *DYSF* sequence variants (including submissions from our laboratory) were referenced in the Leiden Muscular Dystrophy pages database (database update February 27, 2008) (Leiden Muscular Dystrophy pages © www.dmd.nl), including 82 variants classified as non pathogenic polymorphism. Altogether, the disease-causing mutations identified in this cohort account for ~ 25% of known pathogenic *DYSF* variants (30 novel mutations identified in part of this cohort, and 34 already reported (Nguyen et al., 2005; Nguyen et al., 2007)).

A precise determination of the *DYSF* mutational spectrum is important to better define diagnostic strategies, but also to define possible therapeutic strategies using emerging gene-, cell-, or pharmacological-therapy approaches for autosomal recessive muscular disorders (Daniele et al., 2007). The type and distribution of mutations in this cohort were analysed in index cases by counting both disease-causing mutations for proven or possible compound heterozygotes, and only once for homozygotes (total of 152 mutations analysed). This allows an overview of the different disease-causing alleles to better define the *DYSF* mutational spectrum (Figure 1B). Moreover, for this autosomal recessive disease, functional restoration of one deleterious allele is expected to have a therapeutic benefit. Therefore, the data from this analysis also allow better determining the mutations to be possibly targeted in the patients, by mutation-specific therapies for primary dysferlinopathies.

As described in previous reports, we found no "mutational hotspot", mutations being positioned over the entire coding-sequence. However, in comparison to data available from the literature regarding the type of mutations, we found a lower proportion of point mutations (52% vs. 64%; p=0.002). Additionally, we identified a higher proportion of nonsense mutations (26% vs. 18%; p=0.045), but a lower proportion of deleterious missense changes (26% vs. 46%; p<0.001). This difference may at least partially be caused by a mis-interpretation of the pathogenicity for some previously identified missense changes. Interestingly, nonsense mutations, identified in a relatively high proportion in this study, are particular targets for novel therapeutic approaches (Daniele et al., 2007).

From a clinical genetics point of view, our study validated the efficacy of genomic *DYSF* mutational screening for routine diagnosis. By identifying either compound heterozygous mutations, or homozygous mutations (in cases with known familial consanguinity), diagnosis of primary dysferlinopathy was confirmed on a genetic basis in 89 patients (66%).

However, this value should be considered as a careful underestimation of the actual value. In four (F1-8-1-1, F1-67-1-2, F1-139-1-1 and F1-156-1-2) patients, mutations were identified at a homozygous state, without known consanguinity. A pseudo-homozygous state, correlated to a possible, non identified large genomic deletion in *trans*, could not be excluded in these patients, due to lack of available samples from their parents. Moreover, in 4 index patients (F1-29-1-2, F1-55-1-1, F1-82-1-1, F1-83-1-2) with only one identified mutation, clearly considered as disease-causing, we found additional possibly deleterious, missense or isosemantic exonic sequence variants. Also, in patient F1-3-1-2 and her affected relative F1-3-2-2, a novel missense variant in exon 52 (c.5899G>A, p.Gly1967Ser) was identified at a homozygous state, and correlates with absence of dysferlin on Western blot in both patients. Bioinformatics analysis predicts a pathogenicity score of 63, just below the predictive pathogenicity threshold of 65. Therefore, the implication of this variant remains unclear, but is yet likely. In patient F1-123-1-1, the intronic variant c.1180+5G>A (Leiden Muscular Dystrophy pages © www.dmd.nl) is predicted to create a cryptic splice donor site. This has to be further validated at the transcriptional level.

We rely on a simple procedure of genomic DHPLC screening (Nguyen et al., 2005). Nonetheless, for all patients, with only one, or no mutation clearly considered as disease-causing, complementary analysis are necessary. We are currently collecting additional samples from these patients, to analyse possible defects at the transcriptional level (de Luna et al., 2006), and screen for large genomic rearrangements, both of which could be missed using genomic DHPLC screening. In our hands, we consider these techniques as valuable complementary approaches, but difficult to use regarding the important number of samples processed, on a routine basis in our laboratory. Exhaustive molecular screening will also allow, in combination with familial analyses, the identification of possible symptomatic heterozygotes (Illa et al., 2007). Finally, differential diagnosis has to be considered in some cases (Bushby, 1999). In particular, patient F1-54-1-1 presented initially with a MM

phenotype, but without dysferlin-deficiency on Western blot analysis. Even if a functional defect, without any related quantitative effect, could result from one or several variants identified in this patient, this hypothesis is unlikely.

Noteworthy, more than two mutations initially considered as possibly disease-causing (as defined in "Patients, Materials and Methods") were identified in patients F1-18-1-2, F1-47-1-2 and F1-65-1-2.

In patient F1-18-1-2, we previously reported the simultaneous presence in exon 47 of a homozygous non-sense mutation, and a missense change predicted to be pathogenic using bioinformatics analysis. This patient carries in addition a homozygous missense change in exon 6, previously reported as pathogenic (Leiden Muscular Dystrophy pages © www.dmd.nl). Patient F1-47-1-2 is homozygous for a recurrent splice donor site mutation of intron 25 (Leiden Muscular Dystrophy pages © www.dmd.nl), and a novel missence change in exon 42, also predicted to be deleterious. Patient F1-65-1-2 carries a heterozygous frameshifting deletion in exon 50, in addition to two heterozygous missense changes previously reported as pathogenic (Leiden Muscular Dystrophy pages © www.dmd.nl). In these three patients, we could not yet rely on segregation analysis to further evaluate the deleterious, or possibly hypomorphic, implication of the different variants. Even if simultaneous presence of more than two disease-causing mutations cannot be firmly excluded (Drake et al., 2005), further investigation are mandatory before concluding these cases.

Whenever a patient simultaneously carries novel variants, but also two mutations clearly considered as disease-causing, the pathogenicity of the former may be indirectly excluded. In correlation with bioinformatics analyses, this allowed us to exclude in the present series a deleterious effect of 8 exonic variants (7 isosemantic and 1 missense)(Table 3), and 8 intronic variants (Table 4). Two additional novel intronic variants (IVS4: c.343-29A>G, and IVS38: c.4168-40G>A) are candidates for further transcriptional analyses, regarding a possible hypomorphic effect.

As our study was designed for diagnostic purposes, we did not carry out further detailed frequency evaluation of the other variants, classified as most likely non-pathogenic. Such polymorphism analyses are time-consuming and cost-ineffective on a routine basis, and would better suit to large-scale sequencing platforms that should be available in the next future (Mardis, 2006).

Detailed clinical reassessment had been previously done in a subset of the patients described in this study (Nguyen et al., 2007), and identified important variability in the disease phenotype, but without apparent genotype-phenotype correlation. For the 96 additionally included patients, precise clinical data were not available. We therefore compared the types of pathogenic mutations for the two main defined phenotypes, MM and LGMD. In concordance with data from the literature (Glover and Brown, 2007), no significant difference was identified between these two subgroups, further sustaining the possible influence of genetic and/or environmental modifiers on the determination of the primary phenotype. The collection of precise and homogeneous clinical data, and their correlation to genetic findings thus remains an important task, that should take into account the existence of phenotypical "subgroups" among patients affected with primary dysferlinpathy (Nguyen et al., 2007). Moreover, novel large-scale mutational analysis tools, such as genomic micro-array based techniques, should allow to better analyse possible modifying effects of SNPs in the *DYSF* gene, and genes coding for DYSF interacting protein partners.

Table 1: Genomic mutational findings in the patients included in this study.

Mutational data are described using the nomenclature of the Human Genome Variation Society (www.hgvs.org/mutnomen). Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (human *DYSF*, GenBank NM_003494.2), according to journal guidelines. The initiation codon is codon 1.
*As defined in "Patients, Materials and Methods".
** Effects on the amino-acid sequence are predicted from the cDNA sequence.
*** Sequence variants previously reported as non pathogenic polymorphism are not listed. If available, SNP accession numbers are indicated in brackets (from dbSNP; www.ncbi.nlm.nih.gov/SNP/)
# Patients included for analysis of the *DYSF* mutational spectrum, whereas available detailed clinical information and genomic data have been previously evaluated (Nguyen et al., 2007).
Patient identification-data for relatives of index cases are in ***bold-italized***. Novel sequence variants are in **bold**.
Abn. Spl.: abnormal splicing. (CONSANGUINITY): known consanguinity, specified in case of homozygous sequence variants. F: Female. HTZ: heterozygous. HyperCPK: isolated hyperCPKemia. IH: Immunohistochemistry. LGMD: Limb Girdle Muscular Dystrophy. M: Male. MM: Miyoshi myopathy. NA: information not available. (ND-R): not done, relative of an affected index patient. PD: proximo-distal phenotype. PM: pseudo-metabolic phenotype. SPDCM: patients presenting with several mutations considered as disease-causing using the definition detailed in "Patients, Materials and Methods".
UMD: pathogenicity score analysed using "UMD predictor" (Frederic et al., submitted); indicated for all novel missense or isosemantic changes, and in case of SPDCM.
: identification of only one disease-causing mutation using the definition detailed in "Patients, Materials and Methods".

