

Supplementary methods:

Variant classification.

DYSF transcripts ENST00000258104.7 and NM_003494.4 were used for all analyses. The pathogenicity of 176 variants from dysferlinopathy patients referred to our diagnostic laboratory was re-evaluated according to the ACMG/AMP criteria¹ with the modifications described in the Supplementary methods. The variants analyzed in this study are listed in the Supplementary Table S1. The detailed information about the lines of evidence used for variant classification are provided in the Supplementary Table S2. The Supplemental Table S3 lists variants that were re-interpreted as Benign, Likely benign or VUS (variant of uncertain significance). Forty novel variants were submitted to DYSF-LOVD. We also corrected the outdated classifications of variants in our set.

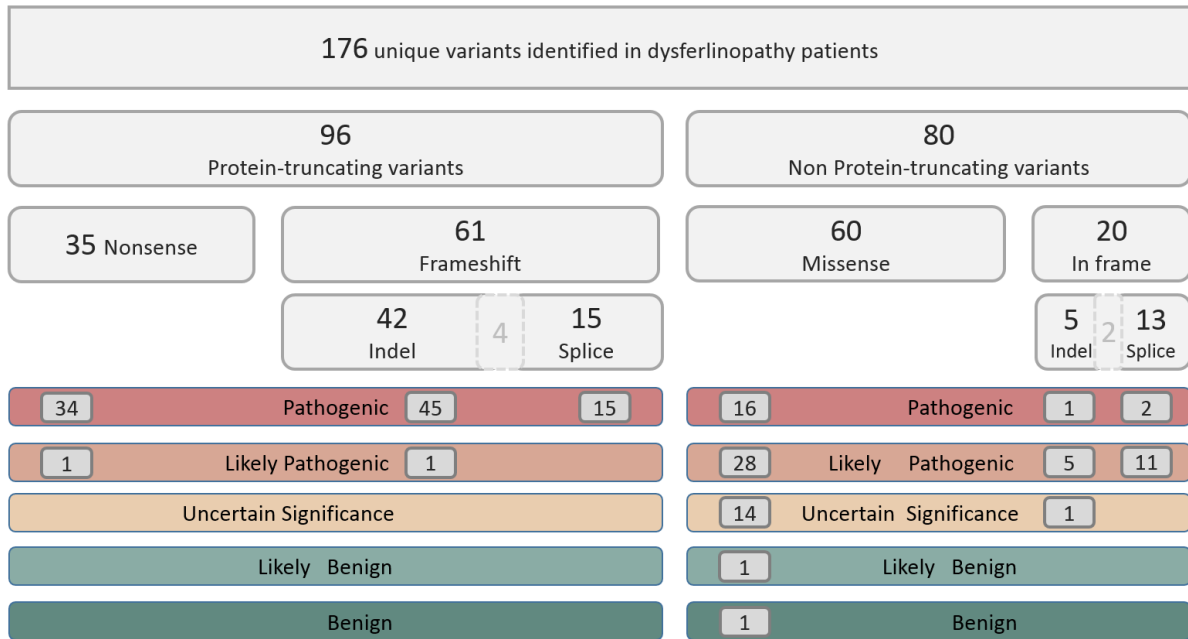
We applied the PVS1 criteria according to ClinGen Sequence Variant Interpretation (SVI) recommendations for Interpreting the Loss of Function PVS1 ACMG/AMP Variant Criteria². When attributing PVS1 to splice variants, presence of cryptic splice sites around the variant was explored using Human Splicing Finder³ (<https://www.genomnis.com/access-hsf>). PS3 score was assigned for variants with a minigene result "Impact on splicing" in Kergoulay *et al.*⁴. This code was downgraded to PS3_supporting for c.565C>G variant since only a trace amount of abnormal transcript was observed in the minigene assay with persistent presence of normally spliced transcript⁴. We applied PP3 score (*in silico* prediction of pathogenicity) if CADD or REVEL score were above 25 and 0.7 respectively. REVEL scores⁵ were obtained from <https://sites.google.com/site/revelgenomics/downloads> and CADD scores⁶ from <https://cadd.gs.washington.edu/snv>, using GRCh37-V1.6 model. HSF³, MaxENT⁷ and SpliceAI⁸ were used to assign PP3 for non-canonical splicing variants using the default thresholds. SpliceAI scores were also obtained for all missense variants in order to identify variants with a possible impact on splicing (no above-threshold variants identified, data not shown). PM3 score (in *trans* with a pathogenic variant) was assigned according to the SVI Recommendation for in *trans* Criterion PM3 (Version 1.0,

https://www.clinicalgenome.org/site/assets/files/3717/svi_proposal_for_pm3_criterion_-_version_1.pdf). PP1 score (segregation data) was assigned according to the recommendations by the Hearing Loss ClinGen Working group that focused in part on recessive disorders⁹. The thresholds for the allele frequency criteria were as following: PM2 - 0.02%, BS1 - 0.5%, BA1 - 5%. The POPMAX Filtering Allele Frequencies were obtained from gnomAD v2.1.1¹⁰. Since clinical presentation of all patients in our cohort was suggestive of Dysferlinopathy (progressive muscle weakness with evidence of proximal or distal muscle involvement confirmed by biopsy or EMG), supporting strength PP4 code was assigned to all rare variants associated with a second pathogenic or likely pathogenic variant. This code was not applied if the variant in question was associated with two pathogenic variants that fully explained the dysferlinopathy phenotype. A moderate strength code (PP4_moderate) was assigned when dysferlin protein was completely absent in muscle tissues or monocytes (as detected by western blot or Immuno-Histo-Chemistry) and the variant in question was associated with a Pathogenic or a Likely pathogenic variant. PP4_moderate code was not assigned if only a partial decrease of Dysferline protein was observed. If the studied variant was associated with two pathogenic variants, PP4_moderate code was not assigned. Indeed, in this case it cannot be ruled out that the absence of the protein was caused by the other two pathogenic variants rather than by the presence of the variant in question. PP4 codes were only assigned to rare variants (PM2 also attributed). PM1 criterion was not used since the functional domains of dysferlin are not devoid of benign variation. BP1/PP2 codes were not used since both benign and pathogenic missense variants are present at high frequency in *DYSF* gene. PP5 and BP6 were not used following the recommendations by ClinGen SVI Working Group¹¹.

Data analysis and visualization was performed with R 4.0.3 using the collection of tidyverse packages (v.1.3.0, <https://www.tidyverse.org/>) and the ggforce package (v.0.3.2). Classification summaries shown in the Supplementary Table S2 were generated by automatic extraction from individual files

containing data for a given variant using an Excel VBA macro. Visualization of variants on the *DYSF* gene structure in the Figure 2 was done with ProteinPaint¹² (<https://pecan.stjude.cloud/proteinpaint>), using the domain positions described by Sula *et al.*¹³.

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Supplementary Figure S1: The numbers of different types of *DYSF* variants identified in dysferlinopathy patients referred to the Medical Genetics Department of Marseille.

Hundred and seventy-six unique variants have been identified in dysferlinopathy patients and returned as pathogenic at the moment of diagnosis since the beginning of diagnostic sequencing of *DYSF* gene at the Medical Genetics Department at La Timone Hospital in Marseille, France – 96 protein-truncating (PTV) and 80 non protein-truncating (non-PTV) variants. Protein-truncating variant group is composed of 35 nonsense variants, 46 frameshift inducing indels and 15 variants with splice impact leading to a frameshift. The non-PTV group consists of 60 missense variants, seven in-frame indels and 13 variants with impact on splicing but leading to inframe splice alterations. Four indel PTVs and 2 indel non-PTVs also had an impact on splicing (listed as “indel” in the Supplementary Table S1 and in Figure 1B). The variants analyzed in this study are listed in the Supplementary Table S1. The detailed information about the lines of evidence used for variant classification is provided in the Supplementary Table S2.

Supplemental Table S1: The list of 176 unique *DYSF* variants identified in dysferlinopathy patients referred to the Medical Genetics Department of Marseille.

Genomic position (hg19)	cDNA (NM_003494.4)	Protein (NP_003485.1)	Exon, Intron	type	Clinical Significance ClinVar	Clinical Significance LOVD	Clinical Significance this study
chr2:71681148	c.20T>C	p.(Leu7Pro)	ex1	missense	-	-	VUS
chr2:71708031_71708032	c.107_108del	p.(Lys36Sfs)	ex2	indel	-	P/LP	pathogenic
chr2:71709018	c.154T>C	p.(Trp52Arg)	ex3	missense	-	P/LP	likely pathogenic
chr2:71730354	c.247del	p.(Glu83fs)	ex4	indel	-	P/LP	pathogenic
chr2:71730358	c.251C>T	p.(Ala84Val)	ex4	missense	Uncertain/conflicting	Benign	VUS
chr2:71730372	c.265C>T	p.(Arg89Ter)	ex4	nonsense	P/LP	P/LP	pathogenic
chr2:71730438	c.331C>T	p.(Gln111Ter)	ex4	nonsense	P/LP	P/LP	pathogenic
chr2:71730454	c.342+5G>A	-	int4	splice	-	-	likely pathogenic
chr2:71730450	c.342+1G>A	-	int4	splice	-	P/LP	pathogenic
chr2:71739052	c.457+1dup	-	int5	indel	-	P/LP	pathogenic
chr2:71740878	c.490G>T	p.(Gly164Ter)	ex6	nonsense	-	P/LP	pathogenic
chr2:71740953	c.565C>G	p.(Leu189Val)	ex6	missense	Benign	Uncertain/conflicting	benign
chr2:71740979	c.591C>G	p.(Tyr197Ter)	ex6	nonsense	-	P/LP	pathogenic
chr2:71740998	c.610C>T	p.(Arg204Ter)	ex6	nonsense	P/LP	Uncertain/conflicting	pathogenic
chr2:71742760	c.671T>G	p.(Val224Gly)	ex7	missense	-	-	VUS
chr2:71742790	c.701G>A	p.(Gly234Glu)	ex7	missense	P/LP	P/LP	likely pathogenic
chr2:71742844	c.755C>T	p.(Thr252Met)	ex7	missense	P/LP	Uncertain/conflicting	pathogenic
chr2:71742846	c.757C>T	p.(Arg253Trp)	ex7	missense	P/LP	Uncertain/conflicting	pathogenic
chr2:71743314	c.797T>C	p.(Leu266Pro)	ex8	missense	-	P/LP	likely pathogenic
chr2:71743316_71743317	c.799_800del	p.(Phe267fs)	ex8	indel	P/LP	P/LP	pathogenic
chr2:71743368	c.851T>C	p.(Ile284Thr)	ex8	missense	Uncertain/conflicting	P/LP	likely pathogenic
chr2:71743373	c.855+1del	-	int8	indel	P/LP	P/LP	pathogenic
chr2:71744120	c.857T>A	p.(Val286Glu)	ex9	missense	Uncertain/conflicting	Uncertain/conflicting	pathogenic
chr2:71744159	c.896G>A	p.(Gly299Glu)	ex9	missense	Uncertain/conflicting	P/LP	VUS
chr2:71744158	c.895G>A	p.(Gly299Arg)	ex9	missense	P/LP	P/LP	pathogenic
chr2:71747318_71747324	c.917_923dup	p.(Ile308fs)	ex10	indel	-	-	pathogenic
chr2:71747940	c.959G>A	p.(Trp320Ter)	ex11	nonsense	-	-	pathogenic
chr2:71748001	c.1020C>A	p.(Ser340Arg)	ex11	missense	P/LP	P/LP	likely pathogenic
chr2:71748001	c.1020C>G	p.(Ser340Arg)	ex11	missense	Uncertain/conflicting	Uncertain/conflicting	likely pathogenic

chr2:71753360_71753361	c.1064_1065del	p.(Lys355fs)	ex12	indel	-	P/LP	pathogenic
chr2:71753451_71753478	c.1155_1180+2del	-	ex12	indel	-	-	pathogenic
chr2:71753453_71753464	c.1157_1168del	p.(Phe386_Asp390delinsTyr)	ex12	indel	-	P/LP	likely pathogenic
chr2:71753464	c.1168G>A	p.(Asp390Asn)	ex12	missense	P/LP	Uncertain/conflicting	pathogenic
chr2:71753473	c.1177C>T	p.(Gln393Ter)	ex12	nonsense	P/LP	P/LP	pathogenic
chr2:71753476_71753483	c.1180_1180+7del	-	ex12	indel	-	P/LP	pathogenic
chr2:71753477	c.1180+1G>A	-	int12	splice	-	P/LP	pathogenic
chr2:71753481	c.1180+5G>A	-	int12	splice	Uncertain/conflicting	Uncertain/conflicting	pathogenic
chr2:71755523	c.1276G>A	p.(Gly426Arg)	ex13	missense	Uncertain/conflicting	P/LP	likely pathogenic
chr2:71755532	c.1284+1G>A	-	int13	splice	-	P/LP	likely pathogenic
chr2:71755533	c.1284+2T>C	-	int13	splice	P/LP	P/LP	pathogenic
chr2:71762412	c.1368C>G	p.(Cys456Trp)	ex15	missense	-	P/LP	likely pathogenic
chr2:71762412	c.1368C>A	p.(Cys456Ter)	ex15	nonsense	P/LP	P/LP	pathogenic
chr2:71762419	c.1375dup	p.(Met459fs)	ex15	indel	P/LP	P/LP	pathogenic
chr2:71762436	c.1392dup	p.(Asp465fs)	ex15	indel	P/LP	P/LP	pathogenic
chr2:71762442	c.1397+1G>C	-	int15	splice	-	-	pathogenic
chr2:71766329_71766332	c.1440_1443del	p.(Ser481fs)	ex16	indel	P/LP	-	pathogenic
chr2:71778203	c.1555G>A	p.(Gly519Arg)	ex18	missense	Uncertain/conflicting	Uncertain/conflicting	pathogenic
chr2:71778265	c.1617C>G	p.(Tyr539Ter)	ex18	nonsense	-	P/LP	pathogenic
chr2:71778287	c.1638+1G>C	-	int18	splice	-	P/LP	pathogenic
chr2:71778740	c.1642dup	p.(Glu548fs)	ex19	indel	P/LP	P/LP	pathogenic
chr2:71778753_71778766	c.1655_1668del	p.(Tyr552fs)	ex19	indel	-	P/LP	pathogenic
chr2:71778761	c.1663C>T	p.(Arg555Trp)	ex19	missense	P/LP	P/LP	pathogenic
chr2:71778797_71778817	c.1699_1719del	p.(His567_Glu573del)	ex19	indel	-	-	likely pathogenic
chr2:71780146	c.1758C>G	p.(Tyr586Ter)	ex20	nonsense	P/LP	P/LP	pathogenic
chr2:71780176_71780178	c.1788_1790dup	p.(Ala597_Phe598insAla)	ex20	indel	-	-	likely pathogenic
chr2:71780183_71780187	c.1795_1799dup	p.(Ala601fs)	ex20	indel	-	P/LP	pathogenic
chr2:71780222	c.1834C>T	p.(Gln612Ter)	ex20	nonsense	P/LP	Uncertain/conflicting	pathogenic
chr2:71780236	c.1848C>A	p.(Ser616Arg)	ex20	missense	-	-	likely pathogenic
chr2:71780240	c.1852G>A	p.(Gly618Arg)	ex40	missense	Uncertain/conflicting	P/LP	likely pathogenic
chr2:71780249	c.1861G>A	p.(Gly621Arg)	ex20	missense	P/LP	P/LP	pathogenic
chr2:71780265	c.1877T>C	p.(Met626Thr)	ex20	missense	Uncertain/conflicting	Uncertain/conflicting	VUS
chr2:71780299	c.1911C>A	p.(Tyr637Ter)	ex20	nonsense	P/LP	P/LP	pathogenic

chr2:71780954	c.1948del	p.(Leu650fs)	ex21	indel	-	P/LP	pathogenic
chr2:71788880	c.2163-2A>G	-	int22	splice	P/LP	P/LP	pathogenic
chr2:71788936	c.2217C>A	p.(Tyr739Ter)	ex23	nonsense	-	P/LP	pathogenic
chr2:71789075	c.2355+1G>A	-	int23	splice	P/LP	P/LP	pathogenic
chr2:71791288	c.2456G>A	p.(Arg819Gln)	ex24	missense	Uncertain/conflicting	Uncertain/conflicting	VUS
chr2:71791328_71791331	c.2496_2499del	p.(Thr833fs)	ex24	indel	P/LP	P/LP	pathogenic
chr2:71791336_71791339	c.2504_2507del	p.(Phe835Ter)	ex24	indel	-	-	pathogenic
chr2:71791344	c.2511+1G>A	-	int24	splice	-	P/LP	likely pathogenic
chr2:71793569_71797845	c.2512-1512_3148del	-	ex25-29	indel	-	-	pathogenic
chr2:71795210	c.2641A>C	p.(Thr881Pro)	ex25	missense	-	Uncertain/conflicting	likely pathogenic
chr2:71795213	c.2643+1G>A	-	int25	splice	P/LP	P/LP	pathogenic
chr2:71795364	c.2706dup	p.(Lys903fs)	ex26	indel	P/LP	P/LP	pathogenic
chr2:71795405_71795425	c.2747_2767delinsTCCA	p.(Asp916fs)	ex26	indel	-	-	pathogenic
chr2:71795430	c.2772G>A	p.(Trp924Ter)	ex26	nonsense	P/LP	P/LP	pathogenic
chr2:71795437	c.2779del	p.(Ala927fs)	ex26	indel	P/LP	P/LP	pathogenic
chr2:71795468	c.2810C>T	p.(Thr937Ile)	ex26	missense	Uncertain/conflicting	-	VUS
chr2:71795473	c.2810+5G>A	-	int26	splice	Uncertain/conflicting	Uncertain/conflicting	likely pathogenic
chr2:71796948	c.2811-2A>C	-	int26	splice	P/LP	P/LP	pathogenic
chr2:71796997	c.2858dup	p.(Phe954fs)	ex27	indel	-	P/LP	pathogenic
chr2:71797014	c.2875C>T	p.(Arg959Trp)	ex27	missense	P/LP	P/LP	pathogenic
chr2:71797033	c.2894G>A	p.(Trp965Ter)	ex27	nonsense	P/LP	P/LP	pathogenic
chr2:71797430	c.2997G>T	p.(Trp999Cys)	ex28	missense	P/LP	P/LP	pathogenic
chr2:71797431	c.2998T>C	p.(Ser1000Pro)	ex28	missense	Uncertain/conflicting	Uncertain/conflicting	likely pathogenic
chr2:71797466	c.3031+2T>C	-	int28	splice	P/LP	P/LP	pathogenic
chr2:71797732	c.3035G>A	p.(Trp1012Ter)	ex29	nonsense	-	P/LP	pathogenic
chr2:71797756	c.3059dup	p.(Glu1021fs)	ex29	indel	-	P/LP	pathogenic
chr2:71797809	c.3112C>G	p.(Arg1038Gly)	ex29	missense	-	-	likely pathogenic
chr2:71797809	c.3112C>T	p.(Arg1038Ter)	ex29	nonsense	P/LP	P/LP	pathogenic
chr2:71797810	c.3113G>A	p.(Arg1038Gln)	ex29	missense	P/LP	Uncertain/conflicting	pathogenic
chr2:71797823	c.3126G>A	p.(Trp1042Ter)	ex29	nonsense	-	P/LP	pathogenic
chr2:71797834	c.3137G>A	p.(Arg1046His)	ex29	missense	P/LP	Uncertain/conflicting	pathogenic
chr2:71801378	c.3225del	p.(Phe1075fs)	ex30	indel	-	P/LP	pathogenic