Patient **Identification and Gender/Age**	**Phenotype** **A= Age of onset** **B=initial phenotype** **C=inflammatory signs on muscle biopsy** **D=CPK level** **E=progression after 10 years**	**DYSF IH or Western blot analysis findings**	**Genomic mutational findings:** **Deleterious sequence variants***	**Deleterious effect****	**Genomic mutational findings:** **Sequence variants of undetermined pathogenicity*****
F1-1-1-1# M 66years	PD#	Absence	IVS8: c.855+1delG HTZ and ex29: c.3126G>A HTZ	Abn.Spl. (p.Trp1042X)	-
F1-100-1-1# M 34years	PD#	Absence	ex47: c.5302C>T HTZ and ex53: c.5979dupA HTZ	(p.Arg1768Trp) (p.Glu1994fsX3)	-
F1-103-1-1 M 22years	A: NA B: LGMD C: NA D: NA E: NA	Absence	ex2: c.107_108delAA HTZ and **ex31: c.3389_3399dupTCTCCACCTTG** HTZ	(p.Lys36SerfsX11) **(p.Phe1135ProfsX3)**	IVS7: c.792+11T>C (rs13428076) HTZ; **IVS38: c.4168-40G>A** HTZ
F1-103-2-1 M NA	A: NA B: HyperCPK C: NA D: NA E: NA	(ND-R)	ex2: c.107_108delAA HTZ and **ex31: c.3389_3399dupTCTCCACCTTG** HTZ	(p.Lys36SerfsX11) **(p.Phe1135ProfsX3)**	-

Patient Identification and Gender/Age	**Phenotype A= Age of onset B=initial phenotype C=inflammatory signs on muscle biopsy D=CPK level E=progression after 10 years**	**DYSF IH or Western blot analysis findings**	**Genomic mutational findings: Deleterious sequence variants***	**Deleterious effect****	**Genomic mutational findings: Sequence variants of undetermined pathogenicity*****
F1-104-1-2# F 21years	LGMD#	Absence	**IVS5: c.457+1dupG** homozygous (CONSANGUINITY)	Abn.Spl.	**IVS1: c.89-29C>G** homozygous
F1-108-1-1 M 54years	A: 23 years B: LGMD C: NA D: NA E: Non ambulant	Absence	ex12: c.1064_1065delAA HTZ and **ex23: c.2217C>A** HTZ	(p.Lys355ArgfsX4) **(p.Tyr739X)**	-
F1-108-2-2 F 51years	A: 23 years B: LGMD C: NA D: NA E: Ambulant	(ND-R)	ex12: c.1064_1065delAA HTZ and **ex23: c.2217C>A** HTZ	(p.Lys355ArgfsX4) **(p.Tyr739X)**	-
F1-11-1-1 M 31years	A: ~20 years B: MM C: No D: NA E: Ambulant	NA	ex11: c.1020C>A HTZ	(p.Ser340Arg)	**IVS6: c.664-17C>T** HTZ; **IVS42: c.4639-38G>A** HTZ
F1-111-2-2 F 47years	A: NA B: MM C: NA D: NA E: NA	NA	ex28: c.2975G>A homozygous (CONSANGUINITY)	(p.Trp992X)	IVS3: c.236+20G>A (rs12470028) homozygous
F1-112-1-2 F 19years	A: 17 years B: MM C: NA D: x35N E: NA	Absence	ex4: c.265C>T HTZ and ex29: c.3126G>A HTZ	(p.Arg89X) (p.Trp1042X)	-
F1-113-1-1# M 64years	HyperCPK#	Absence	ex6: c.509C>A HTZ and ex19: c.1663C>T HTZ	(p.Ala170Glu) (p.Arg555Trp)	**ex11: c.1004G>C (p.Gly335Ala)** homozygous UMD59
F1-114-1-2 F 59years	A: 18 years B: LGMD C: No D: NA E: Non ambulant	NA	ex19: c.1663C>T HTZ and **IVS37: c.4005+1G>A** HTZ	(p.Arg555Trp) Abn.Spl.	-
F1-116-1-1 M 47years	A: NA B: MM C: NA D: NA E: NA	NA	ex46: c.5078G>A HTZ	(p.Arg1693Gln)	-
F1-117-1-1# M 34years	MM#	Absence	ex29: c.3112 C>T HTZ and ex34: c.3832C>T HTZ	(p.Arg1038X) (p.Gln1278X)	-

Patient Identification and Gender/Age	**Phenotype A= Age of onset B=initial phenotype C=inflammatory signs on muscle biopsy D=CPK level E=progression after 10 years**	**DYSF IH or Western blot analysis findings**	**Genomic mutational findings: Deleterious sequence variants***	**Deleterious effect****	**Genomic mutational findings: Sequence variants of undetermined pathogenicity*****
F1-118-1-2# F 37years	PD#	Absence	IVS25: c.2643+1G>A HTZ and IVS38: c.4167+1G>C HTZ	Abn.Spl. Abn.Spl.	-
F1-119-1-2 F 39years	A: NA B: MM C: NA D: NA E: NA	NA	IVS8: c.855+1delG homozygous (CONSANGUINITY)	Abn.Spl.	-
F1-12-1-2 F NA	A: NA B: MM C: NA D: NA E: NA	NA	ex39: c.4200dupC homozygous (CONSANGUINITY)	(p.Ile140HisfsX8)	-
F1-123-1-1 M 40years	A: ~20 years B: MM C: NA D: NA E: NA	Absence	IVS8: c.855+1delG HTZ	Abn.Spl.	IVS12: c.1180+5G>A HTZ**;** **IVS13: c.1285-35G>T** HTZ
F1-125-1-0 (NA)	A: NA B: LGMD C: NA D: NA E: NA	NA	Ex50: c.5594delG HTZ	(p.Gly1865AlafsX101)	-
F1-13-1-1# M 44years	MM#	Absence	IVS8: c.855+1delG HTZ and ex52: c.5813_5821del HTZ	Abn.Spl. (p.Thr1938_Lys1940delinsLys)	-
F1-130-1-1 M 37years	A: NA B: MM C: No D: NA E: NA	(ND-R)	ex34: c.3826C>G HTZ and IVS34: c.3843+1G>A HTZ	(p.Leu1276Val) Abn.Spl.	-
F1-130-2-2# F 47years	PD#	Absence	ex34: c.3826C>G HTZ and IVS34: c.3843+1G>A HTZ	(p.Leu1276Val) Abn.Spl.	-
F1-131-1-1# M 67years	PD#	Absence	ex6: c.490G>T HTZ and ex9: c. 895G>A HTZ	(p.Gly164X) (p.Gly299Arg)	-
F1-133-1-1 M 55years	A: NA B LGMD C: NA D: NA E: NA	Absence	**exon 6: c.591C>G** HTZ	**(p.Tyr197X)**	-

Patient **Identification and Gender/Age**	**Phenotype** **A= Age of onset** **B=initial phenotype** **C=inflammatory signs on muscle biopsy** **D=CPK level** **E=progression after 10 years**	**DYSF IH or Western blot analysis findings**	**Genomic mutational findings: Deleterious sequence variants***	**Deleterious effect****	**Genomic mutational findings: Sequence variants of undetermined pathogenicity*****
F1-135-1-2 F 61years	A: NA B: MM C: NA D: NA E: NA	Absence	IVS8: c.855+1delG HTZ and ex49: c.5509G>A HTZ	Abn.Spl. (p.Asp1837Asn)	-
F1-137-1-2 F 52years	A: NA B: LGMD C: NA D: NA E: NA	Absence	?	-	**ex52: c.5899G>A (p.Gly1967Ser)** homozygous UMD63
F1-138-1-1 M NA	A: NA B: MM C: NA D: NA E: NA	NA	ex50: c.5594delG homozygous (CONSANGUINITY)	(p.Gly1865AlafsX101)	**ex15: c.1362C>T (p.Ser454Ser)** homozygous UMD18
F1-138-2-1 M NA	A: NA B: MM C: NA D: NA E: NA	NA	ex50: c.5594delG homozygous (CONSANGUINITY)	(p.Gly1865AlafsX101)	-
F1-138-3-1 M NA	A: NA B: MM C: NA D: NA E: NA	NA	ex50: c.5594delG homozygous (CONSANGUINITY)	(p.Gly1865AlafsX101)	-
F1-139-1-1 M 47years	A: NA B: MM C: NA D: NA E: NA	Absence	ex51: c.5698_5699delAG homozygous	(p.Ser1900fsX14)	-
F1-14-1-1 M 72years	A: NA B: MM C: NA D: NA E: NA	NA	**ex12: c.1157_1168delTCCGGGCCGAGG** HTZ and ex19: c.1663C>T HTZ	**(p.Phe386_Asp390delinsTyr)** (p.Arg555Trp)	**IVS29: c.3175-61G>C** HTZ
F1-140-1-2 F 51years	A: ~45years B: MM C: NA D: NA E: Ambulant	(ND-R)	**IVS24: c.2511+1G>A** HTZ and ex29: c.3137G>A HTZ	Abn.Spl. (p.Arg1046His)	-

Patient Identification and Gender/Age	**Phenotype A= Age of onset B=initial phenotype C=inflammatory signs on muscle biopsy D=CPK level E=progression after 10 years**	**DYSF IH or Western blot analysis findings**	**Genomic mutational findings: Deleterious sequence variants***	**Deleterious effect****	**Genomic mutational findings: Sequence variants of undetermined pathogenicity*****
F1-140-2-1 M 53years	A: 45 years B: MM C: No D: x50N E: Ambulant	Severely reduced	**IVS24: c.2511+1G>A** HTZ and ex29: c.3137G>A HTZ	Abn.Spl. (p.Arg1046His)	-
F1-142-1-2 F 36years	A: NA B: MM C: NA D: NA E: NA	Absence	?	-	-
F1-143-1-1 M 36years	A: 20 years B: LGMD C: NA D: x100N E: Ambulant	Absence	ex12 : c.1064_1065delAA HTZ	(p.Lys355ArgfsX4)	**IVS41: c.4509+40C>T** homozygous
F1-145-1-1 M 68years	A: NA B: MM C: NA D: NA E: NA	NA	ex39: c.4200dupC HTZ	(p.Ile1401HisfsX8)	**IVS29: c.3175-61G>C** HTZ
F1-148-1-1 M 59years	A: 24 years B: LGMD C: No D: x5N E: Non ambulant	Absence	**IVS51: c.5767+1G>A** homozygous (CONSANGUINITY)	Abn.Spl.	**ex44: c.4867C>T (p.Leu1623Leu)** homozygous UMD23
F1-150-1-2# F 47years	PD#	Severely reduced	ex4: c.247delG HTZ and ex54: c.6124C>T HTZ	(p.Glu83LysfsX68) (p.Arg2042Cys)	-
F1-150-2-2# F 53years	LGMD#	(ND-R)	ex4: c.247delG HTZ and ex54: c.6124C>T HTZ	(p.Glu83LysfsX68) (p.Arg2042Cys)	-
F1-151-1-2# F 65years	LGMD#	Absence	ex42: c.4628 G>A HTZ	(p.Gly1543Asp)	**IVS10: c.938-34T>A** HTZ
F1-153-1-1# M 36years	PD#	Absence	ex11: c.1020C>A HTZ and ex50: c.5594delG HTZ	(p.Ser340Arg) (p.Gly1865AlafsX101)	-
F1-154-1-2 F 36years	A: NA B: LGMD C: NA D: NA E: NA	Absence	**ex8: c.799_800delTT** HTZ and **ex33: c.3687C>A** HTZ	**(p.Phe267LeufsX5)** **(p.Tyr1229X)**	-

Patient Identification and Gender/Age	**Phenotype A= Age of onset B=initial phenotype C=inflammatory signs on muscle biopsy D=CPK level E=progression after 10 years**	**DYSF IH or Western blot analysis findings**	**Genomic mutational findings: Deleterious sequence variants***	**Deleterious effect****	**Genomic mutational findings: Sequence variants of undetermined pathogenicity*****
F1-155-1-2 F 18years	A: 13 years B: HyperCPK C: NA D: x30N E: NA	Absence	ex7: c.757C>T HTZ and ex53: c.5979dupA HTZ	(p.Arg253Trp) (p.Glu1994ArgfsX3)	**ex43: c.4731G>A (p.Glu1577Glu)** HTZ UMD18
F1-156-1-2 F 50years	A: NA B: MM C: NA D: NA E: NA	Severely reduced	**ex52: c.5805delA** homozygous	**(p.Ala1946ProfsX30)**	-
F1-159-1-0 (NA)	A: NA B: LGMD C: NA D: NA E: NA	NA	IVS8: c.855+1delG HTZ and IVS28: c.3031+2T>C HTZ	Abn.Spl. Abn.Spl.	-
F1-159-2-0 (NA)	A: NA B: LGMD C: NA D: NA E: NA	NA	IVS8: c.855+1delG HTZ and IVS28: c.3031+2T>C HTZ	Abn.Spl. Abn.Spl.	-
F1-161-1-1 M 31years	A: NA B: MM C: NA D: NA E: NA	Severely reduced	IVS26: c. 2810+1G>A HTZ	Abn.Spl.	-
F1-162-1-2# F 43years	PM#	NA	ex26: c.2779delG homozygous (CONSANGUINITY)	(p.Ala927LeufsX21)	-
F1-163-1-1# M 57years	LGMD#	Absence	ex20: c.1834C>T HTZ and ex37: c.3967C>T HTZ	(p.Gln612X) (p.Gln1323X)	-
F1-163-2-2# F 31years	PD#	Absence	ex20: c.1834C>T HTZ and ex44: c.4872_4876delGCCCGinsCCCC HTZ	(p.Gln612X) (p.Glu1624AspfsX9)	-
F1-169-1-1 M NA	A: NA B: LGMD C: NA D: NA E: NA	NA	ex6: c.509C>A HTZ	(p.Ala170Glu)	-

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F1-17-1-1# M 51years	PD#	Absence	ex15: c.1392dupA homozygous (CONSANGUINITY)	(p.Asp465ArgfsX9)	-
F1-170-1-2 F 32years	A: 15 years B: MM C: NA D: x20N E: Ambulant	Absence	IVS8: c.855+1delG HTZ	Abn.Spl.	IVS5: c.457+17G>C HTZ
F1-170-2-2 F 32years	A: 15 years B: MM C: NA D: NA E: Ambulant	(ND-R)	IVS8: c.855+1delG HTZ	Abn.Spl.	IVS5: c.457+17G>C HTZ
F1-174-1-1 M 46years	A: 15 years B: NA C: Yes D: x5N E: Non ambulant	Absence	ex26: c.2790G>C HTZ and ex34: c.3832C>T HTZ	(p.Trp930Cys) (p.Gln1278X)	**ex33: c.3702T>C (p.Tyr1234Tyr)** HTZ UMD18
F1-179-1-1 M 49years	A: ~25years B: HyperCPK C: No D: x20N E: Ambulant	Absence	**ex52: c.5871_5872delGT** HTZ	**(p.Ser1958ProfsX3)**	-
F1-18-1-2# F 30years SPCDM	PD#	Absence	ex6: c.565C>G homozygous and ex47: c.5243A>T homozygous and ex47: c. 5296G>T homozygous (CONSANGUINITY)	(p.Leu189Val) UMD29 (p.Glu1748Val) UMD88 (p.Glu1766X)	**ex23: c.2283C>A (p.Gly761Gly)** homozygous UMD18
F1-182-1-2 F 42years	A: NA B: LGMD C: NA D: NA E: NA	Absence	ex51: c.5668-7G>A HTZ and **ex52: c.5908C>T** HTZ	Abn.Spl. **(p.Pro1970Ser)** UMD76	-
F1-183-1-2 F 68years	A: 40 years B: LGMD C: No D: NA E: Non ambulant	Severely reduced	**ex27: c.2894G>A** HTZ and ex39: c.4200dupC HTZ	**(p.Trp965X)** (p.Ile1401HisfsX8)	-
F1-184-1-2 F 30years	A: 27 years B: MM C: Yes D: x52N E: NA	NA	**ex13: c.1276G>A** HTZ and ex27: c.2858dupT HTZ	**(p.Gly426Arg)** UMD100 (p.Phe954ValfsX2)	-