chr2:71801439_71801444	c.3286_3291dup	p.(Arg1097_Met1098insArgArg)	ex30	indel	-	-	VUS
chr2:71801474_71801475	c.3321_3322delinsC	p.(Ala1108fs)	ex30	indel	-	-	pathogenic
chr2:71801474_71801477	c.3321_3324dup	p.(Val1109fs)	ex30	indel	-	P/LP	pathogenic
chr2:71801496	c.3343dup	p.(Ala1115fs)	ex30	indel	-	-	pathogenic
chr2:71801504_71801507	c.3348+3_3348+6del	-	int30	indel	-	P/LP	likely pathogenic
chr2:71816722	c.3349-1G>T	-	int30	splice	-	-	likely pathogenic
chr2:71816747	c.3373del	p.(Glu1125fs)	ex31	indel	-	P/LP	pathogenic
chr2:71816763_71816773	c.3389_3399dup	p.(Phe1135fs)	ex31	indel	-	P/LP	pathogenic
chr2:71817369	c.3471C>A	p.(Tyr1157Ter)	ex32	nonsense	-	-	pathogenic
chr2:71817375	c.3477C>A	p.(Tyr1159Ter)	ex32	nonsense	-	Uncertain/conflicting	pathogenic
chr2:71817414_71817415	c.3516_3517del	p.(Ser1173Ter)	ex32	indel	P/LP	P/LP	pathogenic
chr2:71825770	c.3597G>A	p.(Trp1199Ter)	ex33	nonsense	-	-	pathogenic
chr2:71825856	c.3683T>C	p.(Leu1228Pro)	ex33	missense	-	P/LP	likely pathogenic
chr2:71825860	c.3687C>A	p.(Tyr1229Ter)	ex33	nonsense	P/LP	P/LP	pathogenic
chr2:71827831	c.3703-1G>A	-	int33	splice	-	P/LP	pathogenic
chr2:71825876	c.3702+1G>A	-	int33	splice	-	P/LP	pathogenic
chr2:71827837	c.3708del	p.(Asp1237fs)	ex34	indel	P/LP	P/LP	pathogenic
chr2:71827934	c.3805G>T	p.(Glu1269Ter)	ex34	nonsense	P/LP	P/LP	pathogenic
chr2:71827955	c.3826C>G	p.(Leu1276Val)	ex34	missense	-	P/LP	likely pathogenic
chr2:71827961	c.3832C>T	p.(Gln1278Ter)	ex34	nonsense	P/LP	Uncertain/conflicting	pathogenic
chr2:71827973	c.3843+1G>A	-	int34	splice	P/LP	P/LP	likely pathogenic
chr2:71838438	c.3967C>T	p.(Gln1323Ter)	ex37	nonsense	-	P/LP	pathogenic
chr2:71838477	c.4005+1G>A	-	int37	splice	-	P/LP	likely pathogenic
chr2:71838649	c.4060del	p.(Ser1354fs)	ex38	indel	P/LP	-	pathogenic
chr2:71838670	c.4081T>C	p.(Cys1361Arg)	ex38	missense	P/LP	P/LP	likely pathogenic
chr2:71838757	c.4167+1G>C	-	int38	splice	P/LP	P/LP	likely pathogenic
chr2:71839794	c.4191C>G	p.(Tyr1397Ter)	ex39	nonsense	-	P/LP	pathogenic
chr2:71839803	c.4200del	p.(Ile1401fs)	ex39	indel	-	P/LP	pathogenic
chr2:71839803	c.4200dup	p.(Ile1401fs)	ex39	indel	P/LP	P/LP	pathogenic
chr2:71839804	c.4201dup	p.(Ile1401fs)	ex39	indel	-	P/LP	pathogenic
chr2:71839855	c.4252G>C	p.(Gly1418Arg)	ex39	missense	-	-	likely pathogenic
chr2:71840506	c.4376A>G	p.(Asp1459Gly)	ex40	missense	Benign	-	VUS
chr2:71840541	c.4410+1G>A	-	int40	splice	-	-	pathogenic

chr2:71871117	c.4433G>A	p.(Trp1478Ter)	ex41	nonsense	-	P/LP	pathogenic
chr2:71883332	c.4550del	p.(Gly1517fs)	ex42	indel	-	-	pathogenic
chr2:71883359	c.4577A>C	p.(Lys1526Thr)	ex42	missense	Uncertain/conflicting	Uncertain/conflicting	VUS
chr2:71883398_71883401	c.4616_4619del	p.(Pro1539fs)	ex42	indel	-	-	pathogenic
chr2:71883410	c.4628G>A	p.(Gly1543Asp)	ex42	missense	-	P/LP	likely pathogenic
chr2:71886006	c.4639-2A>G	-	int42	splice	-	-	likely pathogenic
chr2:71886008	c.4639G>C	p.(Gly1547Arg)	ex43	missense	-	P/LP	likely pathogenic
chr2:71886125	c.4756C>T	p.(Arg1586Ter)	ex43	nonsense	P/LP	P/LP	pathogenic
chr2:71886132	c.4763T>G	p.(Phe1588Cys)	ex43	missense	-	-	likely pathogenic
chr2:71887749	c.4854C>G	p.(Tyr1618Ter)	ex44	nonsense	-	-	pathogenic
chr2:71887767_71887771	c.4872_4876delinsCCCC	p.(Glu1624fs)	ex44	indel	P/LP	P/LP	pathogenic
chr2:71891526	c.5015C>T	p.(Ser1672Phe)	ex45	missense	-	P/LP	VUS
chr2:71891544	c.5033G>C	p.(Cys1678Ser)	ex45	missense	Uncertain/conflicting	P/LP	likely pathogenic
chr2:71891547	c.5036G>A	p.(Gly1679Glu)	ex45	missense	-	P/LP	likely pathogenic
chr2:71892312	c.5078G>A	p.(Arg1693Gln)	ex46	missense	Uncertain/conflicting	P/LP	pathogenic
chr2:71892327	c.5093C>T	p.(Pro1698Leu)	ex46	missense	-	-	likely pathogenic
chr2:71892435	c.5200+1G>A	-	int46	splice	P/LP	P/LP	pathogenic
chr2:71894548	c.5243A>T	p.(Glu1748Val)	ex47	missense	-	P/LP	VUS
chr2:71894601	c.5296G>T	p.(Glu1766Ter)	ex47	nonsense	-	P/LP	pathogenic
chr2:71894608	c.5303G>A	p.(Arg1768Gln)	ex47	missense	Uncertain/conflicting	Uncertain/conflicting	likely pathogenic
chr2:71894607	c.5302C>T	p.Arg1768Trp	ex47	missense	P/LP	Uncertain/conflicting	pathogenic
chr2:71894619_71894623	c.5314_5318del	p.(Ser1772fs)	ex47	indel	-	P/LP	pathogenic
chr2:71895882	c.5341-2A>C	-	int47	splice	P/LP	Uncertain/conflicting	likely pathogenic
chr2:71896227_71896243	c.5430-15_5431del	-	ex49	indel	-	-	likely pathogenic
chr2:71896256	c.5444G>A	p.(Cys1815Tyr)	ex49	missense	-	-	likely pathogenic
chr2:71896266	c.5454G>C	p.(Trp1818Cys)	ex49	missense	P/LP	-	VUS
chr2:71896321	c.5509G>A	p.(Asp1837Asn)	ex49	missense	P/LP	P/LP	pathogenic
chr2:71896721	c.5526-14A>G	-	int49	splice	-	Uncertain/conflicting	likely pathogenic
chr2:71896742	c.5533del	p.(Ile1845fs)	ex50	indel	P/LP	-	pathogenic
chr2:71896803	c.5594del	p.(Gly1865fs)	ex50	indel	P/LP	P/LP	pathogenic
chr2:71896835	c.5626G>A	p.(Asp1876Asn)	ex50	missense	Benign	Uncertain/conflicting	Likely benign
chr2:71901320	c.5668-7G>A	-	int50	splice	P/LP	P/LP	pathogenic
chr2:71901372	c.5713C>T	p.(Arg1905Ter)	ex51	nonsense	P/LP	P/LP	pathogenic

chr2:71901427	c.5767+1G>A	-	int51	splice	P/LP	P/LP	pathogenic
chr2:71906232_71906240	c.5813_5821del	p.(Thr1938_Lys1940del)	ex52	indel	-	P/LP	pathogenic
chr2:71906279	c.5860G>T	p.(Glu1954Ter)	ex52	nonsense	-	-	pathogenic
chr2:71906290_71906291	c.5871_5872del	p.(Ser1958fs)	ex52	indel	-	P/LP	pathogenic
chr2:71906318	c.5899G>A	p.(Gly1967Ser)	ex52	missense	-	P/LP	likely pathogenic
chr2:71906322	c.5903G>A	p.(Trp1968Ter)	ex52	nonsense	-	P/LP	pathogenic
chr2:71906327	c.5908C>T	p.(Pro1970Ser)	ex52	missense	P/LP	Uncertain/conflicting	likely pathogenic
chr2:71906339_71906340	c.5920_5921insCCCTGTGTGGCACTGTGTAGGA G	p.(Glu1974fs)	ex52	indel	-	-	pathogenic
chr2:71908163	c.5979dup	p.(Glu1994fs)	ex53	indel	P/LP	P/LP	pathogenic
chr2:71908183	c.5999G>A	p.(Arg2000Gln)	ex53	missense	Uncertain/conflicting	Uncertain/conflicting	VUS
chr2:71908201	c.6017G>A	p.(Arg2006Gln)	ex53	missense	-	-	VUS
chr2:71908213	c.6029A>G	p.(Asn2010Ser)	ex53	missense	-	-	likely pathogenic
chr2:71909722	c.6119G>A	p.(Trp2040Ter)	ex54	nonsense	-	P/LP	pathogenic
chr2:71909727	c.6124C>T	p.(Arg2042Cys)	ex54	missense	P/LP	P/LP	pathogenic
chr2:71913589	c.6210T>G	p.(Tyr2070Ter)	ex55	nonsense	-	-	likely pathogenic
chr2:71913612_71913619	c.6233_6240del	p.(Pro2078fs)	ex55	indel	-	P/LP	likely pathogenic

Supplemental Table S1: The list of 176 unique *DYSF* variants identified in dysferlinopathy patients referred to the Medical Genetics Department of Marseille.

The list of 176 unique *DYSF* variants identified in dysferlinopathy patients referred to our center for diagnostic sequencing of *DYSF* gene since 2001 (see Figure 1A for more details about variant selection). The genomic coordinates (hg19) as well as cDNA and protein position are listed for each variant (columns “Chr”, “Start”, “cDNA”, “Protein”, “Exon/Intron”). The type of each variant is also listed. Indels with a potential effect on splicing are listed as “indel”. Clinical Significance from ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar>) and/or LOVD (<https://databases.lovd.nl/shared/genes/DYSF>) databases is provided if a variant was present in these databases. The pathogenic class after the re-classification of the variant is listed in the “Clinical Significance this study” column. The numbers of re-classified variants by type are represented in the Figure 1B, while the numbers of variants in each pathogenic group are shown in the Supplementary Figure S1. The detailed evidence used to classify each variant according to the modified ACMG/AMP criteria (see Materials and Methods for details) is shown in the “Evidence for each variant” column. The detailed evidence used to classify each variant is shown in the Supplementary Table S2.

Supplemental Table S2: The detailed evidence used for ACMG/AMP classification of *DYSF* variants identified in dysferlinopathy patients referred to the Medical Genetics Department of Marseille.

Variant	Evidence for each variant	ACMG/AMP Classification
<p>DYSF NM_003494.3:c.20T>C p.(Leu7Pro) exon 1 (hg19)chr2:71681148 Canonical Allele Identifier: CA347205953</p>	<p>We have identified this variant in one patient in a homozygous state [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2] and not predicted to affect protein function (CADD = 22,7 (v1.6), REVEL = 0,479). There are no ClinVar or LOVD entries for this variant (submitted to LOVD with ID: 711840). This patient also carried another VUS (c.2810C>T, p.(Thr937Ile)) in a homozygous state.</p>	<p>Uncertain significance: PM2 + PP4 supporting + PM3 supporting</p>
<p>DYSF NM_003494.3:c.107_108del p.(Lys36SerfsTer12) exon 2 (hg19)chr2:71708031_71708032del Canonical Allele Identifier: CA222121</p>	<p>This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.107_108del in 3 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.1758C>G, c.3389_3399dup (<i>trans</i>)), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.</p>	<p>Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate + PP1 supporting</p>
<p>DYSF NM_003494.3:c.154T>C p.(Trp52Arg) exon 3 (hg19)chr2:71709018 Canonical Allele Identifier: CA347216252</p>	<p>We have identified c.154T>C in 1 patient in a heterozygous state (associated with a pathogenic variant: c.1655_1668del) . This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.701G>A, PMID: 27602406), associated in 1 case (PMID: 27602406) with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. It is predicted to affect protein function (CADD = 28 (v1.6), REVEL = 0,736) [PP3].</p>	<p>Likely pathogenic: PM2 + PP4 moderate + PP3 + PM3 supporting</p>
<p>DYSF NM_003494.3:c.247del p.(Glu83LysfsTer) exon 4 (hg19)chr2:71730354del Canonical Allele Identifier: CA1139532912</p>	<p>This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.247del in 2 patients (1 family) in a heterozygous state (associated with a likely pathogenic variant: c.6124C>T) . [PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.</p>	<p>Pathogenic: PVS1 + PM2 + PP1 supporting + PP4 supporting</p>
<p>DYSF NM_003494.3:c.251C>T p.(Ala84Val) exon 4 (hg19)chr2:71730358 Canonical Allele Identifier: CA1705297</p>	<p>We have identified c.251C>T in two patients (two families) in a heterozygous state. In one patient it was associated with two pathogenic variants (c.5594del and c.3113G>A), but it was not possible to confirm if c.251C>T variant was present <i>in cis</i> with one of these two variants. No additional pathogenic variants were identified in the second patient. This variant is present in only 5 individuals in gnomAD v2.1.1 (POPMAX filtering allele frequency: 0,0011 %, 1/24932 in African population) [PM2] and not predicted to affect protein function (CADD = 23,1 (v1.6), REVEL = 0,186). There is one VUS ClinVar entry (ClinVar ID: 284573). This variant has been published by our group as benign (PMID: 18853459, LOVD Individual ID: 215600) before ACMG/AMP classification was established in our laboratory, mostly based on benign <i>in silico</i> predictions.</p>	<p>Uncertain significance: PM2</p>

DYSF NM_003494.3:c.265C>T p.(Arg89Ter) exon 4 (hg19)chr2:71730372 Canonical Allele Identifier: CA275254	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 1 patient in a homozygous state. We have identified c.265C>T in 1 patient in a heterozygous state (associated with a pathogenic variant: c.3126G>A), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is present in only 1 individual in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0009% 1/113746 in European (non-Finnish) population) [PM2] (CADD = 37 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate
DYSF NM_003494.3:c.331C>T p.(Gln111Ter) exon 4 (hg19)chr2:71730438 Canonical Allele Identifier: CA10588346	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 3 patients in a homozygous state, associated in 2 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. We have identified c.331C>T in 1 patient in a heterozygous state (associated with a pathogenic variant: c.2643+1G>A) [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 41 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate
DYSF NM_003494.3:c.342+1G>A: intron 4 (hg19)chr2:71730450 Canonical Allele Identifier: CA347204681	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.342+1G>A in 3 patients (1 family) in a heterozygous state (associated with a pathogenic variant: c.3516-3517del) [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 3 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 moderate] (CADD = 33 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP1 moderate + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.342+5G>A: intron 4 (hg19)chr2:71730454 Canonical Allele Identifier: CA1139532904	We have identified c.342+5G>A in 1 patient in a heterozygous state (associated with a pathogenic variant: c.1663C>T (<i>trans</i>)), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Alteration of the wild type donor site, most probably affecting splicing, is predicted byHSF,MaxEnt, SpliceAI: Donor Loss 0.54) [PP3].	Likely pathogenic: PM2 + PM3 + PP4 moderate + PP3
DYSF NM_003494.3:c.457+1dup: intron 5 (hg19)chr2:71739052dup Canonical Allele Identifier: CA1139532922	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 2 patients in a homozygous state, associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PP1 supporting + PM3 supporting
DYSF NM_003494.3:c.490G>T p.(Gly164Ter) exon 6 (hg19)chr2:71740878 Canonical Allele Identifier: CA347206841	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.490G>T in 3 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.3703-1G>A), associated in these 2 cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting] (CADD = 38 (v1.6)). Even though additional	Pathogenic: PVS1 + PM2 + PP4 moderate + PP1 supporting + PM3 supporting

	evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	
DYSF NM_003494.3:c.565C>G p.(Leu189Val) exon 6 (hg19)chr2:71740953 Canonical Allele Identifier: CA147767	We have identified c.565C>G in four patients (three families). In one patient this variant was present at a homozygous state, associated with a homozygous pathogenic variant c.5296G>T (i.e. present <i>in cis</i> with a pathogenic variant) [BP2]. The second patient was heterozygous for c.565C>G variant, associated with a pathogenic c.2643+1G>A, likely pathogenic c.6029A>G and a VUS c.4577A>C. Two siblings from one additional family were heterozygous for c.565C>G variant, associated with a pathogenic c.2643+1G>A and a VUS c.6017G>A. c.565C>G was confirmed to be in cis with the VUS c.6017G>A. In all three families there was an absence of Dysferlin on western blot. This variant has previously been reported in a heterozygous state in two cases in the literature (associated with c.855+4T>C or c.3516_3517del, PMID: 26088049, 17562833). A minigene assay demonstrated that this variant could create a cryptic 5' splice site, resulting in an abnormal transcript with an out-of-frame deletion of the 103 bp of 3'end of the exon 6 in addition to the normal transcript (PMID: 25312915). The PS3 code was downgraded to PS3_supporting for this variant, since only a trace amount of abnormal transcript was observed in the minigene assay with persistent presence of a normally spliced transcript [PS3 supporting]. This variant is not predicted to affect protein function (CADD = 19,04 (v1.6), REVEL = 0,182). POPMAX filtering allele frequency in gnomAD v2.1.1 is 6,56% (1712/24936 in African population, 62 homozygotes, with only 0.03% in Europeans) [BA1]. This variant has 9 benign and likely benign entries in ClinVar (ClinVar ID: 94343). This variant is listed as benign in five LOVD entries and pathogenic in three LOVD entries (PMIDs:26088049, 17562833)	Benign: BA1 + BP2 + PS3 supporting
DYSF NM_003494.3:c.591C>G p.(Tyr197Ter) exon 6 (hg19)chr2:71740979 Canonical Allele Identifier: CA347207335	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.591C>G in 1 patient in a heterozygous state (associated with a pathogenic variant: c.1155_c.1180+2del) [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 36 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.610C>T p.(Arg204Ter) exon 6 (hg19)chr2:71740998 Canonical Allele Identifier: CA222201	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.610C>T in 2 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.855+1del, c.1663C>T), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is present in only 2 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0029% 1/34588 in Latino population) [PM2] (CADD = 35 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate
DYSF NM_003494.3:c.671T>G p.(Val224Gly) exon 7 (hg19)chr2:71742760 Canonical Allele Identifier: CA347208333	We have identified c.671T>G in one patient in a heterozygous state, associated with two VUS, c.896G>A and c.1877T>C. This variant is absent from gnomAD v2.1.1 [PM2] and is predicted to affect protein function (CADD = 27 (v1.6), REVEL = 0,93) [PP3]. There are no ClinVar or LOVD entries for this variant. The initial LOVD entry for this patient (LOVD Individual ID: 215587) did not include this variant, which was now corrected.	Uncertain significance: PM2 + PP3
DYSF NM_003494.3:c.701G>A p.(Gly234Glu) exon 7 (hg19)chr2:71742790 Canonical Allele Identifier: CA275400	We have identified c.701G>A in 2 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.5903G>A, c.5533del) . This variant has previously been reported in a heterozygous state in 4 cases in the literature (associated with c.154T>C, c.3137G>A, c.1555G>A, c.2200_2205delinsT, PMID: 27602406, LOVD ID 00220647, LOVD ID 00220234, LOVD ID 00215862), associated in 1 case (PMID:	Likely pathogenic: PM3 strong + PM2 + PP4 moderate + PP3