Patient Identification and Gender/Age	**Phenotype A= Age of onset B=initial phenotype C=inflammatory signs on muscle biopsy D=CPK level E=progression after 10 years**	**DYSF IH or Western blot analysis findings**	**Genomic mutational findings: Deleterious sequence variants***	**Deleterious effect****	**Genomic mutational findings: Sequence variants of undetermined pathogenicity*****
F1-187-1-1 M NA	A: NA B: MM C: NA D: NA E: NA	Absence	**ex32: c.3517dupT** HTZ and ex51: c.5713C>T HTZ	**(p.Ser1173PhefsX2)** (p.Arg1905X)	-
F1-188-1-2 F 47years	A: NA B: LGMD C: NA D: NA E: NA	Absence	ex20: c.1852G>A homozygous (CONSANGUINITY)	(p.Gly618Arg)	-
F1-19-1-1 M 36years	A: NA B: MM C: NA D: NA E: NA	Absence	**IVS34: c.3702+1G>A** HTZ and ex49: c.5509G>A	Abn.Spl. (p.Asp1837Asn)	**ex33: c.3702T>C (p.Tyr1234Tyr)** HTZ UMD18
F1-190-1-1 M 30years	A: 18 years B: MM C: Yes D: x125N E: Ambulant	NA	**ex21: c.1948delC** homozygous (CONSANGUINITY)	**(p.Leu650TyrfsX6)**	-
F1-192-1-1 M 26years	A: 18 years B: LGMD C: NA D: x30N E: NA	NA	**ex30: c.3225delT** homozygous (CONSANGUINITY)	**(p.Phe1075LeufsX45)**	-
F1-20-1-2 F 32years	A: NA B: LGMD C: NA D: NA E: NA	NA	**ex6: c.463G>A** HTZ	**(p.Gly155Arg)** UMD82	**IVS29: c.3175-61G>C** HTZ; **IVS42: c.4639-38G>A** HTZ
F1-200-1-1 M 58years	A: NA B: MM C: NA D: NA E: NA	Absence	?	-	**IVS38: c.4168-20G>A** HTZ
F1-201-1-2 F 34years	A: NA B: HyperCPK C: NA D: NA E: NA	Absence	ex12: c.1177C>T HTZ	(p.Gln393X)	IVS21: c.2055+105_2055+106delAC (rs5832058) HTZ
F1-204-1-2 F 36years	A: 33 years B: MM C: Yes D: x20N E: NA	Absence	IVS10: c.937+1G>A HTZ and ex20: c.1758C>G HTZ	Abn.Spl. (p.Tyr586X)	-

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F1-205-1-1 M NA	A: NA B: MM C: NA D: NA E: NA	Absence	**ex9: c.879_883dupGACAG** HTZ	**(p.Asp295GlyfsX45)**	-
F1-21-1-2 F 20years	A: 16 years B: HyperCPK C: No D: x25N E: NA	Absence	ex6: c.490G>T HTZ and IVS33: c.3703-1G>A HTZ	(p.Gly164X) Abn.Spl.	-
F1-21-2-2 F 19years	A: ~ 16 years B: HyperCPK C: NA D: x20N E: NA	(ND-R)	ex6: c.490G>T HTZ and IVS33: c.3703-1G>A HTZ	(p.Gly164X) Abn.Spl.	-
F1-25-1-1 M 46years	A: 23 years B: MM C: NA D: x40N E: Ambulant	(ND-R)	IVS25: c.2643+1G>A homozygous (CONSANGUINITY)	Abn.Spl.	**ex39: c.4323G>A (p.Gln1441Gln)** homozygous UMD29
F1-25-2-2 F 40years	A: 20 years B: MM C: NA D: NA E: Ambulant	(ND-R)	IVS25: c.2643+1G>A homozygous (CONSANGUINITY)	Abn.Spl.	-
F1-25-3-1 M 28years	A: 18 years B: MM C: No D: x25N E: Ambulant	Absence	IVS25: c.2643+1G>A homozygous (CONSANGUINITY)	Abn.Spl.	**IVS35: c.3874-30delG** homozygous; **ex39: c.4323G>A (p.Gln1441Gln)** homozygous UMD29
F1-29-1-2 F 64years	A: 25 years B: LGMD C: No D: x11N E: Ambulant	Severely reduced	ex47: c.5302C>T HTZ	(p.Arg1768Trp)	ex12: c.1168G>A (p.Asp390Asn) HTZ UMD41
F1-3-1-2 F NA	A: NA B: NA C: NA D: NA E: NA	Absence	?	-	**ex52: c.5899G>A (p.Gly1967Ser)** homozygous UMD63; **IVS6: c.664-17C>T** homozygous
F1-3-2-2 F NA	A: NA B: NA C: NA D: NA E: NA	Absence	?	-	**ex52: c.5899G>A (p.Gly1967Ser)** homozygous UMD63

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F1-30-1-2 F 39years	A: NA B: MM C: NA D: NA E: NA	Absence	ex26: c.2779delG HTZ and ex51: c.5713C>T HTZ	(p.Ala927LeufsX21) (p.Arg1905X)	**IVS6: c.664-17C>T** HTZ; **IVS33: c.3703-12C>T** HTZ
F1-31-1-2# F 36years	MM#	Severely reduced	ex9: c.896G>A HTZ	(p.Gly299Glu)	**IVS6: c.664-17C>T** homozygous
F1-32-1-2 F 28years	A: 14 years B: LGMD C: No D: x70N E: Ambulant	Absence	?	-	-
F1-34-1-1 M 34years	A: NA B: MM C: NA D: NA E: NA	Absence	?	-	-
F1-35-1-2# F 43years	MM#	Severely reduced	ex32: c.3516_3517delTT HTZ	(p.Ser1173X)	-
F1-37-1-1# M 37years	MM#	Absence	ex15: c.1368C>G HTZ and ex51: c.5713C>T HTZ	(p.Cys456Trp) (p.Arg1905X)	-
F1-38-1-2 F 33years	A: ~ 20 years B: MM C: NA D: x44N E: Ambulant	NA	ex32: c.3477C>A HTZ and ex53: c.5979dupA HTZ	(p.Tyr1159X) (p.Glu1994fsX3)	**IVS29: c.3175-61G>C** HTZ
F1-40-1-2 F 42years	A: NA B: LGMD C: NA D: x20N E: Non ambulant	Absence	**ex23: c.2217C>A** HTZ and **IVS37: c.4005+1G>A** HTZ	**(p.Tyr739X)** Abn.Spl.	-
F1-44-1-2# F 37years	PD#	Absence	ex39: c.4201dupA homozygous (CONSANGUINITY)	(p.Ile1401AsnfsX8)	-
F1-45-1-1 M 49years	A: NA B: LGMD C: NA D: NA E: NA	Absence	?	-	**IVS35: c.3874-30delG** homozygous

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F1-46-1-2# F 57years	MM#	Absence	ex20: c.1795_1799dupTACTC homozygous (CONSANGUINITY)	(p.Ala601ThrfsX28)	-
F1-46-2-1# M 43years	MM#	Absence	ex20: c.1795_1799dupTACTC homozygous (CONSANGUINITY)	(p.Ala601ThrfsX28)	-
F1-47-1-2 F 27years SPDCM	A: 17 years B: LGMD C: No D: x70N E: Ambulant	Absence	IVS25: c. 2643+1G>A homozygous and **ex42: c.4577A>C** homozygous (CONSANGUINITY)	Abn.Spl. **(p.Lys1526Thr)** UMD80	-
F1-48-1-2 F 36years	A: NA B: MM C: NA D: x20N E: Ambulant	Absence	**ex29: c.3086C>T** HTZ	**(p.Pro1029Leu)** UMD68	-
F1-48-2-2 F 35years	A: NA B: MM C: NA D: x18N E: Ambulant	Absence	**ex29: c.3086C>T** HTZ	**(p.Pro1029Leu)** UMD68	-
F1-49-1-2 F 28years	A: 14 years B: MM C: NA D: x50N E: NA	Absence	ex6: c.610C>T HTZ and ex19: c.1663C>T HTZ	(p.Arg204X) (p.Arg555Trp)	**IVS6: c.664-17C>T** HTZ; **IVS29: c.3175-61G>C** HTZ; **ex52: c.5829C>A (p.Ser1943Ser)** HTZ
F1-5-1-1# M 53years	LGMD#	Severely reduced	ex27: c.2858dupT homozygous (CONSANGUINITY)	(p.Phe954ValfsX2)	-
F1-50-1-2 F 42years	A: ~25 years B: LGMD C: No D: x10N E: Ambulant	Absence	ex7: c.701G>A HTZ and ex52: c.5903G>A HTZ	(p.Gly234Glu) (p.Trp1968X)	-
F1-52-1-2 F NA	A: NA B: LGMD C: NA D: NA E: NA	NA	ex43: c.4756C>T HTZ	(p.Arg1586X)	**IVS30: c.3349-54T>G** homozygous; **IVS31: c.3442+14C>T** homozygous; **IVS32: c.3521-12C>T** HTZ
F1-54-1-1 M 30years	A: 16 years B: MM C: No D: x14N E: NA	Normal	?	-	**ex21: c.1980G>A (p.Val660Val)** HTZ UMD18; **ex24: c.2456G>A (p.Arg819Gln)** HTZ UMD59; **IVS29: c.3175-31G>A** HTZ; **ex37: c.3973 A>G (p.Ile1325Val)** HTZ UMD18