	27602406) with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: LOVD ID 00215626), associated in 1 case (PMID: LOVD ID 00215626) with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. It is present in only 9 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0058% 4/34592 in Latino population) [PM2]. It is predicted to affect protein function (CADD = 27,3 (v1.6), REVEL = 0,642) [PP3].	
DYSF NM_003494.3:c.755C>T p.(Thr252Met) exon 7 (hg19)chr2:71742844 Canonical Allele Identifier: CA222210	We have identified this variant in 2 patients in a homozygous state . We have identified c.755C>T in 1 patient in a heterozygous state (associated with a variant of unknown significance), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 4 cases in the literature (associated with c.5444G>T, c.3789_3796del, c.1464del, c.1392dupA, PMID: 30564623, 27647186, 27602406, 24488599), associated in 1 case (PMID: 27647186) with an absence of Dysferlin on western blot / ImmunoHistoChemistry, and associated in 1 case (PMID: 27602406) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 21522182), associated in 1 case (PMID: 21522182) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. It is present in only 3 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,002% 2/18392 in East Asian population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. It is predicted to affect protein function (CADD = 25,3 (v1.6), REVEL = 0,926) [PP3]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class5/pathogenic) for this variant.	Pathogenic: PM3 strong + PM2 + PP4 moderate + PP1 supporting + PP3
DYSF NM_003494.3:c.757C>T p.(Arg253Trp) exon 7 (hg19)chr2:71742846 Canonical Allele Identifier: CA1705479	We have identified this variant in 1 patient in a homozygous state . We have identified c.757C>T in 5 patients (4 families) in a heterozygous state (associated with pathogenic variants: c.1758C>G, c.5813_5821del, c.5871_5872del, c.5979dupA), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry, and associated in 1 case with a decrease of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 4 cases in the literature (associated with exon 5–10 deletion, c.2248C>T, c.4824del, c.90delA, PMID: 27666772, 25868377, 27602406, 26404900), associated in 2 cases (PMID: 27666772, 27602406) with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 very strong, PP4 moderate]. It is present in only 32 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0126% 23/129172 in European (non-Finnish) population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. It is predicted to affect protein function (CADD = 26,4 (v1.6), REVEL = 0,614) [PP3].	Pathogenic: PM3 very strong + PM2 + PP4 moderate + PP1 supporting + PP3
DYSF NM_003494.3:c.797T>C p.(Leu266Pro) exon 8 (hg19)chr2:71743314 Canonical Allele Identifier: CA347209277	We have identified c.797T>C in 2 patients (1 family) in a heterozygous state (associated with a pathogenic variant: c.4872_4876delinsCCCC), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. It is predicted to affect protein function (CADD = 26,3 (v1.6), REVEL = 0,793) [PP3].	Likely pathogenic: PM2 + PP4 moderate + PP1 supporting + PP3 + PM3 supporting

<p>DYSF NM_003494.3:c.799_800del p.(Phe267LeufsTer5) exon 8 (hg19)chr2:71743316_71743317del Canonical Allele Identifier: CA533253809</p>	<p>This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.799_800del in 2 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.3687C>A), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry, and associated in 1 case with a decrease of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 28403181) [PM3, PP4 moderate]. It is present in only 1 individual in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0054% 1/18394 in East Asian population) [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.</p>	<p>Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate</p>
<p>DYSF NM_003494.3:c.851T>C p.(Ile284Thr) exon 8 (hg19)chr2:71743368 Canonical Allele Identifier: CA347209709</p>	<p>We have identified c.851T>C in 1 patient in a heterozygous state (associated with a pathogenic variant: c.1617C>G), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. It is predicted to affect protein function (CADD = 25,1 (v1.6), REVEL = 0,906) [PP3].</p>	<p>Likely pathogenic: PM2 + PP4 moderate + PP3 + PM3 supporting</p>
<p>DYSF NM_003494.3:c.855+1del: intron 8 (hg19)chr2:71743373del Canonical Allele Identifier: CA253921</p>	<p>This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.855+1del in 8 patients (6 families) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.5594delG, c.3031+2T>C, c.3126G>A, c.610C>T, c.959G>A), associated in 2 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 6 cases in the literature (associated with c.895G>A, c.3031G>C, c.3505_3506insC, c.3112C>T, c.3036G>A, PMID: 16705711, 27666772, 20544924, 27602406), associated in 4 cases (PMID: 16705711, 27602406) with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. It is present in only 2 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0058% 2/34572 in Latino population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class5/pathogenic) for this variant.</p>	<p>Pathogenic: PVS1 + PM3 strong + PM2 + PP4 moderate + PP1 supporting</p>
<p>DYSF NM_003494.3:c.857T>A p.(Val286Glu) exon 9 (hg19)chr2:71744120 Canonical Allele Identifier: CA222220</p>	<p>We have identified c.857T>A in 1 patient in a heterozygous state (associated with a pathogenic variant: c.5860G>T (<i>trans</i>)), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 3 cases in the literature (associated with c.2779del, c.3832C>T, c.4886+1249G>T, PMID: 18832576, 27602406, 25493284), associated in 2 cases (PMID: 18832576, 25493284) with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. It is present in only 1 individual in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0009% 1/113768 in European (non-Finnish) population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 1 affected individual, and 5 unaffected individuals (Marseille cohort) [PP1 supporting]. It is predicted to affect protein function (CADD = 32 (v1.6), REVEL = 0,945) [PP3]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.</p>	<p>Pathogenic: PM3 strong + PM2 + PP4 moderate + PP1 supporting + PP3</p>

<p>DYSF NM_003494.3:c.895G>A p.(Gly299Arg) exon 9 (hg19)chr2:71744158 Canonical Allele Identifier: CA253916</p>	<p>We have identified c.895G>A in 1 patient in a heterozygous state (associated with a pathogenic variant: c.490G>T (<i>trans</i>)), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 2 cases in the literature (associated with c.2997G>T, c.855+1del (<i>trans</i>), PMID: 22297152, 18306167), associated in 1 case (PMID: 18306167) with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 18306167), associated in 1 case (PMID: 18306167) with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. It is present in only 1 individual in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0032% 1/31190 in Genomes count population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 1 unaffected individual (PMID: 18306167) [PP1 supporting]. It is predicted to affect protein function (CADD = 27,8 (v1.6), REVEL = 0,968) [PP3]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.</p>	<p>Pathogenic: PM3 strong + PM2 + PP4 moderate + PP1 supporting + PP3</p>
<p>DYSF NM_003494.3:c.896G>A p.(Gly299Glu) exon 9 (hg19)chr2:71744159 Canonical Allele Identifier: CA347210280</p>	<p>We have identified c.896G>A in two patients (two families) in a heterozygous state. In one case it was associated with a VUS c.1877T>C, confirmed <i>in cis</i> (LOVD Individual:215343, PMIDs:16010686, 18853459), associated with absence of dysferline on western blot performed on monocytes (PP4 not assigned since the variant in question is not associated with a pathogenic or likely pathogenic variant). In the other case, this variant was associated with both VUS c.1877T>C <i>in cis</i> and with VUS c.671T>G <i>in trans</i> (LOVD Individual:215587). This variant has previously been reported in a heterozygous state in one case in the literature (associated with c.1877T>C, PMID: 27602406) associated with a decrease of Dysferlin on western blot. Since the segregation analysis was not performed in this study, it is likely that these two variants (c.1877T>C and c.896G>A) are <i>in cis</i>, as in our two patients, questioning the assumed pathogenicity for one of these variants. This variant is present in only one individual in gnomAD v2.1.1 (POPMAX filtering allele frequency: 0,0065% 1/15424 in European (non-Finnish) population) [PM2]. A different missense variant at the same amino acid position (c.895G>A) has been reported as pathogenic [PM5]. This variant is predicted to affect protein function (CADD = 25,2 (v1.6), REVEL = 0,947) [PP3]. There are two VUS entries in ClinVar (ClinVar ID: 552632). There are two LOVD entries for our two patients, initially listed at Pathogenic, that were now corrected.</p>	<p>Uncertain significance: PM2 + PM5 + PP3</p>
<p>DYSF NM_003494.3:c.917_923dup p.(Ile308MetfsTer22) exon 10 (hg19)chr2:71747318_71747324dup Canonical Allele Identifier: CA1139532927</p>	<p>This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.917_923dup in 1 patient in a heterozygous state (associated with a pathogenic variant: c.4410+1G>A) [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.</p>	<p>Pathogenic: PVS1 + PM2 + PP4 supporting + PM3 supporting</p>
<p>DYSF NM_003494.3:c.959G>A p.(Trp320Ter) exon 11 (hg19)chr2:71747940 Canonical Allele Identifier: CA347211959</p>	<p>This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.959G>A in 1 patient in a heterozygous state (associated with a pathogenic variant: c.855+1del (<i>trans</i>)), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 49 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.</p>	<p>Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate</p>

DYSF NM_003494.3:c.1020C>A p.(Ser340Arg) exon 11 (hg19)chr2:71748001 Canonical Allele Identifier: CA347212168	We have identified c.1020C>A in 2 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.5078G>A, c.5594del), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 4 cases in the literature (associated with c.3504dup, c.1254del, c.4883G>T, c.1168G>A (<i>trans</i>) and c.3181del (<i>trans</i>), PMID: 21522182, 26404900, 19528035, LOVD ID 00215537), associated in 1 case (PMID: 21522182) with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: LOVD ID 00219444) [PM3 strong, PP4 moderate]. It is present in only 2 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0033% 1/30616 in South Asian population) [PM2] (CADD = 23,4 (v1.6), REVEL = 0,695).	Likely pathogenic: PM3 strong + PM2 + PP4 moderate
DYSF NM_003494.3:c.1020C>G p.(Ser340Arg) exon 11 (hg19)chr2:71748001 Canonical Allele Identifier: CA10605994	We have identified this variant in 1 patient in a homozygous state, associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.4883G>T, PMID: 27602406), associated in 1 case (PMID: 27602406) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. Variant with the same amino acid change as a previously established pathogenic variant (c.1020C>A), regardless of nucleotide change [PS1]. It is present in only 1 individual in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0009% 1/113756 in European (non-Finnish) population) [PM2] (CADD = 23,6 (v1.6), REVEL = 0,695).	Likely pathogenic: PS1 + PM2 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.1064_1065del p.(Lys355ArgfsTer4) exon 12 (hg19)chr2:71753360_71753361del Canonical Allele Identifier: CA1139532907	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 1 patient in a homozygous state. We have identified c.1064_1065del in 2 patients (1 family) in a heterozygous state (associated with a pathogenic variant: c.2217C>A), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate + PP1 supporting
DYSF NM_003494.3:c.1155_1180+2del: exon 12 (hg19)chr2:71753451_71753478del Canonical Allele Identifier: CA1139532908	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.1155_1180+2del in 1 patient in a heterozygous state (associated with a pathogenic variant: c.591C>G) [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.1157_1168del p.(Phe386_Asp390delinsTyr) exon 12 (hg19)chr2:71753453_71753464del Canonical Allele Identifier: CA1139532900	We have identified c.1157_1168del in 3 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.5078G>A (<i>trans</i>), c.1663C>T), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Protein-length-changing variant (deletion without frameshift) [PM4]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 1 unaffected individual (Marseille cohort) [PP1 supporting].	Likely pathogenic: PM2 + PM3 + PM4 + PP4 moderate + PP1 supporting
DYSF NM_003494.3:c.1168G>A p.(Asp390Asn) exon 12 (hg19)chr2:71753464 Canonical Allele Identifier: CA10604166	We have identified c.1168G>A in 5 patients (4 families) in a heterozygous state (associated with pathogenic variants: c.5302C>T, c.1177C>T), associated in 2 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.1020C>A (<i>cis</i>) and c.3181del (<i>trans</i>), PMID: LOVD ID	Pathogenic: PM3 strong + PM2 + PP4 moderate + PP1 supporting + PP3

	00215537). This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: not found, LOVD ID 215139) [PM3 strong, PP4 moderate]. It is present in only 2 individuals in gnomAD v2.1.1 (POP MAX Filtering allele frequency: 0,0003% 2/112954 in European (non-Finnish) population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. It is predicted to affect protein function (CADD = 26,7 (v1.6), REVEL = 0,414) [PP3]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	
DYSF NM_003494.3:c.1177C>T p.(Gln393Ter) exon 12 (hg19)chr2:71753473 Canonical Allele Identifier: CA347213670	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.1177C>T in 1 patient in a heterozygous state (associated with a likely pathogenic variant: c.1168G>A) . This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.1180+5G>A, PMID: not found, LOVD ID 00215246) [PM3 supporting, PP4 supporting]. It is present in only 1 individual in gnomAD v2.1.1 (POP MAX Filtering allele frequency: 0,0009% 1/112452 in European (non-Finnish) population) [PM2] (CADD = 46 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.1180_1180+7del: exon 12 (hg19)chr2:71753476_71753483del Canonical Allele Identifier: CA1139532909	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 1 patient in a homozygous state . We have identified c.1180_1180+7del in 1 patient in a heterozygous state (associated with a variant of unknown significance) [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.1180+1G>A: intron 12 (hg19)chr2:71753477 Canonical Allele Identifier: CA347213687	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 1 patient in a homozygous state, associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 33 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.1180+5G>A: intron 12 (hg19)chr2:71753481 Canonical Allele Identifier: CA1705673	We have identified c.1180+5G>A in 1 patient in a heterozygous state (associated with a variant of unknown significance), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 7 cases in the literature (associated with c.3601C>T (<i>trans</i>), c.4194del (<i>trans</i>), c.4941_4942del, c.3988C>T, c.2997G>T, c.6124C>T, c.1177C>T, PMID: 28403181, 27647186, 32400077, 25574751, LOVD ID 00215246), associated in 2 cases (PMID: 32400077, 25574751) with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 28403181) [PM3 very strong, PP4 moderate]. It is present in only 1 individual in gnomAD v2.1.1 (POP MAX Filtering allele frequency: 0,0009% 1/111678 in European (non-Finnish) population) [PM2]. An alteration of the wild type donor site predicted by HSF and SpliceAI (Donor Loss 0.86)) [PP3]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PM3 very strong + PM2 + PP4 moderate + PP3

DYSF NM_003494.3:c.1276G>A p.(Gly426Arg) exon 13 (hg19)chr2:71755523 Canonical Allele Identifier: CA10603801	We have identified c.1276G>A in 1 patient in a heterozygous state (associated with a pathogenic variant: c.2858dup), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.5713C>T, PMID: LOVD ID 00220490). This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 32400077) [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. It is predicted to affect protein function (CADD = 29 (v1.6), REVEL = 0,851) [PP3].	Likely pathogenic: PM2 + PM3 + PP4 moderate + PP3
DYSF NM_003494.3:c.1284+1G>A: intron 13 (hg19)chr2:71755532 Canonical Allele Identifier: CA347215090	We have identified c.1284+1G>A in 1 patient in a heterozygous state (associated with a variant of unknown significance), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 27647186) [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Protein changing length (predicted exon skipping or use of alternate splice sites without frameshift) [PVS1 moderate] (CADD = 35 (v1.6)).	Likely pathogenic: PM2 + PVS1 moderate + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.1284+2T>C: intron 13 (hg19)chr2:71755533 Canonical Allele Identifier: CA222122	We have identified this variant in 1 patient in a homozygous state . This variant has previously been reported in a heterozygous state in 7 cases in the literature (associated with c.2997G>T, c.4756C>T (<i>trans</i>), c.2494C>T, c.4795-2A>G, c.663+1G>C, c.5078G>A, c.823del, PMID: 32400077, 28403181, 25868377, 27363342). This variant has previously been reported in a homozygous state in 2 cases in the literature (PMID: 27363342, 26088049) [PM3 very strong, PP4 supporting]. It is present in only 4 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0047% 4/18360 in East Asian population) [PM2]. Protein changing length (predicted exon skipping or use of alternate splice sites without frameshift) [PVS1 moderate] (CADD = 33 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PM3 very strong + PM2 + PVS1 moderate + PP4 supporting
DYSF NM_003494.3:c.1368C>G p.(Cys456Trp) exon 15 (hg19)chr2:71762412 Canonical Allele Identifier: CA347215771	We have identified c.1368C>G in 1 patient in a heterozygous state (associated with a pathogenic variant: c.5713C>T) [PM3 supporting]. Western blot showed absence of dysferlin in this case [PP4 moderate]. This variant is absent from gnomAD v2.1.1 [PM2]. It is predicted to affect protein function (CADD = 25,6 (v1.6), REVEL = 0,66) [PP3].	Likely pathogenic: PM2 + PP3 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.1368C>A p.(Cys456Ter) exon 15 (hg19)chr2:71762412 Canonical Allele Identifier: CA222125	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.1368C>A in 2 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.5920_5921insCCCTGTGTGGCACTGTGTAGGAG, c.1555G>A), associated in these 2 cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 27602406), associated in 1 case (PMID: 27602406) with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is present in only 5 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0007% 1/24944 in African population) [PM2] (CADD = 38 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate
DYSF NM_003494.3:c.1375dup p.(Met459AsnfsTer15) exon 15 (hg19)chr2:71762419dup Canonical Allele Identifier: CA658822438	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.1375dup in 1 patient in a heterozygous state (associated with a pathogenic variant: c.1638+1G>C), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 5 cases in the	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate

	literature (associated with c.5197A>G, c.2030-?_2439+?del, c.3036G>C, c.3137G>A, c.1464delT, PMID: 28403181, 27647186) [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	
DYSF NM_003494.3:c.1392dup p.(Asp465ArgfsTer9) exon 15 (hg19)chr2:71762436dup Canonical Allele Identifier: CA222127	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 3 patients in a homozygous state. This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.755C>T, PMID: 24488599) [PM3 supporting, PP4 supporting]. It is present in only 1 individual in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0009% 1/113760 in European (non-Finnish) population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 3 affected individuals, and 0 unaffected individual (PMID:) [PP1 moderate]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP1 moderate + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.1397+1G>C: intron 15 (hg19)chr2:71762442 Canonical Allele Identifier: CA347215842	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.1397+1G>C in 1 patient in a heterozygous state (associated with a likely pathogenic variant: c.5533del (<i>trans</i>)), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 34 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate
DYSF NM_003494.3:c.1440_1443del p.(Ser481CysfsTer11) exon 16 (hg19)chr2:71766329_71766332del Canonical Allele Identifier: CA49782801	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.1440_1443del in 1 patient in a heterozygous state (associated with a pathogenic variant: c.3059dup), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.1555G>A p.(Gly519Arg) exon 18 (hg19)chr2:71778203 Canonical Allele Identifier: CA253913	We have identified c.1555G>A in 1 patient in a heterozygous state (associated with a pathogenic variant: c.1368C>A), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 17070050), associated in 1 case (PMID: 17070050) with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. Well-established functional assays demonstrating a variant has abnormal dysferlin function (Creation of a new acceptor splice site resulting in the deletion of 34 bp of the 5' side of the exon 18 PMID: 25312915) [PS3]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (PMID: 17070050) [PP1 supporting]. It is predicted to affect protein function (CADD = 34 (v1.6), REVEL = 0,9) [PP3]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PS3 + PM2 + PM3 + PP4 moderate + PP1 supporting + PP3
DYSF NM_003494.3:c.1617C>G p.(Tyr539Ter) exon 18 (hg19)chr2:71778265 Canonical Allele Identifier: CA347217493	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.1617C>G in 1 patient in a heterozygous state (associated with a variant of unknown significance), associated in this case with an absence of Dysferlin on western blot /	Pathogenic: PVS1 + PM2 + PP4 moderate

	ImmunoHistoChemistry. [PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 35 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	
DYSF NM_003494.3:c.1638+1G>C: intron 18 (hg19)chr2:71778287 Canonical Allele Identifier: CA347217540	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.1638+1G>C in 1 patient in a heterozygous state (associated with a pathogenic variant: c.1375dup), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is present in only 1 individual in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0054% 1/18390 in East Asian population) [PM2] (CADD = 35 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.1642dup p.(Glu548GlyfsTer22) exon 19 (hg19)chr2:71778740dup Canonical Allele Identifier: CA10606691	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.1642dup in 1 patient in a heterozygous state (associated with a pathogenic variant: c.5594del), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is present in only 1 individual in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0036% 1/27766 in South Asian population) [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.1655_1668del p.(Tyr552SerfsTer13) exon 19 (hg19)chr2:71778753_71778766del Canonical Allele Identifier: CA1139532910	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.1655_1668del in 2 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.5979dup (<i>trans</i>)), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate
DYSF NM_003494.3:c.1663C>T p.(Arg555Trp) exon 19 (hg19)chr2:71778761 Canonical Allele Identifier: CA222133	We have identified this variant in 3 patients in a homozygous state, associated in 2 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. We have identified c.1663C>T in 6 patients (6 families) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.610C>T, c.3832C>T (<i>trans</i>), c.1157_1168del, c.4005+1G>A), associated in 3 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 5 cases in the literature (associated with c.3220_3221del, c.2494C>T, c.797T>G, c.2997G>T, c.4497del, PMID: 25591676, 21522182, 27602406, 32400077), associated in 2 cases (PMID: 25591676, 27602406) with an absence of Dysferlin on western blot / ImmunoHistoChemistry, and associated in 1 case (PMID: 21522182) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 27602406), associated in 1 case (PMID: 27602406) with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 very strong, PP4 supporting]. It is present in only 9 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0027% 3/28874 in South Asian population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. It is predicted to affect protein function (CADD = 30 (v1.6), REVEL = 0,94) [PP3]. Even though additional evidence of pathogenicity is	Pathogenic: PM3 very strong + PM2 + PP1 supporting + PP3 + PP4 moderate

	available in the literature for this variant, the evidence listed here allows the maximal classification (pathogenic) for this variant.	
DYSF NM_003494.3:c.1699_1719del p.(His567_Glu573del) exon 19 (hg19)chr2:71778797_71778817del Canonical Allele Identifier: CA1139532901	We have identified c.1699_1719del in 1 patient in a heterozygous state (associated with a pathogenic variant: c.3597G>A), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Protein-length-changing variant (deletion without frameshift) [PM4].	Likely pathogenic: PM2 + PM4 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.1758C>G p.(Tyr586Ter) exon 20 (hg19)chr2:71780146 Canonical Allele Identifier: CA347218306	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.1758C>G in 4 patients (4 families) in a heterozygous state (associated with pathogenic variants: c.107_108del, c.3321_3324dup, c.757C>T, c.937+1G>A), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 40 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM3 strong + PM2 + PP4 moderate
DYSF NM_003494.3:c.1788_1790dup p.(Ala597_Phe598insAla) exon 20 (hg19)chr2:71780176_71780178dup Canonical Allele Identifier: CA1139532902	We have identified this variant in 2 patients in a homozygous state [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. Protein-length-changing variant (duplication without frameshift) [PM4]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (PMID: [PP1 supporting]).	Likely pathogenic: PM2 + PM4 + PP1 supporting + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.1795_1799dup p.(Ala601ThrfsTer28) exon 20 (hg19)chr2:71780183_71780187dup Canonical Allele Identifier: CA915941060	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 2 patients in a homozygous state, associated in these 2 cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PP1 supporting + PM3 supporting
DYSF NM_003494.3:c.1834C>T p.(Gln612Ter) exon 20 (hg19)chr2:71780222 Canonical Allele Identifier: CA277606	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 3 patients in a homozygous state, associated in 2 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. We have identified c.1834C>T in 2 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.3967C>T, c.4872_4876delinsCCCC), associated in these 2 cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. It is present in only 3 individuals in gnomAD v2.1.1 (POP MAX Filtering allele frequency: 0,0003% 3/113748 in European (Non-Finnish) population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting] (CADD = 45 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM3 strong + PM2 + PP4 moderate + PP1 supporting
DYSF NM_003494.3:c.1848C>A p.(Ser616Arg) exon 20 (hg19)chr2:71780236 Canonical Allele Identifier: CA347218592	We have identified c.1848C>A in 2 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.4854C>G (<i>trans</i>), c.4550del and c.1351A>G), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry, and associated in 1 case with a decrease of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. It is predicted to affect protein function (CADD = 26,6 (v1.6), REVEL = 0,914) [PP3].	Likely pathogenic: PM2 + PM3 + PP4 moderate + PP3

<p>DYSF NM_003494.3:c.1852G>A p.(Gly618Arg) exon 40 (hg19)chr2:71780240 Canonical Allele Identifier: CA241938</p>	<p>We have identified this variant in 2 patients in a homozygous state, associated in these 2 cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.4985C>G, PMID: 15469449), associated in 1 case (PMID: 15469449) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 3 cases in the literature (PMID: 32400077, 17828519, 26088049), associated in 1 case (PMID: 32400077) with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is present in only 2 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0046% 1/21646 in European (Finnish) population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (PMID: 15469449) [PP1 supporting]. It is predicted to affect protein function (CADD = 28,2 (v1.6), REVEL = 0,925) [PP3].</p>	<p>Likely pathogenic: PM2 + PM3 + PP4 moderate + PP1 supporting + PP3</p>
<p>DYSF NM_003494.3:c.1861G>A p.(Gly621Arg) exon 20 (hg19)chr2:71780249 Canonical Allele Identifier: CA10606091</p>	<p>We have identified this variant in 1 patient in a homozygous state, associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 6 cases in the literature (associated with 5509G>A, c.827del (<i>trans</i>), c.1180+2T>C, c.5668-7G>A, c.533del, c.2643+1G>A, PMID: 21522182, 19528035, 17994539, LOVD ID 00215864, LOVD ID 00220795), associated in 4 cases (PMID: 21522182, 19528035, 17994539) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 27602406) [PM3 very strong, PP4 moderate]. It is present in only 1 individual in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0029% 1/34588 in Latino population) [PM2]. It is predicted to affect protein function (CADD = 24,6 (v1.6), REVEL = 0,88) [PP3]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.</p>	<p>Pathogenic: PM3 very strong + PM2 + PP4 moderate + PP3</p>
<p>DYSF NM_003494.3:c.1877T>C p.(Met626Thr) exon 20 (hg19)chr2:71780265 Canonical Allele Identifier: CA1705983</p>	<p>We have identified c.1877T>C in two patients (two families) in a heterozygous state. In one case it was associated with a VUS c.896G>A, confirmed <i>in cis</i> (LOVD Individual: 215343, PMIDs:16010686, 18853459). In the other case, this variant was associated with both VUS c.896G>A <i>in cis</i> and with VUS c.671T>G <i>in trans</i> (LOVD Individual:215587). This variant has previously been reported in a heterozygous state in one case in the literature (also carrying c.896G>A, PMID: 27602406), associated with a decrease of Dysferlin on western blot. Since the segregation analysis was not performed in this study, it is likely that these two variants (c.1877T>C and c.896G>A) were <i>in cis</i>, as in our two patients, questioning the assumed pathogenicity for one of these variants. c.1877T>C variant is present in 356 individuals in gnomAD v2.1.1 (POPMAX filtering allele frequency: 0,23% 312/129084 in European (non-Finnish) population, 2 homozygotes). This variant is not predicted to affect protein function (CADD = 8,856 (v1.6), REVEL = 0,122). This variant has six ClinVar entries (ClinVar ID: 281014, three likely benign and three VUS) and one VUS entry in LOVD (in addition to our two patients).</p>	<p>Uncertain significance: no codes attributed</p>
<p>DYSF NM_003494.3:c.1911C>A p.(Tyr637Ter) exon 20 (hg19)chr2:71780299 Canonical Allele Identifier: CA347218736</p>	<p>This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.1911C>A in 1 patient in a heterozygous state (associated with a variant of unknown significance), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 17994539), associated in 1 case (PMID: 17994539) with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1</p>	<p>Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting</p>

	[PM2] (CADD = 38 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	
DYSF NM_003494.3:c.1948del p.(Leu650TyrfsTer6) exon 21 (hg19)chr2:71780954del Canonical Allele Identifier: CA1139532911	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 1 patient in a homozygous state [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.2163-2A>G: intron 22 (hg19)chr2:71788880 Canonical Allele Identifier: CA1706103	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.2163-2A>G in 1 patient in a heterozygous state (associated with a pathogenic variant: c.5668-7G>A), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is present in only 1 individual in gnomAD v2.1.1 (POP MAX Filtering allele frequency: 0,0062% 1/16254 in African population) [PM2] (CADD = 34 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.2217C>A p.(Tyr739Ter) exon 23 (hg19)chr2:71788936 Canonical Allele Identifier: CA347209063	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.2217C>A in 3 patients (2 families) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.4005+1G>A, c.1064_1065del), associated in these 2 cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting] (CADD = 38 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PP1 supporting + PM3 supporting
DYSF NM_003494.3:c.2355+1G>A: intron 23 (hg19)chr2:71789075 Canonical Allele Identifier: CA10604289	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 2 patients in a homozygous state, associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. We have identified c.2355+1G>A in 1 patient in a heterozygous state (associated with a pathogenic variant: c.5399_5400dup), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is present in only 1 individual in gnomAD v2.1.1 (POP MAX Filtering allele frequency: 0,0033% 1/30418 in South Asian population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting] (CADD = 34 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate + PP1 supporting
DYSF NM_003494.3:c.2456G>A p.(Arg819Gln) exon 24 (hg19)chr2:71791288 Canonical Allele Identifier: CA1706192	We have identified c.2456G>A in one patient in a heterozygous state without additional pathogenic or likely pathogenic variants. Western blot showed normal levels of dysferline. This variant has previously been reported in a heterozygous state in one LGMD case in the literature (associated with VUS c.1980G>A, PMID: 30564623). It is present in only 11 individuals in gnomAD v2.1.1 (POP MAX filtering allele frequency: 0,0029% 3/30616 in South Asian population) [PM2]. This variant is not predicted to affect protein function (CADD = 22,6 (v1.6), REVEL = 0,078). There are two VUS entries for this variant in ClinVar (ClinVar ID: 497312) and one VUS entry in LOVD (PMID: 30564623, in addition to our patient 215595).	Uncertain significance: PM2

DYSF NM_003494.3:c.2496_2499del p.(Thr833SerfsTer3) exon 24 (hg19)chr2:71791328_71791331del Canonical Allele Identifier: CA1706197	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.2496_2499del in 2 patients (1 family) in a heterozygous state (associated with a likely pathogenic variant: c.5036G>A (<i>trans</i>)), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is present in only 1 individual in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0009% 1/113764 in European (non-Finnish) population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate + PP1 supporting
DYSF NM_003494.3:c.2504_2507del p.(Phe835Ter) exon 24 (hg19)chr2:71791336_71791339del Canonical Allele Identifier: CA892695933	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 1 patient in a homozygous state, associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.2511+1G>A: intron 24 (hg19)chr2:71791344 Canonical Allele Identifier: CA347211915	We have identified c.2511+1G>A in 2 patients (1 family) in a heterozygous state (associated with a pathogenic variant: c.3137G>A) [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. Protein-length-changing variant (Predicted exon 24 skipping without frameshift) [PVS1 moderate]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting] (CADD = 34 (v1.6)).	Likely pathogenic: PM2 + PVS1 moderate + PP1 supporting + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.2512-1512_3148del: exon 25_29del (hg19)chr2:71793569_71797845del Canonical Allele Identifier: CA1139532913	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 1 patient in a homozygous state, associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.2641A>C p.(Thr881Pro) exon 25 (hg19)chr2:71795210 Canonical Allele Identifier: CA347214045	We have identified c.2641A>C in 1 patient in a heterozygous state (associated with a pathogenic variant: c.1911C>A), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 2 cases in the literature (associated with c.907-3C>A, c.4756C>T, PMID: 24488599, LOVD ID 00221032), associated in 1 case (PMID: 24488599) with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Protein-length-changing variant (Exon 25 skipping without frameshift PMID: 25312915) [PM4] (CADD = 29,9 (v1.6), REVEL = 0,736).	Likely pathogenic: PM2 + PM3 + PM4 + PP4 moderate
DYSF NM_003494.3:c.2643+1G>A: intron 25 (hg19)chr2:71795213 Canonical Allele Identifier: CA222141	We have identified this variant in 8 patients in a homozygous state, associated in 4 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. We have identified c.2643+1G>A in 5 patients (4 families) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.4167+1G>C, c.3349-1G>T, c.331C>T), associated in 2 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 10 cases in the literature (associated with c.3327_3328del, c.4194C>A, c.827del, c.3112C>T, c.2790G>C, c.937+1G>A, c.4497del, c.2997G>T, c.3959delinsGA, c.1566C>G, PMID: 24488599, 21522182, 27602406, 32400077), associated in 4 cases (PMID: 21522182, 32400077) with an absence of Dysferlin on western blot / ImmunoHistoChemistry, and associated in 3 cases (PMID: 24488599, 27602406) with a decrease of	Pathogenic: PM3 very strong + PVS1 moderate + PP4 moderate + PP1 moderate

	Dysferlin on western blot / ImmunoHistoChemistry [PM3 very strong, PP4 moderate]. This variant has a POPMAX filtering frequency of 0.11% (44/24964 alleles with no homozygous in African population) in gnomAD v2.1.1. Despite absence of PM2 code, we still assigned PP4_moderate code to this variant since this variant can be confidently included in the allele frequency exception list (i.e. relatively frequent but pathogenic variant). Protein-length-changing variant (Exon 25 skipping without frameshift PMID: 25312915) [PM4]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 3 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 moderate]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class5/pathogenic) for this variant.	
DYSF NM_003494.3:c.2706dup p.(Lys903GlnfsTer4) exon 26 (hg19)chr2:71795364dup Canonical Allele Identifier: CA913189537	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 1 patient in a homozygous state [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.2747_2767delinsTCCA p.(Asp916ValfsTer20) exon 26 (hg19)chr2:71795405_71795425delinsTCCA Canonical Allele Identifier: CA1139532914	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 3 patients in a homozygous state [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 3 affected individuals, and 2 unaffected individuals (Marseille cohort) [PP1 moderate]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP1 moderate + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.2772G>A p.(Trp924Ter) exon 26 (hg19)chr2:71795430 Canonical Allele Identifier: CA347215119	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.2772G>A in 1 patient in a heterozygous state (associated with a pathogenic variant: c.4872_4876delinsCCCC), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 44 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.2779del p.(Ala927LeufsTer21) exon 26 (hg19)chr2:71795437del Canonical Allele Identifier: CA233930	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 3 patients in a homozygous state . We have identified c.2779del in 5 patients (3 families) in a heterozygous state (associated with pathogenic variants: c.5713C>T, c.4390G>T and c.3624C>G, c.5979dupA), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. It is present in only 7 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0198% 7/35434 in Latino population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM3 strong + PM2 + PP4 moderate + PP1 supporting
DYSF NM_003494.3:c.2810C>T p.(Thr937Ile) exon 26 (hg19)chr2:71795468 Canonical Allele Identifier: CA1706297	We have identified this variant in 1 patient in a homozygous state [PM3 supporting, PP4 supporting]. It is present in 33 individuals (1 homozygote) in gnomAD v2.1.1 (POPMAX filtering allele frequency: 0,0091% 3/19954 in East Asian population) [PM2]. It is predicted to affect protein function (CADD = 28,8 (v1.6), REVEL = 0,438) [PP3]. This variant contains 5 VUS entries in ClinVar (ClinVar ID: 336962) and no previous	Uncertain significance: PM2 + PP3 + PP4 supporting + PM3 supporting