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F1-55-1-1 M NA	A: NA B: MM C: NA D: NA E: NA	NA	**ex39: c.4191C>G** HTZ	**(p.Tyr1397X)**	**IVS6: c.664-17C>T** HTZ; IVS21: c.2055+105_2055+106delAC (rs5832058) HTZ; **ex52: c.5899G>A (p.Gly1967Ser)** HTZ UMD63
F1-56-1-2 F 57years	A: NA B: MM C: NA D: NA E: NA	Absence	?	-	-
F1-57-1-1 M 38years	A: NA B: LGMD C: NA D: NA E: NA	Absence	**ex8: c.851T>C** HTZ and **ex18: c.1617C>G** HTZ	**(p.Ile284Thr)** UMD93 **(p.Tyr593X)**	**IVS52: c.5947-33G>A** HTZ
F1-58-1-1# M deceased	LGMD#	Severely reduced	ex19: c.1663C>T HTZ and ex34: c.3832C>T HTZ	(p.Arg555Trp) (p.Gln1278X)	-
F1-58-2-2 F 42years	A: NA B: Myalgia C: NA D: NA E: NA	(ND-R)	ex34: c.3832C>T HTZ	(p.Gln1278X)	**IVS28: c.3032-16G>A** HTZ; **IVS29: c.3175-61G>C** HTZ
F1-6-1-1# M 34years	MM#	Absence	ex19: c.1663C>T homozygous (CONSANGUINITY)	(p.Arg555Trp)	-
F1-61-1-2 F 45years	A: 28 years B: LGMD C: Yes D: x10N E: Ambulant	Absence	**ex23: c.2192C>G** HTZ and ex39: c.4200dupC HTZ	**(p.Pro731Arg)** UMD82 (p.Ile1401HisfsX8)	**IVS4: c.343-29A>G** HTZ
F1-63-1-2# F 36years	PD#	Absence	IVS8: c.855+1delG HTZ	Abn.Spl.	-
F1-64-1-2 F 44years	A: 20 years B: MM C: Yes D: x20N E: Non ambulant	Absence	?	-	-
F1-65-1-2 F 60years SPDCM	A: NA B: MM C: NA D: NA E: NA	NA	ex12: c.1120G>C HTZ and ex29: c.3113G>A HTZ and ex50: c.5594delG HTZ	(p.Val374Leu) UMD29 (p.Arg1038Gln) UMD59 (p.Gly1865AlafsX101)	**ex4: c.251C>T (p.Ala84Val)** HTZ UMD41; **ex15: c.1362C>T (p.Ser454Ser)** HTZ UMD18

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F1-65-2-1 M 50years	A: NA B: MM C: NA D: NA E: NA	NA	ex50: c.5594delG homozygous (CONSANGUINITY)	(p.Gly1865AlafsX101)	**IVS2: c.144+46delG** homozygous; **ex15: c.1362C>T (p.Ser454Ser)** homozygous UMD18
F1-65-3-2 F 54years	A: NA B: MM C: NA D: NA E: NA	NA	ex50: c.5594delG homozygous (CONSANGUINITY)	(p.Gly1865AlafsX101)	-
F1-67-1-2 F 43years	A: 25 years B: MM C: Yes D: x20N E: Ambulant	Absence	ex34: c.3832C>T homozygous	(p.Gln1278X)	-
F1-68-1-2# F 35years	HyperCPK#	Absence	ex20: c.1758C>G HTZ and ex30: c.3321_3324dupAGCT HTZ	(p.Tyr586X) (p.Val1109SerfsX6)	-
F1-7-1-2 F 37years	A: 24 years B: MM C: Yes D: x9N E: Ambulant	NA	ex4: c.265C>T homozygous (CONSANGUINITY)	(p.Arg89X)	-
F1-70-1-2# F 51years	PM#	Absence	ex39: c.4200dupC HTZ and ex41: c.4433G>A HTZ	(p.Ile1401HisfsX8) (p.Trp1478X)	-
F1-71-1-2 F 53years	A: ~ 40 years B: LGMD C: No D: x20N E: Ambulant	Absence	**ex33: c.3683T>C** HTZ and ex44: c.4872_4876delGCCCGinsCCCC HTZ	**(p.Leu1228Pro)** UMD71 (p.Glu1624AspfsX9)	**IVS29: c.3175-61G>C** HTZ
F1-73-1-1 M 38years	A: NA B: MM C: NA D: NA E: NA	NA	ex54: c.6124C>T HTZ	(p.Arg2042Cys)	-
F1-74-1-2# F 49years	PM#	Severely reduced	ex8: c.797T>C HTZ and ex44: c.4876delG HTZ	(p.Leu266Pro) (p.Val1626TyrfsX8)	**IVS33: c.3521-12C>T** HTZ
F1-75-1-1# M 32years	LGMD#	Severely reduced	ex29: c.3035G>A homozygous (CONSANGUINITY)	(p.Trp1012X)	-
F1-75-2-1# M 27years	LGMD#	Severely reduced	ex29: c.3035G>A homozygous (CONSANGUINITY)	(p.Trp1012X)	-

Patient **Identification and Gender/Age**	**Phenotype** **A= Age of onset** **B=initial phenotype** **C=inflammatory signs on muscle biopsy** **D=CPK level** **E=progression after 10 years**	**DYSF IH or Western blot analysis findings**	**Genomic mutational findings:** **Deleterious sequence variants***	**Deleterious effect****	**Genomic mutational findings:** **Sequence variants of undetermined pathogenicity*****
F1-76-1-1# M 34years	MM#	Severely reduced	ex6: c.610C>T HTZ and IVS8: c.855+1delG HTZ	(p.Arg204X) Abn.Spl.	-
F1-8-1-1 M 68years	A: NA B: MM C: NA D: NA E: NA	Absence	IVS50: c.5668-7G>A homozygous	(p.Asp1890ValfsX78)	-
F1-80-1-0 (NA)	A: NA B: LGMD C: NA D: NA E: NA	NA	ex29: c.3065G>A HTZ	(p.Arg1022Gln)	-
F1-82-1-1# M 38years	PM#	Absence	ex52: c.5903G>A HTZ	(p.Trp1968X)	**IVS31: c.3442+4A>G** HTZ; **ex38: c.4089C>T (p.Gly1363Gly)** HTZ UMD18
F1-82-2-2 F NA	A: NA B: LGMD C: NA D: NA E: NA	(ND-R)	ex52: c.5903G>A HTZ	(p.Trp1968X)	-
F1-83-1-2 F 46years	A: 16 years B: LGMD C: NA D: x8N E: Ambulant	Absence	ex12: c.1177C>T HTZ	(p.Glu393X)	**IVS2: c.144+46delG** homozygous; ex12: c.1168G>A (p.Asp390Asn) HTZ UMD41; **IVS33: c.3703-12C>T** HTZ
F1-84-1-2# F 51years	LGMD#	Absence	ex39: c.4200delC homozygous (CONSANGUINITY)	(p.Ile1401SerfsX47)	-
F1-85-1-2 F NA	A: NA B: MM C: NA D: NA E: NA	NA	ex4: c.331C>T HTZ and ex25: c.2643+1G>A HTZ	(p.Gln111X) Abn.Spl.	-
F1-86-1-2# F 35years	MM#	Absence	IVS30: c.3348+1_3348+4delGTAT HTZ and ex47: c.5314_5318delAGCCC HTZ	Abn.Spl. (p.Ser1772delfsX51)	-
F1-88-1-2 F 38years	A: 17 years B: LGMD C: No D: x10 E: Non ambulant	NA	ex26: c.2779delG homozygous (CONSANGUINITY)	(p.Ala927LeufsX21)	-

Patient Identification and Gender/Age	**Phenotype A= Age of onset B=initial phenotype C=inflammatory signs on muscle biopsy D=CPK level E=progression after 10 years**	**DYSF IH or Western blot analysis findings**	**Genomic mutational findings: Deleterious sequence variants***	**Deleterious effect****	**Genomic mutational findings: Sequence variants of undetermined pathogenicity*****
F1-9-1-2 F 36years	A: NA B: LGMD C: NA D: NA E: NA	Absence	ex53: c.5979dupA homozygous (CONSANGUINITY)	(p.Glu1994ArgfsX3)	**IVS29: c.3175-61G>C** homozygous
F1-90-1-0 (NA)	A: NA B: MM C: NA D: NA E: NA	Absence	ex29: c.3137G>A HTZ and ex32: c.3477C>A HTZ	(p.Arg1046His) (p.Tyr1159X)	-
F1-91-1-1# M 29years	PD#	Absence	ex50: c.5594delG homozygous (CONSANGUINITY)	(p.Gly1865AlafsX101)	-
F1-92-1-2 F 33years	A: 14 years B: LGMD C: NA D: x50N E: Ambulant	NA	ex3: c.154 T>C HTZ and **ex19: c.1655_1668delATCGTGGCCGGCTT** HTZ	(p.Trp52Arg) **(p.Tyr552SerfsX13)**	-
F1-96-1-2 F 33years	A: 16 years B: MM C: No D: x15N E: Ambulant	NA	ex2: c.107_108delAA HTZ and ex20: c.1758C>G HTZ	(p.Lys36SerfsX11) (p.Tyr586X)	-

Table 2: Novel nonsense-, frameshifting-, splice-donor-site- and splice-acceptor-site mutations identified in this study.

DNA sequence variation	Deleterious effect *	Localisation	Number of alleles	Patient
c.457+1insG	Motif: donor site WT: CAGgtgggt (CV 90.50) Mut: CAGggtggg (CV 57.87)	IVS 5	2	F1-104-1-2 (HOZ)
c.591C>G	p.Tyr197X	Exon 6	1	F1-133-1-1
c.799_800delTT	p.Phe267LeufsX5	Exon 8	1	F1-154-1-2
c.879_883dup	p.Asp295GlyfsX45	Exon 9	1	F1-205-1-1
c.1157_1168delTCCGGGCCGAGG	p.Phe386_Asp390delinsTyr	Exon 12	1	F1-14-1-1
c.1617C>G	p.Tyr539X	Exon 18	1	F1-57-1-1
c.1655_1668delATCGTGGCCGGCTT	p.Tyr552SerfsX13	Exon 19	1	F1-92-1-2
c.1948delC	p.Leu650TyrfsX6	Exon 21	2	F1-190-1-1 (HOZ)
c.2217C>A	**p.Tyr739X**	**Exon 23**	**3**	**F1-40-1-2, F1-108-1-1, F1-108-2-2**
c.2511+1G>A	Motif: donor site WT: AAAgtgagt (CV 87.31) Mut: AAAatgagt (CV 60.47)	IVS 24	2	F1-140-1-2, F1-140-2-1
c.2894G>A	p.Trp965X	Exon 27	1	F1-183-1-2
c.2975G>A	p.Trp992X	Exon 28	2	F1-111-2-2 (HOZ)
c.3225delT	p.Phe1075LeufsX45	Exon 30	2	F1-192-1-1
c.3389_3399dupTCTCCACCTTG	p.Phe1135ProfsX3	Exon 31	2	F1-103-1-1, F1-103-2-1
c.3517dupT	p.Ser1173PhefsX2	Exon 32	1	F1-187-1-1
c.3687C>A	p.Tyr1229X	Exon 33	1	F1-154-1-2
c.3702+1G>A	Motif: donor site WT: TATgtgagt (CV 85.99) Mut: TATatgagt (CV 59.16)	IVS 33	1	F1-19-1-1
c.4005+1G>A	Motif: donor site WT: GAGgtgagc (CV 94.91) Mut: GAGatgagc (CV 68.07)	**IVS 37**	**2**	**F1-40-1-2, F1-114-1-2**
c.4191C>G	p.Tyr1397X	Exon 39	1	F1-55-1-1
c.5767+1G>A	Motif: donor site WT: TGGgtaagc (CV 90.99) Mut: TGGataagc (CV 64.15)	IVS 51	2	F1-148-1-1 (HOZ)
c.5805delA	p.Ala1936ProfsX30	Exon 52	2	F1-156-1-2 (HOZ)
c.5871_5872delGT	p.Ser1958ProfsX3	Exon 52	1	F1-179-1-1

Mutation numbering is based on cDNA sequence (human *DYSF*, GenBank NM_003494.2) according to journal guidelines (www.hgvs.org/mutnomen), and as detailed in "Patients, Materials and Methods".

* Deleterious effects at the protein level are predicted from the DNA sequence variation. Recurrent mutations are in bold.

WT:wild type motif. Mut: mutated motif. CV:calculated consensus site value (treshhold=70) using Splicing Sequences Finder (www.umd.be/SSF/). HOZ: homozygous.

Table 3: Previously reported disease-causing missense mutations, and novel missense and isosemantic *(italized)* exonic variants identified in this study.

Mutation numbering is based on cDNA sequence (human *DYSF*, GenBank NM_003494.2) according to journal guidelines (www.hgvs.org/mutnomen), and as detailed in "Patients, Materials and Methods".
: Mutations/Variants identified in patients presenting more than two mutations initially considered as possibly disease-causing

* Leiden Muscular Dystrophy pages database (www.dmd.nl)
** Exclusion based on simultaneous identification of two additional mutations clearly considered as disease-causing, in the same patient
*** Pathogenicity score using UMDpredictor (Frederic et al., submitted)
**** Predicted effect determined as follows depending on the calculated UMDpredictor score:
<50: polymorphism; ≥50 and <65: probable polymorphism; ≥65 and <75: probably pathogenic; ≥75: pathogenic

Patient	Sequence variation	Predicted amino-acid variation	Exon	Number of patients	Reports of the variant and information on pathogenicity	Domain	Conservation	SIFT score	BLOSUM62 score	Bio chemical Value	ESE modif	Splice site	Pathogenicity score***	Conclusion of predictive Analysis****
F1-92-1-2	c.154T>C	p.Trp52Arg	3	1	Previously reported* as pathogenic	C2 Domain	1	0.01	-3.00	0.38	SRp40 [3.69]	No impact	93	Pathogenic
F1-65-1-2	c.251C>T	p.Ala84Val	4	1	This study	C2 Domain	0.93	0.53	0.00	0.75		No impact	41	Polymorphism
F1-20-1-2	c.463G>A	p.Gly155Arg	6	1	This study		0.5	0.58	-2.00	0.13		No impact	82	Pathogenic
F1-169-1-1, F1-113-1-1	c.509C>A	p.Ala170Glu	6	2	Previously reported* as pathogenic/ unclear		0.5	0.11	-1.00	0.21		No impact	71	Probably Pathogenic
F1-18-1-2 (HOZ)	c.565C>G	p.Leu189Val	6	1	Previously reported* as pathogenic/ unclear		0.64	0.69	1.00	0.88		Potential donor splice site [81.82]	29	Polymorphism
F1-50-1-2	c.701G>A	p.Gly234Glu	7	1	Previously reported* as pathogenic	C2 Domain	0.71	0.03	-2.00	0.21		No impact	86	Pathogenic
F1-155-1-2	c.757C>T	p.Arg253Trp	7	1	Previously reported* as pathogenic	C2 Domain	0.79	0.02	-3.00	0.38	SRp55 [4.13]	No impact	93	Pathogenic
F1-74-1-2	c.797T>C	p.Leu266Pro	8	1	Previously reported* as pathogenic	C2 Domain	0.79	0.02	-3.00	0.67		No impact	69	Probably Pathogenic
F1-57-1-1	c.851T>C	p.Ile284Thr	8	1	This study	C2 Domain	0.79	0.01	-3.00	0.42	SF2/ASF [2.23] SRp40 [2.71]	No impact	93	Pathogenic

Patient	Sequence variation	Predicted amino-acid variation	Exon	Number of patients	Reports of the variant and information on pathogenicity	Domain	Conservation	SIFT score	BLOSUM62 score	Bio chemical Value	ESE modif	Splice site	Pathogenicity score***	Conclusion of predictive Analysis****
F1-131-1-1	c.895G>A	p.Gly299Arg	9	1	Previously reported* as pathogenic	C2 Domain	0.86	0.01	-2.00	0.13	SRp40 [2.7]	Potential acceptor splice site [91.47]	100	Pathogenic
F1-31-1-2	c.896G>A	p.Gly299Glu	9	1	Previously reported* as pathogenic	C2 Domain	0.86	0.02	-2.00	0.21		Potential acceptor splice site [81.74]	99	Pathogenic
F1-113-1-1	c.1004G>C	p.Gly335Ala	11	1	This study, exclusion of pathogenicity in patient F1-113-1-1**	Ferlin family domain	0.71	0.00	0.00	0.75	SF2/ASF [3.53] SC35 [2.84]	No impact	59	Probable polymorphism
F1-153-1-1, F1-11-1-1	c.1020C>A	p.Ser340Arg	11	2	Previously reported* as pathogenic	Ferlin family domain	0.71	0.10	-1.00	0.29		No impact	71	Probably Pathogenic
F1-65-1-2	c.1120G>C	p.Val374Leu	12	1	Previously reported* as pathogenic	Ferlin family domain	0.79	0.40	1.00	0.88		No impact	29	Polymorphism
F1-83-1-2	c.1168G>A	p.Asp390Asn	12	2	Previously reported* as unclear	C2 Domain	0.93	0.28	1.00	0.75	SF2/ASF [4.01]	No impact	41	Polymorphism
F1-184-1-2	c.1276G>A	p.Gly426Arg	13	1	This study	C2 Domain	1	0.03	-2.00	0.13	SRp55 [3.56]	Potential acceptor splice site [70.48]	100	Pathogenic
F1-65-1-2, F1-65-2-1 (HOZ), F1-138-1-1 (HOZ)	***c.1362C>T***	***p.Ser454Ser***	15	3	This study, exclusion of pathogenicity in patient F1-138-1-1**	C2 Domain	0.86	1.00	4.00	1.00		No impact	18	Polymorphism
F1-37-1-1	c.1368C>G	p.Cys456Trp	15	1	Previously reported* as pathogenic	C2 Domain	0.93	0.03	-2.00	0.38	SRp55 [3.17]	No impact	86	Pathogenic
F1-58-1-1, F1-6-1-1 (HOZ), F1-113-1-1, F1-114-1-2, F1-14-1-1, F1-49-1-2	c.1663C>T	p.Arg555Trp	19	6	Previously reported* as pathogenic		0.71	0.01	-3.00	0.38		No impact	88	Pathogenic
F1-188-1-2 (HOZ)	c.1852G>A	p.Gly618Arg	20	1	Previously reported* as pathogenic		0.71	0.00	-2.00	0.13		No impact	94	Pathogenic
F1-54-1-1	***c.1980G>A***	***p.Val660Val***	21	1	This study		0.71	1.00	4.00	1.00		No impact	18	Polymorphism
F1-61-1-2	c.2192C>G	p.Pro731Arg	23	1	This study	Ferlin family domain	0.71	0.45	-2.00	0.17	SRp40 [3.82]	No impact	82	Pathogenic