	LOVD entries (submitted with ID: 711841). Our patient also carried another VUS (c.20T>C, p.(Leu7Pro)) in a homozygous state.	
DYSF NM_003494.3:c.2810+5G>A: intron 26 (hg19)chr2:71795473 Canonical Allele Identifier: CA10606385	We have identified c.2810+5G>A in 1 patient in a heterozygous state (associated with a pathogenic variant: c.2875C>T (<i>trans</i>)), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 2 cases in the literature (associated with c.5651G>T, c.1906C>T, PMID: 30564623, 27647186) [PM3, PP4 moderate]. It is present in only 1 individual in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0009% 1/112156 in European (non-Finnish) population) [PM2]. An alteration of the wild-type donor site predicted by HSF and SpliceAI (Donor loss 0.92)) [PP3].	Likely pathogenic: PM2 + PM3 + PP4 moderate + PP3
DYSF NM_003494.3:c.2811-2A>C: intron 26 (hg19)chr2:71796948 Canonical Allele Identifier: CA10606168	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 3 patients in a homozygous state [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 3 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 moderate]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP1 moderate + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.2858dup p.(Phe954ValfsTer2) exon 27 (hg19)chr2:71796997dup Canonical Allele Identifier: CA915941059	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 1 patient in a homozygous state . We have identified c.2858dup in 2 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.526C>T) [PM3, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 supporting
DYSF NM_003494.3:c.2875C>T p.(Arg959Trp) exon 27 (hg19)chr2:71797014 Canonical Allele Identifier: CA1706331	We have identified this variant in 1 patient in a homozygous state, associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. We have identified c.2875C>T in 4 patients (3 families) in a heterozygous state (associated with likely pathogenic variants: c.3112C>G (<i>trans</i>), c.2810+5G>A (<i>trans</i>)), associated in these 3 cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.5606G>A, PMID: 21522182), associated in 1 case (PMID: 21522182) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 21522182), associated in 1 case (PMID: 21522182) with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. It is present in only 11 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0035% 9/129146 in European (non-Finnish) population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 1 unaffected individual (Marseille cohort) [PP1 supporting]. It is predicted to affect protein function (CADD = 31 (v1.6), REVEL = 0,773) [PP3]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PM3 strong + PM2 + PP4 moderate + PP1 supporting + PP3
DYSF NM_003494.3:c.2894G>A p.(Trp965Ter) exon 27 (hg19)chr2:71797033 Canonical Allele Identifier: CA347216158	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.2894G>A in 2 patients (2 families) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.6233_6240del, c.4200dup), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is present in only 1	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting

	individual in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0009% 1/113692 in European (non-Finnish) population) [PM2] (CADD = 45 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	
DYSF NM_003494.3:c.2997G>T p.(Trp999Cys) exon 28 (hg19)chr2:71797430 Canonical Allele Identifier: CA222147	We have identified c.2997G>T in 2 patients (1 family) in a heterozygous state (associated with a pathogenic variant: c.3373del), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 11 cases in the literature (associated with c.1180+5G>A, c.1284+2T>C, c.2974T>C, c.4497del, c.3137G>A, c.1663C>T, c.356T>C, c.5668-7G>A, c.3827del, c.6135G>A, c.1566C>G, PMID: 32400077), associated in 4 cases (PMID: 32400077) with an absence of Dysferlin on western blot / ImmunoHistoChemistry, and associated in 1 case (PMID: 32400077) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 2 cases in the literature (PMID: 32400077), associated in 1 case (PMID: 32400077) with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 very strong, PP4 moderate]. It is present in only 3 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0047% 3/18394 in East Asian population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. It is predicted to affect protein function (CADD = 32 (v1.6), REVEL = 0,876) [PP3]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PM3 very strong + PM2 + PP4 moderate + PP1 supporting + PP3
DYSF NM_003494.3:c.2998T>C p.(Ser1000Pro) exon 28 (hg19)chr2:71797431 Canonical Allele Identifier: CA10604386	We have identified this variant in 1 patient in a homozygous state, associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. It is predicted to affect protein function (CADD = 24,6 (v1.6), REVEL = 0,732) [PP3].	Likely pathogenic: PM2 + PP4 moderate + PP3 + PM3 supporting
DYSF NM_003494.3:c.3031+2T>C: intron 28 (hg19)chr2:71797466 Canonical Allele Identifier: CA10604913	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 2 patients in a homozygous state, associated in these 2 cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. We have identified c.3031+2T>C in 2 patients (1 family) in a heterozygous state (associated with a pathogenic variant: c.855+1del) [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting] (CADD = 32 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate + PP1 supporting
DYSF NM_003494.3:c.3035G>A p.(Trp1012Ter) exon 29 (hg19)chr2:71797732 Canonical Allele Identifier: CA347216891	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 2 patients in a homozygous state [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting] (CADD = 38 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP1 supporting + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.3059dup p.(Glu1021GlyfsTer11) exon 29 (hg19)chr2:71797756dup	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.3059dup in 1 patient in a heterozygous state (associated with a pathogenic variant: c.1440_1443del), associated in this case with an absence of Dysferlin on western blot /	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting

Canonical Allele Identifier: CA1706427	ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is present in only 2 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0018% 2/113020 in European (non-Finnish) population) [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	
DYSF NM_003494.3:c.3112C>G p.(Arg1038Gly) exon 29 (hg19)chr2:71797809 Canonical Allele Identifier: CA347217057	We have identified c.3112C>G in 2 patients (1 family) in a heterozygous state (associated with a pathogenic variant: c.2875C>T (<i>trans</i>)), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Novel missense variant at amino acid with different pathogenic (c.3113G>A) missense change (PM5). This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 1 unaffected individual (Marseille cohort) [PP1 supporting] (CADD = 24,6 (v1.6), REVEL = 0,747).	Likely pathogenic: PM2 + PM3 + PM5 + PP4 moderate + PP1 supporting
DYSF NM_003494.3:c.3112C>T p.(Arg1038Ter) exon 29 (hg19)chr2:71797809 Canonical Allele Identifier: CA277609	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.3112C>T in 4 patients (3 families) in a heterozygous state (associated with pathogenic variants: c.5594del, c.3832C>T, c.3805G>T), associated in 2 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is present in only 6 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0011% 1/10060 in Ashkenazi Jewish population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting] (CADD = 38 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate + PP1 supporting
DYSF NM_003494.3:c.3113G>A p.(Arg1038Gln) exon 29 (hg19)chr2:71797810 Canonical Allele Identifier: CA1706448	We have identified this variant in 1 patient in a homozygous state, associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. We have identified c.3113G>A in 1 patient in a heterozygous state (associated with a pathogenic variant: c.5594del), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 5 cases in the literature (associated with c.1464del, c.5979dup, c.4756C>T, c.2071C>T, c.2643+5G>A, PMID: 27363342, 17994539, 27602406, 24488599), associated in 2 cases (PMID: 27602406, 24488599) with an absence of Dysferlin on western blot / ImmunoHistoChemistry, and associated in 1 case (PMID: 17994539) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 30564623) [PM3 strong, PP4 moderate]. It is present in only 13 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0055% 11/113392 in European (non-Finnish) population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (PMID: 17994539) [PP1 supporting]. It is predicted to affect protein function (CADD = 31 (v1.6), REVEL = 0,935) [PP3]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PM3 strong + PM2 + PP4 moderate + PP1 supporting + PP3
DYSF NM_003494.3:c.3126G>A p.(Trp1042Ter) exon 29 (hg19)chr2:71797823 Canonical Allele Identifier: CA347217077	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.3126G>A in 2 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.265C>T, c.855+1del), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 41 (v1.6)).	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate

	Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	
DYSF NM_003494.3:c.3137G>A p.(Arg1046His) exon 29 (hg19)chr2:71797834 Canonical Allele Identifier: CA222152	We have identified this variant in 4 patients in a homozygous state, associated in 2 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. We have identified c.3137G>A in 4 patients (3 families) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.3477C>A, c.2511+1G>A), associated in 2 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 4 cases in the literature (associated with c.1375dupA, c.2997G>T, c.965T>C, c.353del, PMID: 27647186, 32400077, 24488599), associated in 1 case (PMID: 27647186) with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 2 cases in the literature (PMID: 27647186, 11468312), associated in 1 case (PMID: 27647186) with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. It is present in only 6 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,004% 4/35346 in Latino population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. It is predicted to affect protein function (CADD = 29,6 (v1.6), REVEL = 0,826) [PP3]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class5/pathogenic) for this variant.	Pathogenic: PM3 strong + PM2 + PP4 moderate + PP1 supporting + PP3
DYSF NM_003494.3:c.3225del p.(Phe1075LeufsTer45) exon 30 (hg19)chr2:71801378del Canonical Allele Identifier: CA1139532915	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 1 patient in a homozygous state [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.3286_3291dup p.(Arg1097_Met1098insArgArg) exon 30 (hg19)chr2:71801439_71801444dup Canonical Allele Identifier: CA1139532899	We have identified c.3286_3291dup in one patient in a heterozygous state (associated with a pathogenic variant: c.755C>T without confirmation of <i>in trans</i> occurrence) [PM3 supporting]. Dysferlin was absent on western blot in this case [PP4 moderate]. This variant is absent from gnomAD v2.1.1 [PM2]. The effect of this variant on the protein function is not clear since it adds two Arginine residues to a stretch of three Arginine. There are no ClinVar or LOVD entries for this variants (submitted to LOVD with Individual ID: 326923).	Uncertain significance: PM2 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.3321_3322delinsC p.(Ala1108LeufsTer12) exon 30 (hg19)chr2:71801474_71801475delinsC Canonical Allele Identifier: CA1139532916	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 2 patients in a homozygous state, associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PP1 supporting + PM3 supporting
DYSF NM_003494.3:c.3321_3324dup p.(Val1109SerfsTer6) exon 30 (hg19)chr2:71801474_71801477dup Canonical Allele Identifier: CA533655769	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.3321_3324dup in 1 patient in a heterozygous state (associated with a pathogenic variant: c.1758C>G) [PM3 supporting, PP4 supporting]. It is present in only 1 individual in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0009% 1/113572 in European (non-Finnish) population) [PM2]. Even though	Pathogenic: PVS1 + PM2 + PP4 supporting + PM3 supporting

	additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	
DYSF NM_003494.3:c.3343dup p.(Ala1115GlyfsTer8) exon 30 (hg19)chr2:71801496dup Canonical Allele Identifier: CA1139532917	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.3343dup in 1 patient in a heterozygous state (associated with a variant of unknown significance), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. [PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate
DYSF NM_003494.3:c.3348+3_3348+6del: intron 30 (hg19)chr2:71801504_71801507del Canonical Allele Identifier: CA1139532903	We have identified c.3348+3_3348+6del in 1 patient in a heterozygous state (associated with a pathogenic variant: c.5314_5318del (<i>trans</i>)) [PM3, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. An alteration of the wild-type donor site is predicted by HSF and SpliceAI (Donor loss 0.95) [PP3].	Likely pathogenic: PM2 + PM3 + PP3 + PP4 supporting
DYSF NM_003494.3:c.3349-1G>T: intron 30 (hg19)chr2:71816722 Canonical Allele Identifier: CA347220232	We have identified c.3349-1G>T in 1 patient in a heterozygous state (associated with a pathogenic variant: c.2643+1G>A), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is present in only 1 individual in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0062% 1/16256 in African population) [PM2]. Protein changing length (predicted exon skipping or use of alternate splice sites without frameshift) [PVS1 moderate] (CADD = 33 (v1.6)).	Likely pathogenic: PM2 + PVS1 moderate + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.3373del p.(Glu1125LysfsTer9) exon 31 (hg19)chr2:71816747del Canonical Allele Identifier: CA1139532918	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.3373del in 2 patients (1 family) in a heterozygous state (associated with a pathogenic variant: c.2997G>T), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PP1 supporting + PM3 supporting
DYSF NM_003494.3:c.3389_3399dup p.(Phe1135ProfsTer3) exon 31 (hg19)chr2:71816763_71816773dup Canonical Allele Identifier: CA1139532919	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.3389_3399dup in 2 patients (1 family) in a heterozygous state (associated with a pathogenic variant: c.107_108del (<i>trans</i>)), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate + PP1 supporting
DYSF NM_003494.3:c.3471C>A p.(Tyr1157Ter) exon 32 (hg19)chr2:71817369 Canonical Allele Identifier: CA347220779	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 2 patients in a homozygous state, associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 1 unaffected individual (Marseille cohort) [PP1 supporting] (CADD = 45 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PP1 supporting + PM3 supporting

DYSF NM_003494.3:c.3477C>A p.(Tyr1159Ter) exon 32 (hg19)chr2:71817375 Canonical Allele Identifier: CA347220794	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.3477C>A in 2 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.3137G>A, c.5979dup (<i>trans</i>)), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 46 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate
DYSF NM_003494.3:c.3516_3517del p.(Ser1173Ter) exon 32 (hg19)chr2:71817414_71817415del Canonical Allele Identifier: CA279106	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.3516_3517del in 4 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.342+1G>A) [PM3 supporting, PP4 supporting]. It is present in only 7 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0054% 7/129162 in European (non-Finnish) population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 3 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 moderate]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP1 moderate + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.3597G>A p.(Trp1199Ter) exon 33 (hg19)chr2:71825770 Canonical Allele Identifier: CA347223846	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.3597G>A in 1 patient in a heterozygous state (associated with a likely pathogenic variant: c.1699_1719del), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. [PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 47 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate
DYSF NM_003494.3:c.3683T>C p.(Leu1228Pro) exon 33 (hg19)chr2:71825856 Canonical Allele Identifier: CA347224316	We have identified c.3683T>C in 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.4872_4876delinsCCCC), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. It is predicted to affect protein function (CADD = 31 (v1.6), REVEL = 0,9) [PP3].	Likely pathogenic: PM2 + PP4 moderate + PP3 + PM3 supporting
DYSF NM_003494.3:c.3687C>A p.(Tyr1229Ter) exon 33 (hg19)chr2:71825860 Canonical Allele Identifier: CA347224324	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.3687C>A in 1 patient in a heterozygous state (associated with a pathogenic variant: c.799_800del), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 37 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.3702+1G>A: intron 33 (hg19)chr2:71825876 Canonical Allele Identifier: CA347224359	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.3702+1G>A in 1 patient in a heterozygous state (associated with a pathogenic variant: c.5509G>A), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 34 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.3703-1G>A: intron 33 (hg19)chr2:71827831 Canonical Allele Identifier: CA347224709	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy (Abolition of the 3' splice site of intron 33 and use of a cryptic 3' splice site resulting in the deletion of a G at the beginning of the exon 34. A weak exon 34 skipping is also present PMID: 25312915) [PVS1]. We have identified c.3703-1G>A in 2 patients (1 family) in a heterozygous state (associated with a pathogenic	Pathogenic: PVS1 + PM2 + PP4 moderate + PP1 supporting + PM3 supporting

	variant: c.490G>T), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting] (CADD = 33 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	
DYSF NM_003494.3:c.3708del p.(Asp1237ThrfsTer24) exon 34 (hg19)chr2:71827837del Canonical Allele Identifier: CA1706701	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 2 patients in a homozygous state, associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate
DYSF NM_003494.3:c.3805G>T p.(Glu1269Ter) exon 34 (hg19)chr2:71827934 Canonical Allele Identifier: CA1706733	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.3805G>T in 2 patients (1 family) in a heterozygous state (associated with a pathogenic variant: c.3112C>T), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is present in only 1 individual in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0009% 1/113044 in European (non-Finnish) population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting] (CADD = 41 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PP1 supporting + PM3 supporting
DYSF NM_003494.3:c.3826C>G p.(Leu1276Val) exon 34 (hg19)chr2:71827955 Canonical Allele Identifier: CA347225630	We have identified c.3826C>G in 2 patients (1 family) in a heterozygous state (associated with a likely pathogenic variant: c.3843+1G>A), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. [PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. It is predicted to affect protein function (CADD = 25,8 (v1.6), REVEL = 0,801) [PP3].	Likely pathogenic: PM2 + PP4 moderate + PP1 supporting + PP3
DYSF NM_003494.3:c.3832C>T p.(Gln1278Ter) exon 34 (hg19)chr2:71827961 Canonical Allele Identifier: CA233937	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 1 patient in a homozygous state . We have identified c.3832C>T in 4 patients (4 families) in a heterozygous state (associated with pathogenic variants: c.5979dup, c.1663C>T (<i>trans</i>), c.3112C>T), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. It is present in only 4 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0023% 2/24904 in African population) [PM2] (CADD = 42 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM3 strong + PM2 + PP4 moderate
DYSF NM_003494.3:c.3843+1G>A: intron 34 (hg19)chr2:71827973 Canonical Allele Identifier: CA347225730	We have identified c.3843+1G>A in 2 patients (1 family) in a heterozygous state (associated with a likely pathogenic variant: c.3826C>G), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. [PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Protein-length-changing variant (Exon 34 skipping without frameshift PMID: 25312915) [PM4]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting] (CADD = 35 (v1.6)).	Likely pathogenic: PM2 + PM4 + PP4 moderate + PP1 supporting

DYSF NM_003494.3:c.3967C>T p.(Gln1323Ter) exon 37 (hg19)chr2:71838438 Canonical Allele Identifier: CA347228293	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.3967C>T in 1 patient in a heterozygous state (associated with a pathogenic variant: c.1834C>T), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 50 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.4005+1G>A: intron 37 (hg19)chr2:71838477 Canonical Allele Identifier: CA347228368	We have identified c.4005+1G>A in 3 patients (3 families) in a heterozygous state (associated with pathogenic variants: c.1663C>T, c.2217C>A, c.855+1del), associated in 2 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Protein-length-changing variant (Predicted exon 17 skipping without frameshift) [PVS1 moderate] (CADD = 33 (v1.6)).	Likely pathogenic: PM2 + PM3 + PVS1 moderate + PP4 moderate
DYSF NM_003494.3:c.4060del p.(Ser1354ProfsTer6) exon 38 (hg19)chr2:71838649del Canonical Allele Identifier: CA1139532920	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 1 patient in a homozygous state, associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.4081T>C p.(Cys1361Arg) exon 38 (hg19)chr2:71838670 Canonical Allele Identifier: CA1706900	We have identified this variant in 1 patient in a homozygous state, associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.2200_2205delinsT, PMID: 21522182), associated in 1 case (PMID: 21522182) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 17994539), associated in 1 case (PMID: 17994539) with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is present in only 2 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0003% 2/113660 in European (non-Finnish) population) [PM2]. It is predicted to affect protein function (CADD = 27,2 (v1.6), REVEL = 0,914) [PP3].	Likely pathogenic: PM2 + PM3 + PP4 moderate + PP3
DYSF NM_003494.3:c.4167+1G>C: intron 38 (hg19)chr2:71838757 Canonical Allele Identifier: CA347228990	We have identified c.4167+1G>C in 1 patient in a heterozygous state (associated with a pathogenic variant: c.2643+1G>A) [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. Protein-length-changing variant (Predicted exon 38 skipping without frameshift) [PVS1 moderate] (CADD = 35 (v1.6)).	Likely pathogenic: PM2 + PVS1 moderate + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.4191C>G p.(Tyr1397Ter) exon 39 (hg19)chr2:71839794 Canonical Allele Identifier: CA347229228	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.4191C>G in 1 patient in a heterozygous state (associated with a likely pathogenic variant: c.5899G>A) . [PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 41 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 supporting
DYSF NM_003494.3:c.4200del p.(Ile1401SerfsTer47) exon 39 (hg19)chr2:71839803del Canonical Allele Identifier: CA1706939	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 1 patient in a homozygous state, associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is present in only 4 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0163% 3/18380 in East Asian population) [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting

DYSF NM_003494.3:c.4200dup p.(Ile1401HisfsTer8) exon 39 (hg19)chr2:71839803dup Canonical Allele Identifier: CA222163	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy (PVS1). We have identified this variant in 8 patients in a homozygous state, associated in 2 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. We have identified c.4200dup in 3 patients (3 families) in a heterozygous state (associated with pathogenic variants: c.2894G>A, c.4433G>A), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.3307A>T, PMID: 16891820) (PM3 strong, PP4 supporting). It is present in only 11 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0065% 2/30580 in South Asian population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 3 affected individuals, and unaffected individual (Marseille cohort) [PP1 moderate]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class5/pathogenic) for this variant.	Pathogenic: PVS1 + PM3 strong + PM2 + PP1 moderate + PP4 moderate
DYSF NM_003494.3:c.4201dup p.(Ile1401AsnfsTer8) exon 39 (hg19)chr2:71839804dup Canonical Allele Identifier: CA645519992	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 1 patient in a homozygous state [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.4252G>C p.(Gly1418Arg) exon 39 (hg19)chr2:71839855 Canonical Allele Identifier: CA347229338	We have identified this variant in 2 patients in a homozygous state, associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. It is predicted to affect protein function (CADD = 29,9 (v1.6), REVEL = 0,975) [PP3].	Likely pathogenic: PM2 + PP4 moderate + PP1 supporting + PP3 + PM3 supporting
DYSF NM_003494.3:c.4376A>G p.(Asp1459Gly) exon 40 (hg19)chr2:71840506 Canonical Allele Identifier: CA152669	We have identified c.4376A>G in one patient in a heterozygous state (associated with two pathogenic variants: c.917_923dup and c.4410+1G>A). It is predicted to affect protein function (CADD = 26,9 (v1.6), REVEL = 0,586) [PP3]. POPMAX filtering allele frequency in gnomAD v2.1.1 is 2,3% (607/24780 in African population, and four homozygotes) [BS1]. There are three Benign and one Likely benign entries in ClinVar (ClinVar ID : 128949), and no LOVD entries (submitted to LOVD with Individual ID: 326977).	Uncertain significance: PP3 + BS1
DYSF NM_003494.3:c.4410+1G>A: intron 40 (hg19)chr2:71840541 Canonical Allele Identifier: CA347229685	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.4410+1G>A in 1 patient in a heterozygous state (associated with a pathogenic variant: c.917_923dup) [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 34 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.4433G>A p.(Trp1478Ter) exon 41 (hg19)chr2:71871117 Canonical Allele Identifier: CA347217851	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.4433G>A in 1 patient in a heterozygous state (associated with a pathogenic variant: c.4200dup) [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 53 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.4550del p.(Gly1517AlafsTer25) exon 42 (hg19)chr2:71883332del	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.4550del in 1 patient in a heterozygous state (associated with a likely pathogenic variant: c.1351A>G and c.1848C>A) . [PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. Even though	Pathogenic: PVS1 + PM2 + PP4 supporting

Canonical Allele Identifier: CA1139532921	additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	
DYSF NM_003494.3:c.4577A>C p.(Lys1526Thr) exon 42 (hg19)chr2:71883359 Canonical Allele Identifier: CA207041	We have identified c.4577A>C in four patients (four families) in a homozygous state, associated with a homozygous Pathogenic variant c.2643+1G>A in each one of these cases [BP2]. One additional patient was heterozygous for VUS c.4577A>C variant, associated with the same pathogenic variant c.2643+1G>A and a likely pathogenic c.6029A>G. Dysferlin was absent in muscle biopsies in all five patients. This variant has been reported as VUS in three additional patients also associated with c.2643+1G>A (PMID:30564623). It is thus very likely that c.4577A>C and c.2643+1G>A are always found <i>in cis</i> . In another study, c.4577A>C was reported as pathogenic associated with c.2643+1G>A, however the phase of the variants was not determined (PMID: 20544924). c.4577A>C is predicted to affect protein function (CADD = 25,1 (v1.6), REVEL = 0,712) [PP3]. POPMAX filtering allele frequency in gnomAD v2.1.1 is 0.2% (56/24966 alleles with no homozygotes in African population). There are five ClinVar entries for this variant (one VUS and four Benign/Likely benign, ClinVar ID: 94327) and one VUS entry in LOVD in addition to the published cases described above.	Uncertain significance: PP3 + BP2
DYSF NM_003494.3:c.4616_4619del p.(Pro1539LeufsTer2) exon 42 (hg19)chr2:71883398_71883401del Canonical Allele Identifier: CA1139532923	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 2 patients in a homozygous state, associated in 1 case with a decrease of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP1 supporting + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.4628G>A p.(Gly1543Asp) exon 42 (hg19)chr2:71883410 Canonical Allele Identifier: CA347219550	We have identified this variant in 1 patient in a homozygous state, associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. We have identified c.4628G>A in 1 patient in a heterozygous state (associated with a pathogenic variant: c.1180+5G>A), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. It is predicted to affect protein function (CADD = 27,7 (v1.6), REVEL = 0,892) [PP3].	Likely pathogenic: PM2 + PM3 + PP4 moderate + PP3
DYSF NM_003494.3:c.4639-2A>G: intron 42 (hg19)chr2:71886006 Canonical Allele Identifier: CA347219583	We have identified this variant in 1 patient in a homozygous state [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. Protein changing length (predicted exon skipping or use of alternate splice sites without frameshift) [PVS1 moderate] (CADD = 35 (v1.6)).	Likely pathogenic: PM2 + PVS1 moderate + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.4639G>C p.(Gly1547Arg) exon 43 (hg19)chr2:71886008 Canonical Allele Identifier: CA347219589	We have identified this variant in 3 patients in a homozygous state, associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 3 affected individuals, and 0 unaffected individual (PMID: 23530687) [PP1 moderate]. It is predicted to affect protein function (CADD = 32 (v1.6), REVEL = 0,94) [PP3].	Likely pathogenic: PM2 + PP4 moderate + PP1 moderate + PP3 + PM3 supporting
DYSF NM_003494.3:c.4756C>T p.(Arg1586Ter) exon 43 (hg19)chr2:71886125 Canonical Allele Identifier: CA222174	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 2 patients in a homozygous state, associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.1284+2T>C, PMID: 27647186) [PM3, PP4 moderate]. It is present in only 5 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency:	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate

	0,0011% 2/30616 in South Asian population) [PM2] (CADD = 41 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	
DYSF NM_003494.3:c.4763T>G p.(Phe1588Cys) exon 43 (hg19)chr2:71886132 Canonical Allele Identifier: CA347219853	We have identified c.4763T>G in 1 patient in a heterozygous state (associated with a pathogenic variant: c.3343dup), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. It is predicted to affect protein function (CADD = 27,8 (v1.6), REVEL = 0,319) [PP3].	Likely pathogenic: PM2 + PP4 moderate + PP3 + PM3 supporting
DYSF NM_003494.3:c.4854C>G p.(Tyr1618Ter) exon 44 (hg19)chr2:71887749 Canonical Allele Identifier: CA1707200	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.4854C>G in 1 patient in a heterozygous state (associated with a likely pathogenic variant: c.1848C>A (<i>trans</i>)), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is present in only 1 individual in gnomAD v2.1.1 (POP MAX Filtering allele frequency: 0,0062% 1/16256 in African population) [PM2] (CADD = 47 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate
DYSF NM_003494.3:c.4872_4876delinsCCCC p.(Glu1624AspfsTer10) exon 44 (hg19)chr2:71887767_71887771delinsCCCC Canonical Allele Identifier: CA253904	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 2 patients in a homozygous state, associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. We have identified c.4872_4876delinsCCCC in 5 patients (4 families) in a heterozygous state (associated with pathogenic variants: c.2772G>A, c.1834C>T (<i>trans</i>)), associated in 3 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM3 strong + PM2 + PP4 moderate
DYSF NM_003494.3:c.5015C>T p.(Ser1672Phe) exon 45 (hg19)chr2:71891526 Canonical Allele Identifier: CA347220545	We have identified c.5015C>T in one patient in a heterozygous state (associated with a pathogenic variant: c.799_800del) [PM3 supporting, PP4 supporting]. Dysferlin was severely decreased on western blot in this case. It is absent from gnomAD v2.1.1 [PM2]. This variant is predicted to affect protein function (CADD = 29,8 (v1.6), REVEL = 0,592) [PP3]. There are no ClinVar and one Likely Pathogenic LOVD entry for this variant without information on the second variant (PMID: 32528171) (submitted to LOVD with Individual ID: 327046).	Uncertain significance: PM2 + PP3 + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.5033G>C p.(Cys1678Ser) exon 45 (hg19)chr2:71891544 Canonical Allele Identifier: CA1707259	We have identified c.5033G>C in 1 patient in a heterozygous state (associated with a pathogenic variant: c.3137G>A), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.1120G>C, PMID: 17994539), associated in 1 case (PMID: 17994539) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is present in only 3 individuals in gnomAD v2.1.1 (POP MAX Filtering allele frequency: 0,0007% 3/113730 in European (non-Finnish) population) [PM2]. It is predicted to affect protein function (CADD = 27,4 (v1.6), REVEL = 0,959) [PP3].	Likely pathogenic: PM2 + PP4 moderate + PP3 + PM3 supporting
DYSF NM_003494.3:c.5036G>A p.(Gly1679Glu) exon 45 (hg19)chr2:71891547 Canonical Allele Identifier: CA347220615	We have identified c.5036G>A in 2 patients (1 family) in a heterozygous state (associated with a pathogenic variant: c.2496_2499del (<i>trans</i>)), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.4720G>T, PMID: 32400077) [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected	Likely pathogenic: PM2 + PM3 + PP4 moderate + PP1 supporting + PP3

	family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. It is predicted to affect protein function (CADD = 26,2 (v1.6), REVEL = 0,853) [PP3].	
DYSF NM_003494.3:c.5078G>A p.(Arg1693Gln) exon 46 (hg19)chr2:71892312 Canonical Allele Identifier: CA351294	We have identified c.5078G>A in 3 patients (2 families) in a heterozygous state (associated with a likely pathogenic variant: c.1157_1168del (<i>trans</i>)), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 3 cases in the literature (associated with c.1284+2T>C, c.1175del, c.2997G>T, PMID: 25868377, 32400077, 26060040), associated in 1 case (PMID: 32400077) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 27066573), associated in 1 case (PMID: 27066573) with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. It is present in only 2 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0055% 1/18260 in East Asian population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 1 unaffected individual (Marseille cohort) [PP1 supporting]. It is predicted to affect protein function (CADD = 31 (v1.6), REVEL = 0,925) [PP3]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PM3 strong + PM2 + PP4 moderate + PP1 supporting + PP3
DYSF NM_003494.3:c.5093C>T p.(Pro1698Leu) exon 46 (hg19)chr2:71892327 Canonical Allele Identifier: CA347220971	We have identified c.5093C>T in 1 patient in a heterozygous state (associated with a pathogenic variant: c.2875C>T), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. It is predicted to affect protein function (CADD = 29,7 (v1.6), REVEL = 0,874) [PP3].	Likely pathogenic: PM2 + PP4 moderate + PP3 + PM3 supporting
DYSF NM_003494.3:c.5200+1G>A: intron 46 (hg19)chr2:71892435 Canonical Allele Identifier: CA1707318	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy (RNA mutation c.5201_5340del resulting in protein p (Glu1734GlyfsX47)) [PVS1]. We have identified this variant in 1 patient in a homozygous state . We have identified c.5200+1G>A in 1 patient in a heterozygous state (associated with a pathogenic variant: c.5314_5318del) . This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 21522182), associated in 1 case (PMID: 21522182) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 supporting]. It is present in only 1 individual in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0009% 1/113762 in European (non-Finnish) population) [PM2] (CADD = 35 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 supporting
DYSF NM_003494.3:c.5243A>T p.(Glu1748Val) exon 47 (hg19)chr2:71894548 Canonical Allele Identifier: CA347221296	We have identified c.5243A>T in one patient in a homozygous state, associated with a homozygous pathogenic variant c.5296G>T (i.e. present <i>in cis</i> with a pathogenic variant) [BP2]. Dysferlin was absent on western blot in this case. This variant has previously been reported in a homozygous state in one case in the literature (PMID: 27671536), associated with an absence of Dysferlin on ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. It is predicted to affect protein function (CADD = 28,9 (v1.6), REVEL = 0,921) [PP3]. This variant was returned before ACMG/AMP classification was established in our laboratory, using terms that were consistent with VUS category. There are no ClinVar entries for this variant, no additional LOVD entries besides our patient (LOVD ID: 215219) and the patient from PMID: 27671536.	Uncertain significance: PM2 + PM3 supporting + PP3 + PP4 moderate + BP2

DYSF NM_003494.3:c.5296G>T p.(Glu1766Ter) exon 47 (hg19)chr2:71894601 Canonical Allele Identifier: CA347221400	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 1 patient in a homozygous state, associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 49 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.5302C>T p.Arg1768Trp exon 47 (hg19)chr2:71894607 Canonical Allele Identifier: CA1707356	We have identified c.5302C>T in 5 patients (4 families) in a heterozygous state (associated with pathogenic variants: c.5594delG, c.5979dupA), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry, and associated in 1 case with a decrease of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 3 cases in the literature (associated with c.3116G>T, c.2997G>T, PMID: 17070050,, 32400077), associated in 1 case (PMID: 17070050) with an absence of Dysferlin on western blot / ImmunoHistoChemistry, and associated in 1 case (PMID: 32400077) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 6 cases in the literature (PMID: 21522182,, 27647186, 28403181), associated in 1 case (PMID: 27647186) with an absence of Dysferlin on western blot / ImmunoHistoChemistry, and associated in 2 cases (PMID: 21522182, 28403181) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry (PM3 strong, PP4 moderate). This variant was shown to co-segregate with dysferlinopathy in one affected family, with 4 affected individuals, and 0 unaffected individual (PMID: 21522182) [PP1 strong]. It is present in only 3 individuals in gnomAD v2.1.1 (POP MAX Filtering allele frequency: 0,001% 2/35424 in Latino population) [PM2]. It is predicted to affect protein function (CADD = 32 (v1.6), REVEL = 0,704) [PP3]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class5/pathogenic) for this variant.	Pathogenic: PM3 strong + PP1 strong + PM2 + PP4 moderate + PP3
DYSF NM_003494.3:c.5303G>A p.(Arg1768Gln) exon 47 (hg19)chr2:71894608 Canonical Allele Identifier: CA1707357	We have identified this variant in 1 patient in a homozygous state, associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is present in only 11 individuals in gnomAD v2.1.1 (POP MAX Filtering allele frequency: 0,004% 3/35424 in Latino population) [PM2]. Novel missense variant at amino acid with different pathogenic (c.5302C>T) missense change (PM5) (CADD = 32 (v1.6), REVEL = 0,843).	Likely pathogenic: PM2 + PM5 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.5314_5318del p.(Ser1772ProfsTer54) exon 47 (hg19)chr2:71894619_71894623del Canonical Allele Identifier: CA533264201	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.5314_5318del in 2 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.5200+1G>A) [PM3 supporting, PP4 supporting]. It is present in only 1 individual in gnomAD v2.1.1 (POP MAX Filtering allele frequency: 0,0009% 1/113542 in European (non-Finnish) population) [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.5341-2A>C: intron 47 (hg19)chr2:71895882 Canonical Allele Identifier: CA222184	We have identified this variant in 2 patients in a homozygous state, associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is present in only 3 individuals in gnomAD v2.1.1 (POP MAX Filtering allele frequency: 0,0026% 3/30396 in South Asian population) [PM2]. Protein changing length (predicted exon skipping or use of alternate splice sites without frameshift) [PVS1 moderate] (CADD = 33 (v1.6)).	Likely pathogenic: PM2 + PM3 + PVS1 moderate + PP4 moderate

DYSF NM_003494.3:c.5430-15_5431del: exon 49 (hg19)chr2:71896227_71896243del Canonical Allele Identifier: CA1139532905	We have identified this variant in 1 patient in a homozygous state [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. Protein-length-changing variant (predicted exon 49 skipping or use of a cryptic splice site without frameshift) [PM4].	Likely pathogenic: PM2 + PVS1 moderate + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.5444G>A p.(Cys1815Tyr) exon 49 (hg19)chr2:71896256 Canonical Allele Identifier: CA347222684	We have identified this variant in 1 patient in a homozygous state [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2]. Novel missense variant at amino acid with different pathogenic (c.5444G>T) missense change (PM5) (CADD = 25,9 (v1.6), REVEL = 0,641).	Likely pathogenic: PM2 + PM5 + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.5454G>C p.(Trp1818Cys) exon 49 (hg19)chr2:71896266 Canonical Allele Identifier: CA347222737	We have identified c.5454G>C in one patient in a heterozygous state (associated with a likely pathogenic variant: c.1284+1G>A without confirmation of <i>in trans</i> occurrence). Dysferlin was absent on western blot in this case [PP4 moderate]. This variant is absent from gnomAD v2.1.1 [PM2] and is predicted to affect protein function (CADD = 32 (v1.6), REVEL = 0,885) [PP3]. There is one Likely pathogenic entry for this variant in ClinVar (ClinVar ID: 693999) and no LOVD entries (submitted with individual ID: 327048).	Uncertain significance: PM2 + PP4 moderate + PP3
DYSF NM_003494.3:c.5509G>A p.(Asp1837Asn) exon 49 (hg19)chr2:71896321 Canonical Allele Identifier: CA222190	We have identified this variant in 1 patient in a homozygous state . We have identified c.5509G>A in 2 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.3702+1G>A, c.855+1del), associated in these 2 cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 4 cases in the literature (associated with c.5903G>A, c.6124C>T, 1861G>A, c.265C>T, PMID: 22194990, 27666772, 21522182, 28403181), associated in 1 case (PMID: 22194990) with an absence of Dysferlin on western blot / ImmunoHistoChemistry, and associated in 1 case (PMID: 21522182) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 3 cases in the literature (PMID: 21522182, 32400077, 20544924), associated in 1 case (PMID: 20544924) with an absence of Dysferlin on western blot / ImmunoHistoChemistry, and associated in 1 case (PMID: 21522182) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 4 affected individuals, and 0 unaffected individual (PMID: 11257469) [PP1 strong]. It is present in only 3 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0056% 1/17910 in East Asian population) [PM2]. It is predicted to affect protein function (CADD = 32 (v1.6), REVEL = 0,819) [PP3]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PM3 strong + PP1 strong + PM2 + PP4 moderate + PP3
DYSF NM_003494.3:c.5526-14A>G: intron 49 (hg19)chr2:71896721 Canonical Allele Identifier: CA892680374	We have identified c.5526-14A>G in 1 patient in a heterozygous state (associated with a pathogenic variant: c.1663C>T), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.3112C>T, PMID: not found, LOVD individual ID 00300627) [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. An activation of a cryptic Acceptor site 13bp before natural splice site is predicted by HSF and SpliceAI (Acceptor gain 0.83) [PP3].	Likely pathogenic: PM2 + PM3 + PP4 moderate + PP3
DYSF NM_003494.3:c.5533del p.(Ile1845LeufsTer121) exon 50 (hg19)chr2:71896742del Canonical Allele Identifier: CA658821130	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.5533del in 2 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.1397+1G>C (<i>trans</i>)), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Even though	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate

	additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	
DYSF NM_003494.3:c.5594del p.(Gly1865AlafsTer101) exon 50 (hg19)chr2:71896803del Canonical Allele Identifier: CA212859	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 9 patients in a homozygous state, associated in 2 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. We have identified c.5594del in 7 patients (6 families) in a heterozygous state (associated with pathogenic variants: c.3112C>T, c.855+1del, c.3113G>A, c.1642dup, c.5302C>T), associated in 4 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. It is present in only 1 individual in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0009% 1/113770 in European (non Finnish) population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 3 affected individuals, and 0 unaffected individual (2 families with 3 cases in Marseille cohort) [PP1 moderate]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class5/pathogenic) for this variant.	Pathogenic: PVS1 + PM3 strong + PM2 + PP4 moderate + PP1 moderate
DYSF NM_003494.3:c.5626G>A p.(Asp1876Asn) exon 50 (hg19)chr2:71896835 Canonical Allele Identifier: CA222191	We have identified c.5626G>A in 1 patient in a heterozygous state (without second variant), associated in this case with an absence of Dysferlin on western blot. This variant has been observed in a homozygous state and reported as pathogenic in two patients (PMIDs:21522182, 26404900), including one case with a greatly reduced dysferlin. In another report, this variant was initially reported as pathogenic and then reclassified as Likely benign after it was confirmed to be <i>in cis</i> with a pathogenic variant (c.796_797del p.(Leu266PhefsTer6) PMID:17897828) [BP2]. c.5626G>A was reported as VUS in two additional patients (PMID: 29970176). Overall, there are eight entries for this variant in LOVD (three Benign/Likely benign, three VUS and four pathogenic). There are six Benign or Likely benign entries for this variant in ClinVar (ClinVar ID: 94341). POPMAX filtering allele frequency in gnomAD v2.1.1 is 0,77% (265/30616 in South Asian population, 6 homozygotes) [BS1]. It is not predicted to affect protein function (CADD = 24,1 (v1.6), REVEL = 0,377).	Likely benign: BS1 + BP2
DYSF NM_003494.3:c.5668-7G>A: intron 50 (hg19)chr2:71901320 Canonical Allele Identifier: CA1707510	We have identified this variant in 1 patient in a homozygous state, associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. We have identified c.5668-7G>A in 2 patients (2 families) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.2163-2A>G, c.5908C>T (<i>trans</i>)), associated in these 2 cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 3 cases in the literature (associated with c.2997G>T, c.4817_4820delinsA, c.2974T>C, PMID: 32400077), associated in 1 case (PMID: 32400077) with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 32400077) [PM3 strong, PP4 moderate]. Well-established functional assays demonstrating a variant has abnormal dysferlin function (Alteration of the natural 3' splice site and use of a cryptic acceptor site in intron 51 resulting in an insertion of GTCAG before exon 51 PMID: 25312915) [PS3]. It is present in only 3 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0003% 1/30614 in South Asian population) [PM2] (CADD = 24,5 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PS3 + PM3 strong + PM2 + PP4 moderate