Patient	Sequence variation	Predicted amino-acid variation	Exon	Number of patients	Reports of the variant and information on pathogenicity	Domain	Conservation	SIFT score	BLOSUM62 score	Bio chemical Value	ESE modif	Splice site	Pathogenicity score***	Conclusion of predictive Analysis****
F1-18-1-2 (HOZ)	***c.2283C>A***	***p.Gly761GLY***	23	1	This study, pathogenicity excluded in patient F-18-1-2**		0.64	0.43	6.00	1.00		No impact	18	Polymorphism
F1-54-1-1	c.2456G>A	p.Arg819Gln	24	1	This study	Ferlin family domain	0.64	0.60	1.00	0.50		Potential acceptor splice site [98.99]	59	Probable polymorphism
F1-174-1-1	c.2790G>C	p.Trp930Cys	26	1	Previously reported* as pathogenic	Repeated Dysf domain ?	0.71	0.00	-2.00	0.38		No impact	82	Pathogenic
F1-80-1-0	c.3065G>A	p.Arg1022Gln	29	1	Previously reported* as unclear	Repeated Dysf domain ?	0.64	0.62	1.00	0.50	SF2/ASF [3.16] SRp55 [3.08]	Potential acceptor splice site [81.31]	65	Probably Pathogenic
F1-48-1-2, F1-48-2-2	c.3086C>T	p.Pro1029Leu	29	2	This study	Repeated Dysf domain ?	0.64	0.04	-3.00	0.67		No impact	68	Probably Pathogenic
F1-65-1-2	c.3113G>A	p.Arg1038Gln	29	1	Previously reported* as pathogenic	Repeated Dysf domain ?	0.64	0.00	1.00	0.50		No impact	59	Probable polymorphism
F1-90-1-0, F1-140-2-1, F1-140-1-2	c.3137G>A	p.Arg1046His	29	3	Previously reported* as pathogenic/ unclear	Repeated Dysf domain ?	0.64	0.00	0.00	0.58	SRp55 [3.17]	No impact	65	Probably Pathogenic
F1-71-1-2	c.3683T>C	p.Leu1228Pro	33	1	This study	C2 Domain	1	0.00	-3.00	0.67		No impact	71	Probably Pathogenic
F1-174-1-1, F1-19-1-1	***c.3702T>C***	***p.Tyr1234Tyr***	33	2	This study, pathogenicity excluded in patient F1-174-1-1**	C2 Domain	1	1.00	7.00	1.00		No impact	18	Polymorphism
F1-130-1-1, F1-130-2-2	c.3826C>G	p.Leu1276Val	34	2	Previously reported* as pathogenic		1	0.02	1.00	0.88		Potential donor splice site [70.91]	40	Polymorphism
F1-54-1-1	c.3973A>G	p.Ile1325Val	37	1	This study		1	0.12	3.00	0.88		No impact	18	Polymorphism
F1-82-1-1	***c.4089C>T***	***p.Gly1363Gly***	38	1	This study	Possible C2 Domain	1	1.00	6.00	1.00		Potential donor splice site [76.91]	18	Polymorphism
F1-25-1-1 (HOZ), F1-25-3-1 (HOZ)	***c.4323G>A***	***p.Gln1441Gln***	39	1	This study, pathogenicity excluded in patient F1-25-1-1 and F1-25-3-1**		1	1.00	5.00	1.00		Potential acceptor splice site [81.04]	29	Polymorphism
F1-47-1-2 (HOZ)	c.4577A>C	p.Lys1526Thr	42	1	This study		1	0.04	-1.00	0.21		No impact	80	Pathogenic

Patient	Sequence variation	Predicted amino-acid variation	Exon	Number of patients	Reports of the variant and information on pathogenicity	Domain	Conservation	SIFT score	BLOSUM62 score	Bio chemical Value	ESE modif	Splice site	Pathogenicity score***	Conclusion of predictive Analysis****
F1-151-1-2	c.4628G>A	p.Gly1543Asp	42	1	Previously reported* as pathogenic		1	0.00	-1.00	0.29	SRp40 [2.88]	No impact	88	Pathogenic
F1-155-1-2	***c.4731G>A***	***p.Glu1577Glu***	43	1	This study, pathogenicity excluded in patient F1-155-1-2**	Possible C2 Domain	1	1.00	5.00	1.00		No impact	18	Polymorphism
F1-148-1-1 (HOZ)	***c.4867C>T***	***p.Leu1623Leu***	44	1	This study, pathogenicity excluded in patient F1-148-1-1**	C2 Domain	1	1.00	4.00	1.00	SF2/ASF [3.08] SC35 [2.51]	No impact	23	Polymorphism
F1-116-1-1	c.5078G>A	p.Arg1693Gln	46	1	Previously reported* as pathogenic		1	0.00	1.00	0.50		Potential acceptor splice site [70.39]	71	Probably Pathogenic
F1-18-1-2	c.5243A>T	p.Glu1748Val	47	1	Previously reported* as unclear/ polymorphism		1	0.01	-2.00	0.21		Potential donor splice site [85.82]	88	Pathogenic
F1-29-1-2, F1-100-1-1	c.5302C>T	p.Arg1768Trp	47	2	Previously reported* as pathogenic/ unclear		1	0.00	-3.00	0.38	SF2/ASF [3.16] SRp40 [3.1] SRp40 [2.93] SRp55 [3.89]	No impact	94	Pathogenic
F1-135-1-2, F1-19-1-1	c.5509G>A	p.Asp1837Asn	49	2	Previously reported* as pathogenic	Possible C2 Domain	1	0.00	1.00	0.75		No impact	47	Polymorphism
F1-49-1-2	***c.5829C>A***	***p.Ser1943Ser***	52	1	This study, pathogenicity excluded in patient F1-49-1-2**		1	0.79	4.00	1.00		No impact	18	Polymorphism
F1-137-1-2, F1-3-2-2, F1-3-1-2, F1-55-1-1	c.5899G>A	p.Gly1967Ser	52	4	This study		0.93	0.03	0.00	0.50		No impact	63	Probable polymorphism
F1-182-1-2	c.5908C>T	p.Pro1970Ser	52	1	This study		0.93	0.00	-1.00	0.38		No impact	76	Pathogenic
F1-150-1-2, F1-150-2-2, F1-150-2-2	c.6124C>T	p.Arg2042Cys	54	3	Previously reported* as pathogenic		0.86	0.02	-3.00	0.25		No impact	93	Pathogenic

Table 4: Previously reported intronic variants with unknown pathogenicity, and novel intronic variants identified in this study.

Patient	Intronic sequence variant	Reports of the variant and information on pathogenicity	SSF predictive analysis****
F1-104-1-2	IVS1: c.89-29C>G	This study, exclusion of pathogenicity in patient F1.104.1**	no effect
F1-65-2-1	IVS2: c.144+46delG	This study, exclusion of pathogenicity in patient F1.65.2.1**	no effect
F1-111-2-2	IVS3: c.236+20G>A	Previously reported in dbSNP*** (rs12470028). Exclusion of pathogenicity in patient F1.111.2.2**	no effect
F1-61-1-2	IVS4: c.343-29A>G	This study, exclusion of pathogenicity in patient F1.61.1.2**	Branch Point inactivation WT: caccaAc (CV 72.27) Mut: caccaGc (CV 42.65)
F1-170-1-2, F1-170-2-2	IVS5: c.457+17G>C	Previously reported* as unclear	no effect
F1-3-1-2, F1-11-1-1, F1-30-1-2, F1-31-1-2, F1-49-1-2, F1-55-1-1	IVS6: c.664-17C>T	This study, exclusion of pathogenicity in patient F1.30.1.2**	no effect
F1-103-1-1	IVS7: c.792+11T>C	Previously reported in dbSNP*** (rs13428076). Exclusion of pathogenicity in patient F1.103.1.1**	no effect
F1-151-1-2	IVS10: c.938-34T>A	This study	no effect
F1-123-1-1	IVS12: c.1180+5G>A	Previously reported* as unclear	Donor Site inactivation WT: AGAgtgcgt (CV 73.65) Mut: AGAgtgcat (CV 61.48)
F1-123-1-1	IVS13: c.1285-35G>T	This study	no effect
F1-55-1-1, F1-201-1-2	IVS21: c.2055+105_2055+106delAC	Previously reported in dbSNP*** (rs5832058)	no effect
F1-58-2-2	IVS28: c.3032-16G>A	This study	no effect
F1-9-1-2, F1-49-1-2, F1-58-2-2, F1-145-1-1	IVS29: c.3175-61G>C	This study, exclusion of pathogenicity in patient F1.9.1.2**	no effect
F1-54-1-1	IVS29: c.3175-31G>A	This study	no effect
F1-52-1-2	IVS30: c.3349-54T>G	This study	no effect
F1-82-1-1	IVS31: c.3442+4A>G	This study	no effect

Patient	Intronic sequence variant	Reports of the variant and information on pathogenicity	SSF predictive analysis****
F1-52-1-2	IVS31: c.3442+14C>T	This study	no effect
F1-52-1-2	IVS32: c.3521-12C>T	This study	no effect
F1-30-1-2, F1-83-1-2	IVS33: c.3703-12C>T	This study, exclusion of pathogenicity in patient F1.30.1.2**	no effect
F1-25-3-1, F1-45-1-1	IVS35: c.3874-30delG	This study, exclusion of pathogenicity in patient F1.25.3.1**	no effect
F1-103-1-1	IVS38: c.4168-40G>A	This study, exclusion of pathogenicity in patient F1.103.1.1**	Branch Point inactivation WT: CCCAGAG (CV 77.25) Mut: CCCAGGG (CV 47.63)
F1-200-1-1	IVS38: c.4168-20G>A	This study	no effect
F1-143-1-1	IVS41: c.4509+40C>T	This study	no effect
F1-11-1-1	IVS42: c.4639-38A>G	This study	no effect

(With exception of intronic mutations affecting the consensus AG acceptor, or GT donor sites: see Table 1)
Mutation numbering is based on cDNA sequence (human *DYSF*, GenBank NM_003494.2) according to journal guidelines (www.hgvs.org/mutnomen), and as detailed in "Patients, Materials and Methods". WT: wild type motif. Mut: mutated motif. CV: calculated consensus site value (treshhold=70). HOZ: homozygous.

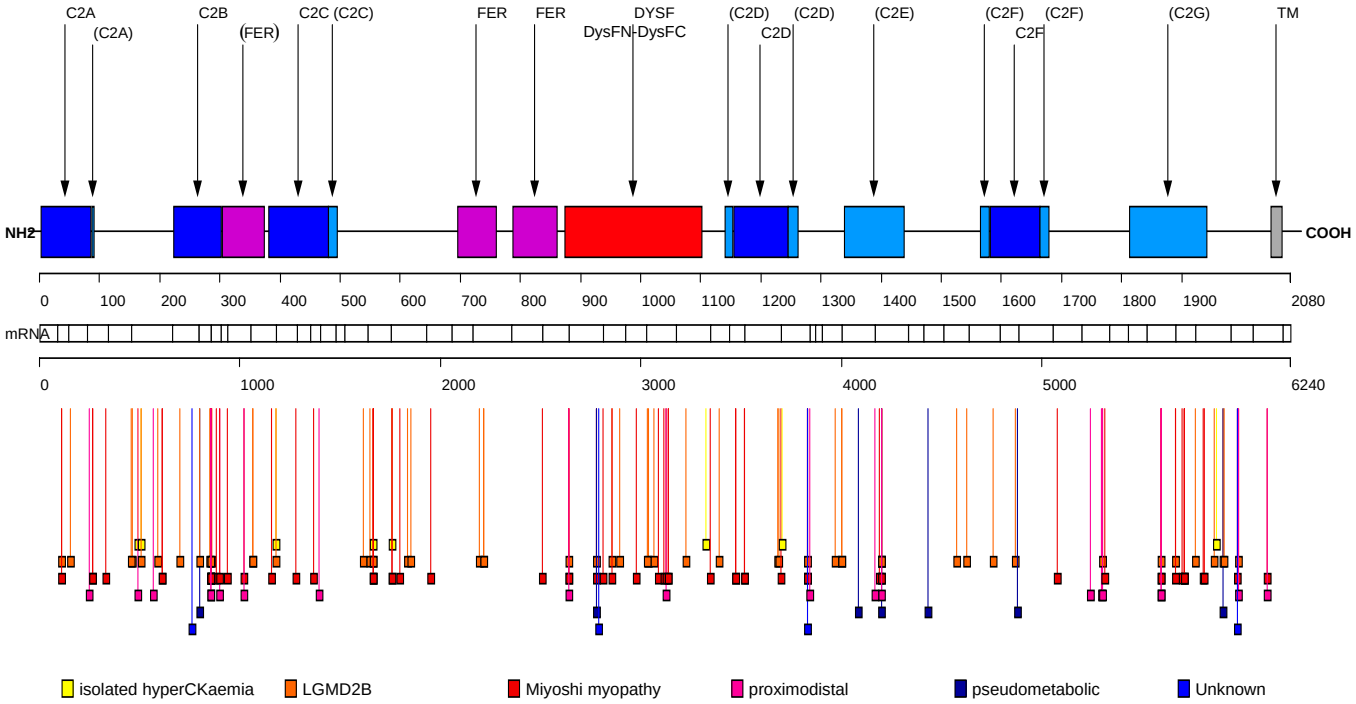
* Leiden Muscular Dystrophy pages database (www.dmd.nl)

** Exclusion based on simultaneous identification of two additional mutations clearly considered as disease-causing, in the same patient

*** dbSNP: www.ncbi.nlm.nih.gov/SNP/

**** Splicing Sequences Finder: www.umd.be/SSF/

A



B

	This Series All phenotypes	This series MM	This series LGMD	Literature All phenotypes
TOTAL	152	62	51	246
Deletions and Insertions	45 (29.60%)	18 (29.03%)	15 (29.41%)	60 (24.39%)
Deletions	27 (17.76%)	11 (17.74%)	10 (19.60%)	42 (17.07%)
Out of frame deletions	25 (16.44%)	9 (14.52%)	10 (19.60%)	42 (17.07%)
In frame deletions	2 (1.31%)	2 (3.23%)	0 (0%)	0 (0%)
Insertions	18 (11.84%)	7 (11.29%)	5 (9.80%)	18 (7.32%)
Out of frame insertions	18 (11.84%)	7 (11.29%)	5 (9.80%)	18 (7.32%)
In frame insertions	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Point mutations	79 (51.97%)	30 (48.39%)	28 (54.90%)	158 (64.23%)
Missenses	39 (25.66%)	12 (19.35%)	15 (29.41%)	114 (46.34%)
Nonsense	40 (26.31%)	18 (29.03%)	13 (25.49%)	44 (17.89%)
Intronic mutations	28 (18.42%)	14 (22.58%)	8 (15.67%)	28 (11.38%)

Figure 1. A. Distribution of mutations identified in this study on the *DYSF* coding sequence, and corresponding protein domains. **B.** Different types of disease-causing mutations identified in index cases included in this study. For heterozygotes, both disease-causing mutations are counted; for homozygotes, the disease-causing mutation is counted once.

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REFERENCES

Anderson LV, Davison K. 1999. Multiplex Western blotting system for the analysis of muscular dystrophy proteins. Am J Pathol 154:1017-22.

Aoki M, Liu J, Richard I, Bashir R, Britton S, Keers SM, Oeltjen J, Brown HE, Marchand S, Bourg N, Beley 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 57:271-8.

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 20:37-42.

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 15:86-94.

Beroud C, Hamroun D, Collod-Beroud G, Boileau C, Soussi T, Claustres M. 2005. UMD (Universal Mutation Database): 2005 update. Hum Mutat 26:184-91.

Bushby KM. 1999. Making sense of the limb-girdle muscular dystrophies. Brain 122 (Pt 8):1403-20.

Daniele N, Richard I, Bartoli M. 2007. Ins and outs of therapy in limb girdle muscular dystrophies. Int J Biochem Cell Biol 39:1608-24.

de Luna N, Gallardo E, Soriano M, Dominguez-Perles R, de la Torre C, Rojas-Garcia R, Garcia-Verdugo JM, Illa I. 2006. Absence of dysferlin alters myogenin expression and delays human muscle differentiation "in vitro". J Biol Chem 281:17092-8.

Drake JW, Bebenek A, Kissling GE, Peddada S. 2005. Clusters of mutations from transient hypermutability. Proc Natl Acad Sci U S A 102:12849-54.

Glover L, Brown RH, Jr. 2007. Dysferlin in Membrane Trafficking and Patch Repair. Traffic.

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 68:1284-9.

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 20:31-6.

Mardis ER. 2006. Anticipating the 1,000 dollar genome. Genome Biol 7:112.

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 26:165.

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 64:1176-82.

Ueyama H, Kumamoto T, Horinouchi H, Fujimoto S, Aono H, Tsuda T. 2002. Clinical heterogeneity in dysferlinopathy. Intern Med 41:532-6.