<p>DYSF NM_003494.3:c.5713C>T p.(Arg1905Ter) exon 51 (hg19)chr2:71901372 Canonical Allele Identifier: CA118412</p>	<p>This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 1 patient in a homozygous state, associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. We have identified c.5713C>T in 3 patients (3 families) in a heterozygous state (associated with pathogenic variants: c.2779del, c.3517dup (<i>trans</i>)), associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.1276G>A, PMID: not found, LOVD ID 00220490) [PM3 strong, PP4 moderate]. It is present in only 3 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0003% 1/34592 in Latino population) [PM2] (CADD = 45 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.</p>	<p>Pathogenic: PVS1 + PM3 strong + PM2 + PP4 moderate</p>
<p>DYSF NM_003494.3:c.5767+1G>A: intron 51 (hg19)chr2:71901427 Canonical Allele Identifier: CA49771597</p>	<p>This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 1 patient in a homozygous state, associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 34 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.</p>	<p>Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting</p>
<p>DYSF NM_003494.3:c.5813_5821del p.(Thr1938_Lys1940del) exon 52 (hg19)chr2:71906232_71906240del Canonical Allele Identifier: CA1139532924</p>	<p>We have identified c.5813_5821del in 2 patients (2 families) in a heterozygous state (associated with pathogenic variants: c.855+1del, c.757C>T), associated in 1 case with a decrease of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 3 cases in the literature (associated with c.2875C>T, c.2779del, no RNA expression 2nd allele, PMID: 17994539, 21522182), associated in 1 case (PMID: 21522182) with an absence of Dysferlin on western blot / ImmunoHistoChemistry, and associated in 2 cases (PMID: 17994539, 21522182) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Protein-length-changing variant (deletion without frameshift) [PM4]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (PMID: 17994539) [PP1 supporting]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.</p>	<p>Pathogenic: PM3 strong + PM2 + PM4 + PP4 moderate + PP1 supporting</p>
<p>DYSF NM_003494.3:c.5860G>T p.(Glu1954Ter) exon 52 (hg19)chr2:71906279 Canonical Allele Identifier: CA347226407</p>	<p>This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.5860G>T in 1 patient in a heterozygous state (associated with a likely pathogenic variant: c.857T>A (<i>trans</i>)), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 52 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.</p>	<p>Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate</p>
<p>DYSF NM_003494.3:c.5871_5872del p.(Ser1958ProfsTer3) exon 52 (hg19)chr2:71906290_71906291del Canonical Allele Identifier: CA1139532925</p>	<p>This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.5871_5872del in 2 patients (1 family) in a heterozygous state (associated with a likely pathogenic variant: c.757C>T), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. [PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. Even though additional evidence of pathogenicity is</p>	<p>Pathogenic: PVS1 + PM2 + PP4 moderate + PP1 supporting</p>

	available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	
DYSF NM_003494.3:c.5899G>A p.(Gly1967Ser) exon 52 (hg19)chr2:71906318 Canonical Allele Identifier: CA347226496	We have identified this variant in 4 patients in a homozygous state, associated in these 4 cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. We have identified c.5899G>A in 1 patient in a heterozygous state (associated with a pathogenic variant: c.4191C>G) [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. It is predicted to affect protein function (CADD = 29,8 (v1.6), REVEL = 0,648) [PP3].	Likely pathogenic: PM2 + PM3 + PP4 moderate + PP1 supporting + PP3
DYSF NM_003494.3:c.5903G>A p.(Trp1968Ter) exon 52 (hg19)chr2:71906322 Canonical Allele Identifier: CA347226504	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.5903G>A in 1 patient in a heterozygous state (associated with a variant of unknown significance), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 2 cases in the literature (associated with c.3444_3445delinsAA, c.5509G>A, PMID: 27602406, 22194990) [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 51 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate
DYSF NM_003494.3:c.5908C>T p.(Pro1970Ser) exon 52 (hg19)chr2:71906327 Canonical Allele Identifier: CA16604254	We have identified c.5908C>T in 1 patient in a heterozygous state (associated with a pathogenic variant: c.5668-7G>A (<i>trans</i>)), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 2 cases in the literature (associated with c.353del, c.3512_3513del, PMID: 27602406, 27666772), associated in 1 case (PMID: 27666772) with an absence of Dysferlin on western blot / ImmunoHistoChemistry, and associated in 1 case (PMID: 27602406) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. It is predicted to affect protein function (CADD = 27,4 (v1.6), REVEL = 0,947) [PP3].	Likely pathogenic: PM3 strong + PM2 + PP4 moderate + PP3
DYSF NM_003494.3:c.5920_5921insCCCTGTGTGGCACTGTGTAGGAG p.(Glu1974AlafsTer7) exon 52 (hg19)chr2:71906339_71906340insAGCCCTGTGTGGCACTGTGTAGG Canonical Allele Identifier: CA1139532926	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.5920_5921insCCCTGTGTGGCACTGTGTAGGAG in 1 patient in a heterozygous state (associated with a pathogenic variant: c.1368C>A), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.5979dup p.(Glu1994ArgfsTer3) exon 53 (hg19)chr2:71908163dup Canonical Allele Identifier: CA222200	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 4 patients in a homozygous state, associated in 1 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. We have identified c.5979dup in 7 patients (6 families) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.2779del, c.1655_1668del (<i>trans</i>), c.757C>T, c.3477C>A (<i>trans</i>), c.3832C>T), associated in 2 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 very strong, PP4 moderate]. It is present in only 14 individuals in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0095% 8/35436 in Latino population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. Even	Pathogenic: PVS1 + PM3 very strong + PM2 + PP4 moderate + PP1 supporting

	though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	
DYSF NM_003494.3:c.5999G>A p.(Arg2000Gln) exon 53 (hg19)chr2:71908183 Canonical Allele Identifier: CA1707599	We have identified c.5999G>A in one patient in a heterozygous state (associated with two pathogenic variants: c.857T>A (trans) and c.5860G>T (cis)) [BP2]. There was an absence of Dysferlin on western blot in our case. This variant has previously been reported in a heterozygous state in two case in the literature (in both cases associated with a pathogenic variant, c.5194G>T) (PMID: 17070050, PMID: 11468312). In one of these two cases there was an absence of Dysferlin on western blot (PMID: 17070050). It has also been reported as VUS in three LGMD patients since no second variant was identified (PMID: 30564623). This variant has a POPMAX filtering allele frequency in gnomAD v2.1.1 of 0.029% (16/35436 alleles in Latino population, no homozygous). It is predicted to affect protein function (CADD = 32 (v1.6), REVEL = 0,734) [PP3]. This variant has seven ClinVar entries (four VUS and three Likely benign, ClinVar ID: 287816) and seven LOVD entries in addition to our case (two pathogenic described above and five VUS, we submitted our case with LOVD ID: 327391).	Uncertain significance: PP3 + BP2
DYSF NM_003494.3:c.6017G>A p.(Arg2006Gln) exon 53 (hg19)chr2:71908201 Canonical Allele Identifier: CA1707602	We have identified c.6017G>A in 2 patients (1 family) in a heterozygous state (associated with a pathogenic variant c.2643+1G>A; confirmed <i>in trans</i>). There was an absence of Dysferlin on western blot. [PM3 and PP4 moderate were not assigned since this variant is not rare]. This variant was shown to co-segregate with dysferlinopathy in this family with 2 affected individuals [PP1 supporting]. This variant is present in 39 individuals (0 homozygotes) in gnomAD v2.1.1 (POPMAX filtering allele frequency: 0,12% 38/30604 in South Asian population). It is predicted to affect protein function (CADD = 28 (v1.6), REVEL = 0,715) [PP3]. There are no ClinVar or LOVD entries for this variant (submitted LOVD ID: 711848).	Uncertain significance: PP1 supporting + PP3
DYSF NM_003494.3:c.6029A>G p.(Asn2010Ser) exon 53 (hg19)chr2:71908213 Canonical Allele Identifier: CA347226863	We have identified c.6029A>G in 1 patient in a heterozygous state (associated with a pathogenic variant: c.2643+1G>A and c.4577A>C), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Novel missense variant at amino acid with different pathogenic (c.6029A>C p.(Asn2010Thr)) missense change (PM5). It is predicted to affect protein function (CADD = 26,7 (v1.6), REVEL = 0,609).	Likely pathogenic: PM2 + PM5 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.6119G>A p.(Trp2040Ter) exon 54 (hg19)chr2:71909722 Canonical Allele Identifier: CA347227156	This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified this variant in 2 patients in a homozygous state [PM3, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2] (CADD = 51 (v1.6)). Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	Pathogenic: PVS1 + PM2 + PM3 + PP4 supporting
DYSF NM_003494.3:c.6124C>T p.(Arg2042Cys) exon 54 (hg19)chr2:71909727 Canonical Allele Identifier: CA222203	We have identified c.6124C>T in 4 patients (3 families) in a heterozygous state (associated with pathogenic variants: c.247del, c.5077C>T), associated in 2 of these cases with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 6 cases in the literature (associated with c.1180+5G>A, c.4872_4876delinsCCCC, c.3444_3445delinsAA, c.5979dup, c.4408C>T, c.2352_2355+1del, PMID: 25574751, 17994539, 27602406), associated in 3 cases (PMID: 25574751, 27602406) with an absence of Dysferlin on western blot / ImmunoHistoChemistry, and associated in 3 cases (PMID: 17994539, 27602406) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 21522182), associated in 1 case (PMID: 21522182) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry [PM3 very strong, PP4 moderate]. It is present in only 8 individuals	Pathogenic: PM3 very strong + PM2 + PP4 moderate + PP1 supporting + PP3

	in gnomAD v2.1.1 (POPMAX Filtering allele frequency: 0,0029% 6/113770 in European (non-Finnish) population) [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 2 affected individuals, and 0 unaffected individual (Marseille cohort) [PP1 supporting]. It is predicted to affect protein function (CADD = 29,5 (v1.6), REVEL = 0,859) [PP3]. Even though additional evidence of pathogenicity is available in the literature for this variant, the evidence listed here allows the maximal classification (Class 5/pathogenic) for this variant.	
DYSF NM_003494.3:c.6210T>G p.(Tyr2070Ter) exon 55 (hg19)chr2:71913589 Canonical Allele Identifier: CA347227857	We have identified c.6210T>G in 1 patient in a heterozygous state without second variant, associated with an absence of Dysferlin on western blot / ImmunoHistoChemistry. It is absent from gnomAD v2.1.1 [PM2]. This protein truncating variant is present in the last exon (NMD not predicted) and deletes a region critical for protein function, since several pathogenic protein-truncating variants have been previously described downstream of c.6210T>G [PVS1 strong].	Likely pathogenic: PM2 + PM4 + PVS1 strong
DYSF NM_003494.3:c.6233_6240del p.(Pro2078LeufsTer?) exon 55 (hg19)chr2:71913612_71913619del Canonical Allele Identifier: CA1139532906	We have identified c.6233_6240del in 1 patient in a heterozygous state (associated with a pathogenic variant: c.2894G>A), associated in this case with an absence of Dysferlin on western blot / ImmunoHistoChemistry. This variant has previously been reported in a homozygous state in 1 case in the literature (PMID: 21522182), associated in 1 case (PMID: 21522182) with a decrease of Dysferlin on western blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. Since this protein truncating variant is present in the last exon (NMD not predicted), [PVS1 moderate] was assigned.	Likely pathogenic: PM2 + PM3 + PVS1 moderate + PP4 moderate

Supplemental Table S2: The detailed evidence used for ACMG/AMP classification of *DYSF* variants identified in dysferlinopathy patients referred to the Medical Genetics Department of Marseille.

All the 176 variants analyzed in this study are listed. The detailed evidence used to classify each variant according to the modified ACMG/AMP criteria (see the Supplementary Methods for details) is shown in the “Evidence for each variant” column. The column “ACMG/AMP classification” shows the classification into ACMG/AMP pathogenic classes (“Pathogenic”, “Likely Pathogenic”, “VUS”, “Likely Benign” and “Benign”) as well the codes attributed to each variant.

Supplemental Table S3: ACMG/AMP classification of 17 *DYSF* variants assigned to VUS, Likely benign or Benign categories.

Variant	Evidence for each variant and reasons for downgrading	ACMG/AMP Classification
DYSF NM_003494.3:c.20T>C p.(Leu7Pro) exon 1 (hg19)chr2:71681148 Canonical Allele Identifier: CA347205953	We have identified this variant in one patient in a homozygous state [PM3 supporting, PP4 supporting]. It is absent from gnomAD v2.1.1 [PM2] and not predicted to affect protein function (CADD = 22,7 (v1.6), REVEL = 0,479). There are no ClinVar or LOVD entries for this variant (submitted to LOVD with ID: 711840). This patient also carried another VUS (c.2810C>T, p.(Thr937Ile)) in a homozygous state. Both of these variants of uncertain significance were returned before ACMG/AMP classification was established in our laboratory, using terms that were consistent with VUS category.	Uncertain significance: PM2 + PP4 supporting + PM3 supporting

	<p><i>Major reasons for not reaching the Likely pathogenic classification: no other patients described with this variant [PM3 at supporting level]; no protein expression data available [PP4 at supporting level]; in silico algorithms do not predict this variant as deleterious [no PP3].</i></p>	
<p>DYSF NM_003494.3:c.251C>T p.(Ala84Val) exon 4 (hg19)chr2:71730358 Canonical Allele Identifier: CA1705297</p>	<p>We have identified c.251C>T in two patients (two families) in a heterozygous state. In one patient it was associated with two pathogenic variants (c.5594del and c.3113G>A), but it was not possible to confirm if c.251C>T variant was present <i>in cis</i> with one of these two variants. No additional pathogenic variants were identified in the second patient. This variant is present in only 5 individuals in gnomAD v2.1.1 (POPMAX filtering allele frequency: 0,0011 %, 1/24932 in African population) [PM2] and not predicted to affect protein function (CADD = 23,1 (v1.6), REVEL = 0,186). There is one VUS ClinVar entry (ClinVar ID: 284573). This variant has been published by our group as benign (PMID: 18853459, LOVD Individual ID: 215600) before ACMG/AMP classification was established in our laboratory, mostly based on benign <i>in silico</i> predictions. Clinical record were not available to determine the exact terms used to return this variant to the referring clinician.</p> <p><i>Major reasons for not reaching the Likely pathogenic classification: association with two pathogenic variants probably explaining phenotype [no PP4]; no other patients described with this variant [no PM3]; not predicted as deleterious by in silico tools [no PP3].</i></p>	<p>Uncertain significance: PM2</p>
<p>DYSF NM_003494.3:c.565C>G p.(Leu189Val) exon 6 (hg19)chr2:71740953 Canonical Allele Identifier: CA147767</p>	<p>We have identified c.565C>G in four patients (three families). In one patient this variant was present at a homozygous state, associated with a homozygous pathogenic variant c.5296G>T (i.e. present <i>in cis</i> with a pathogenic variant) [BP2]. The second patient was heterozygous for c.565C>G variant, associated with a pathogenic c.2643+1G>A, likely pathogenic c.6029A>G and a VUS c.4577A>C. Two siblings from one additional family were heterozygous for c.565C>G variant, associated with a pathogenic c.2643+1G>A and a VUS c.6017G>A. c.565C>G was confirmed to be in <i>cis</i> with the VUS c.6017G>A. In all three families there was an absence of Dysferlin on western blot. This variant has previously been reported in a heterozygous state in two cases in the literature (associated with c.855+4T>C or c.3516_3517del, PMID: 26088049, 17562833). A minigene assay demonstrated that this variant could create a cryptic 5' splice site, resulting in an abnormal transcript with an out-of-frame deletion of the 103 bp of 3'end of the exon 6 in addition to the normal transcript (PMID: 25312915). The PS3 code was downgraded to PS3_supporting for this variant, since only a trace amount of abnormal transcript was observed in the minigene assay with persistent presence of a normally spliced transcript [PS3 supporting]. This variant is not predicted to affect protein function (CADD = 19,04 (v1.6), REVEL = 0,182). POPMAX filtering allele frequency in gnomAD v2.1.1 is 6,56% (1712/24936 in African population, 62 homozygotes, with only 0.03% in Europeans) [BA1]. This variant has 9 benign and likely benign entries in ClinVar (ClinVar ID: 94343). This variant is listed as benign in five LOVD entries and pathogenic in three LOVD entries (PMIDs:26088049, 17562833)</p> <p>Initially, before ACMG/AMP classification was established in our laboratory, this variant was returned using terms that were consistent with VUS category. After minigene analysis showed an effect on splicing, but before the 6.8% allele frequency in African population became available, this variant was considered as "at least partially pathogenic". The current re-classification of this variant as Benign according to the ACMG/AMP criteria does not affect the diagnosis for two patients already carrying two pathogenic DYSF</p>	<p>Benign: BA1 + BP2 + PS3 supporting</p>

	<p>variants explaining the phenotype. We are in the process of contacting the referring clinician of the two patients from the third family, since these patients did not have a clear molecular diagnosis (a VUS <i>in trans</i> with a pathogenic variant).</p> <p><i>Major reasons for not reaching the Likely pathogenic classification: this variant has an allelic frequency incompatible with pathogenicity [BA1 attributed, no PM3, no PP4].</i></p>	
<p>DYSF NM_003494.3:c.671T>G p.(Val224Gly) exon 7 (hg19)chr2:71742760 Canonical Allele Identifier: CA347208333</p>	<p>We have identified c.671T>G in one patient in a heterozygous state, associated with two VUS, c.896G>A and c.1877T>C. This variant is absent from gnomAD v2.1.1 [PM2] and is predicted to affect protein function (CADD = 27 (v1.6), REVEL = 0,93) [PP3]. There are no ClinVar or LOVD entries for this variant. The initial LOVD entry for this patient (LOVD Individual ID: 215587) did not include this variant, which was now corrected. This variant was returned before ACMG/AMP classification was established in our laboratory, using terms that were consistent with VUS category.</p> <p><i>Major reasons for not reaching the Likely pathogenic classification: not associated with a pathogenic or likely pathogenic variant in reported cases, absence of other patients with this variant [no PM3, no PP4]</i></p>	<p>Uncertain significance: PM2 + PP3</p>
<p>DYSF NM_003494.3:c.896G>A p.(Gly299Glu) exon 9 (hg19)chr2:71744159 Canonical Allele Identifier: CA347210280</p>	<p>We have identified c.896G>A in two patients (two families) in a heterozygous state. In one case it was associated with a VUS c.1877T>C, confirmed <i>in cis</i> (LOVD Individual:215343, PMIDs:16010686, 18853459), associated with absence of dysferline on western blot performed on monocytes (PP4 not assigned since the variant in question is not associated with a pathogenic or likely pathogenic variant). In the other case, this variant was associated with both VUS c.1877T>C <i>in cis</i> and with VUS c.671T>G <i>in trans</i> (LOVD Individual:215587). This variant has previously been reported in a heterozygous state in one case in the literature (associated with c.1877T>C, PMID: 27602406) associated with a decrease of Dysferlin on western blot. Since the segregation analysis was not performed in this study, it is likely that these two variants (c.1877T>C and c.896G>A) are <i>in cis</i>, as in our two patients, questioning the assumed pathogenicity for one of these variants. This variant is present in only one individual in gnomAD v2.1.1 (POPMAX filtering allele frequency: 0,0065% 1/15424 in European (non-Finnish) population) [PM2]. A different missense variant at the same amino acid position (c.895G>A) has been reported as pathogenic [PM5]. This variant is predicted to affect protein function (CADD = 25,2 (v1.6), REVEL = 0,947) [PP3]. There are two VUS entries in ClinVar (ClinVar ID: 552632). There are two LOVD entries for our two patients, initially listed at Pathogenic, that were now corrected. This variant was returned as pathogenic before the ACMG/AMP classification was established in our laboratory. In light of the current re-classification, we are in the process of contacting the referring clinician of two patients in order to update the molecular diagnoses in these cases.</p> <p><i>Major reasons for not reaching the Likely pathogenic classification: not associated with a pathogenic or likely pathogenic variant in reported cases [no PM3, no PP4].</i></p>	<p>Uncertain significance: PM2 + PM5 + PP3</p>
<p>DYSF NM_003494.3:c.1877T>C p.(Met626Thr) exon 20 (hg19)chr2:71780265 Canonical Allele Identifier: CA1705983</p>	<p>We have identified c.1877T>C in two patients (two families) in a heterozygous state. In one case it was associated with a VUS c.896G>A, confirmed <i>in cis</i> (LOVD Individual: 215343, PMIDs:16010686, 18853459). In the other case, this variant was associated with both VUS c.896G>A <i>in cis</i> and with VUS c.671T>G <i>in trans</i> (LOVD Individual:215587). This variant has previously been reported in a heterozygous state in one case in the literature (also carrying c.896G>A, PMID: 27602406), associated with a decrease of Dysferlin on</p>	<p>Uncertain significance: no codes attributed</p>

	<p>western blot. Since the segregation analysis was not performed in this study, it is likely that these two variants (c.1877T>C and c.896G>A) were <i>in cis</i>, as in our two patients, questioning the assumed pathogenicity for one of these variants. c.1877T>C variant is present in 356 individuals in gnomAD v2.1.1 (POPMAX filtering allele frequency: 0,23% 312/129084 in European (non-Finnish) population, 2 homozygotes). This variant is not predicted to affect protein function (CADD = 8,856 (v1.6), REVEL = 0,122). This variant has six ClinVar entries (ClinVar ID: 281014, three likely benign and three VUS) and one VUS entry in LOVD (in addition to our two patients). This variant was returned before ACMG/AMP classification was established in our laboratory, using terms that were consistent with VUS category.</p> <p><i>Major reasons for not reaching the Likely pathogenic classification: not associated with a pathogenic or likely pathogenic variant in reported cases [no PM3, no PP4]; not rare in the general population [no PM2], not predicted as deleterious by in silico tools [no PP3].</i></p>	
<p>DYSF NM_003494.3:c.2456G>A p.(Arg819Gln) exon 24 (hg19)chr2:71791288 Canonical Allele Identifier: CA1706192</p>	<p>We have identified c.2456G>A in one patient in a heterozygous state without additional pathogenic or likely pathogenic variants. Western blot showed normal levels of dysferline. This variant has previously been reported in a heterozygous state in one LGMD case in the literature (associated with VUS c.1980G>A, PMID: 30564623). It is present in only 11 individuals in gnomAD v2.1.1 (POPMAX filtering allele frequency: 0,0029% 3/30616 in South Asian population) [PM2]. This variant is not predicted to affect protein function (CADD = 22,6 (v1.6), REVEL = 0,078). There are two VUS entries for this variant in ClinVar (ClinVar ID: 497312) and one VUS entry in LOVD (PMID: 30564623, in addition to our patient 215595). This variant was returned before ACMG/AMP classification was established in our laboratory, using terms that were consistent with Likely benign category.</p> <p><i>Major reasons for not reaching the Likely pathogenic classification: not associated with a pathogenic or likely pathogenic variant in reported cases [no PM3, no PP4]; not predicted as deleterious by in silico tools [no PP3].</i></p>	<p>Uncertain significance: PM2</p>
<p>DYSF NM_003494.3:c.2810C>T p.(Thr937Ile) exon 26 (hg19)chr2:71795468 Canonical Allele Identifier: CA1706297</p>	<p>We have identified this variant in 1 patient in a homozygous state [PM3 supporting, PP4 supporting]. It is present in 33 individuals (1 homozygote) in gnomAD v2.1.1 (POPMAX filtering allele frequency: 0,0091% 3/19954 in East Asian population) [PM2]. It is predicted to affect protein function (CADD = 28,8 (v1.6), REVEL = 0,438) [PP3]. This variant contains 5 VUS entries in ClinVar (ClinVar ID: 336962) and no previous LOVD entries (submitted with ID: 711841). Our patient also carried another VUS (c.20T>C, p.(Leu7Pro)) in a homozygous state. Both of these variants of uncertain significance were returned before ACMG/AMP classification was established in our laboratory, using terms that were consistent with VUS category.</p> <p><i>Major reasons for not reaching the Likely pathogenic classification: no other patients described with this variant [PM3 at supporting level]; no protein expression data available [PP4 at supporting level].</i></p>	<p>Uncertain significance: PM2 + PP3 + PP4 supporting + PM3 supporting</p>
<p>DYSF NM_003494.3:c.3286_3291dup p.(Arg1097_Met1098insArgArg) exon 30 (hg19)chr2:71801439_71801444dup Canonical Allele Identifier: CA1139532899</p>	<p>We have identified c.3286_3291dup in one patient in a heterozygous state (associated with a pathogenic variant: c.755C>T without confirmation of <i>in trans</i> occurrence) [PM3 supporting]. Dysferlin was absent on western blot in this case [PP4 moderate]. This variant is absent from gnomAD v2.1.1 [PM2]. The effect of this variant on the protein function is not clear since it adds two Arginine residues to a stretch of three Arginine. There are no ClinVar or LOVD entries for this variants (submitted to LOVD with Individual ID:</p>	<p>Uncertain significance: PM2 + PP4 moderate + PM3 supporting</p>

	<p>326923). This variant was returned as pathogenic before the ACMG/AMP classification was established in our laboratory. In light of the current re-classification, we are in the process of contacting the clinicians following this patient in order to update the molecular diagnosis for this patient.</p> <p><i>Major reasons for not reaching the Likely pathogenic classification: no other patients described with this variant [PM3 at supporting level]; not predicted as deleterious by in silico tools [no PP3].</i></p>	
<p>DYSF NM_003494.3:c.4376A>G p.(Asp1459Gly) exon 40 (hg19)chr2:71840506 Canonical Allele Identifier: CA152669</p>	<p>We have identified c.4376A>G in one patient in a heterozygous state (associated with two pathogenic variants: c.917_923dup and c.4410+1G>A). It is predicted to affect protein function (CADD = 26,9 (v1.6), REVEL = 0,586) [PP3]. POPMAX filtering allele frequency in gnomAD v2.1.1 is 2,3% (607/24780 in African population, and four homozygotes) [BS1]. There are three Benign and one Likely benign entries in ClinVar (ClinVar ID : 128949), and no LOVD entries (submitted to LOVD with Individual ID: 326977). This variant was returned before ACMG/AMP classification was established in our laboratory, using terms that were consistent with VUS category.</p> <p><i>Major reasons for not reaching the Likely pathogenic classification: high allelic frequency in one subpopulation [BS1, no PM3]; association with two pathogenic variants probably explaining phenotype [no PP4].</i></p>	<p>Uncertain significance: PP3 + BS1</p>
<p>DYSF NM_003494.3:c.4577A>C p.(Lys1526Thr) exon 42 (hg19)chr2:71883359 Canonical Allele Identifier: CA207041</p>	<p>We have identified c.4577A>C in four patients (four families) in a homozygous state, associated with a homozygous Pathogenic variant c.2643+1G>A in each one of these cases [BP2]. One additional patient was heterozygous for VUS c.4577A>C variant, associated with the same pathogenic variant c.2643+1G>A and a likely pathogenic c.6029A>G. Dysferlin was absent in muscle biopsies in all five patients. This variant has been reported as VUS in three additional patients also associated with c.2643+1G>A (PMID:30564623). It is thus very likely that c.4577A>C and c.2643+1G>A are always found in cis. In another study, c.4577A>C was reported as pathogenic associated with c.2643+1G>A, however the phase of the variants was not determined (PMID: 20544924). c.4577A>C is predicted to affect protein function (CADD = 25,1 (v1.6), REVEL = 0,712) [PP3]. POPMAX filtering allele frequency in gnomAD v2.1.1 is 0.2% (56/24966 alleles with no homozygotes in African population). There are five ClinVar entries for this variant (one VUS and four Benign/Likely benign, ClinVar ID: 94327) and one VUS entry in LOVD in addition to the published cases described above. This variant was returned as pathogenic before the ACMG/AMP classification was established in our laboratory. In light of the current re-classification, we are in the process of contacting the clinicians following this patient in order to update the molecular diagnosis for this patient.</p> <p><i>Major reasons for not reaching the Likely pathogenic classification: association with two pathogenic variants probably explaining phenotype [no PP4, no PM3]; association in cis with a pathogenic variant [BP2]; not rare in the general population [no PM2].</i></p>	<p>Uncertain significance: PP3 + BP2</p>
<p>DYSF NM_003494.3:c.5015C>T p.(Ser1672Phe) exon 45 (hg19)chr2:71891526 Canonical Allele Identifier: CA347220545</p>	<p>We have identified c.5015C>T in one patient in a heterozygous state (associated with a pathogenic variant: c.799_800del) [PM3 supporting, PP4 supporting]. Dysferlin was severely decreased on western blot in this case. It is absent from gnomAD v2.1.1 [PM2]. This variant is predicted to affect protein function (CADD = 29,8 (v1.6), REVEL = 0,592) [PP3]. There are no ClinVar and one Likely Pathogenic LOVD entry for this variant without information on the second variant (PMID: 32528171) (submitted to LOVD with Individual</p>	<p>Uncertain significance: PM2 + PP3 + PP4 supporting + PM3 supporting</p>

	<p>ID: 327046). This variant was returned as pathogenic before the ACMG/AMP classification was established in our laboratory. In light of the current re-classification, we are in the process of contacting the clinicians following this patient in order to update the molecular diagnosis for this patient.</p> <p><i>Major reasons for not reaching the Likely pathogenic classification: only one patient with this variant was counted towards PM3, as no information about the second variant was available for the patient from PMID: 32528171 (PM3 at supporting level). Clinical records were not available to determine if the severely decreased dysferlin on western blot could be considered as absent (PP4 at supporting level).</i></p>	
<p>DYSF NM_003494.3:c.5243A>T p.(Glu1748Val) exon 47 (hg19)chr2:71894548 Canonical Allele Identifier: CA347221296</p>	<p>We have identified c.5243A>T in one patient in a homozygous state, associated with a homozygous pathogenic variant c.5296G>T (i.e. present <i>in cis</i> with a pathogenic variant) [BP2]. Dysferlin was absent on western blot in this case. This variant has previously been reported in a homozygous state in one case in the literature (PMID: 27671536), associated with an absence of Dysferlin on ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomAD v2.1.1 [PM2]. It is predicted to affect protein function (CADD = 28,9 (v1.6), REVEL = 0,921) [PP3]. This variant was returned before ACMG/AMP classification was established in our laboratory, using terms that were consistent with VUS category. There are no ClinVar entries for this variant, no additional LOVD entries besides our patient (LOVD ID: 215219) and the patient from PMID: 27671536. Clinical records were not available to determine the exact terms used to return this variant to the referring clinician.</p> <p><i>Major reasons for not reaching the Likely pathogenic classification: only the previously reported patient (PMID: 27671536) was counted towards PM3 and PP4 since an additional pathogenic variant was present in our patient in a homozygous state [PM3 at supporting level]; association in cis with a pathogenic variant [BP2].</i></p>	<p>Uncertain significance: PM2 + PM3 supporting + PP3 + PP4 moderate + BP2</p>
<p>DYSF NM_003494.3:c.5454G>C p.(Trp1818Cys) exon 49 (hg19)chr2:71896266 Canonical Allele Identifier: CA347222737</p>	<p>We have identified c.5454G>C in one patient in a heterozygous state (associated with a likely pathogenic variant: c.1284+1G>A without confirmation of <i>in trans</i> occurrence). Dysferlin was absent on western blot in this case [PP4 moderate]. This variant is absent from gnomAD v2.1.1 [PM2] and is predicted to affect protein function (CADD = 32 (v1.6), REVEL = 0,885) [PP3]. There is one Likely pathogenic entry for this variant in ClinVar (ClinVar ID: 693999) and no LOVD entries (submitted with individual ID: 327048). This variant was returned as pathogenic before the ACMG/AMP classification was established in our laboratory. In light of the current re-classification, we are in the process of contacting the clinicians following this patient in order to update the molecular diagnoses or to get additional segregation data in order to classify this variant as Likely Pathogenic.</p> <p><i>Major reasons for not reaching the Likely pathogenic classification: only one patient with this variant, associated with a likely pathogenic variant without confirmation of in trans occurrence [PM3 not assigned].</i></p>	<p>Uncertain significance: PM2 + PP4 moderate + PP3</p>
<p>DYSF NM_003494.3:c.5626G>A p.(Asp1876Asn) exon 50 (hg19)chr2:71896835 Canonical Allele Identifier: CA222191</p>	<p>We have identified c.5626G>A in 1 patient in a heterozygous state (without second variant), associated in this case with an absence of Dysferlin on western blot. This variant has been observed in a homozygous state and reported as pathogenic in two patients (PMIDs:21522182, 26404900), including one case with a greatly reduced dysferlin. In another report, this variant was initially reported as pathogenic and then reclassified as Likely benign after it was confirmed to be <i>in cis</i> with a pathogenic variant (c.796_797del</p>	<p>Likely benign: BS1 + BP2</p>

	<p>p.(Leu266PhefsTer6) PMID:17897828) [BP2]. c.5626G>A was reported as VUS in two additional patients (PMID: 29970176). Overall, there are eight entries for this variant in LOVD (three Benign/Likely benign, three VUS and four pathogenic). There are six Benign or Likely benign entries for this variant in ClinVar (ClinVar ID: 94341). POPMAX filtering allele frequency in gnomAD v2.1.1 is 0,77% (265/30616 in South Asian population, 6 homozygotes) [BS1]. It is not predicted to affect protein function (CADD = 24,1 (v1.6), REVEL = 0,377). This variant was returned before ACMG/AMP classification was established in our laboratory, using terms that were consistent with VUS category.</p> <p><u>Major reasons for not reaching the Likely pathogenic classification:</u> <i>high allelic frequency in one subpopulation [BS1, therefore no PP4, no PM3]; not predicted as deleterious by in silico tools [no PP3]</i></p>	
<p>DYSF NM_003494.3:c.5999G>A p.(Arg2000Gln) exon 53 (hg19)chr2:71908183 Canonical Allele Identifier: CA1707599</p>	<p>We have identified c.5999G>A in one patient in a heterozygous state (associated with two pathogenic variants: c.857T>A (trans) and c.5860G>T (cis)) [BP2]. There was an absence of Dysferlin on western blot in our case. This variant has previously been reported in a heterozygous state in two cases in the literature (in both cases associated with a pathogenic variant, c.5194G>T) (PMID: 17070050, PMID: 11468312). In one of these two cases there was an absence of Dysferlin on western blot (PMID: 17070050). It has also been reported as VUS in three LGMD patients since no second variant was identified (PMID: 30564623). This variant has a POPMAX filtering allele frequency in gnomAD v2.1.1 of 0.029% (16/35436 alleles in Latino population, no homozygous). It is predicted to affect protein function (CADD = 32 (v1.6), REVEL = 0,734) [PP3]. This variant has seven ClinVar entries (four VUS and three Likely benign, ClinVar ID: 287816) and seven LOVD entries in addition to our case (two pathogenic described above and five VUS, we submitted our case with LOVD ID: 327391. Given the <i>in cis</i> occurrence with a pathogenic variant in our patient, c.5999G>A was returned (before ACMG/AMP classification was established in our laboratory) using terms that were consistent with Likely benign category.</p> <p><u>Major reasons for not reaching the Likely pathogenic classification:</u> <i>observed in cis with a pathogenic variant [BP2]; the variant is associated with two pathogenic variants in our case, the variant is not very rare in the general population [no PP4, no PM2, no PM3].</i></p>	<p>Uncertain significance: PP3 + BP2</p>
<p>DYSF NM_003494.3:c.6017G>A p.(Arg2006Gln) exon 53 (hg19)chr2:71908201 Canonical Allele Identifier: CA1707602</p>	<p>We have identified c.6017G>A in 2 patients (1 family) in a heterozygous state (associated with a pathogenic variant c.2643+1G>A; confirmed <i>in trans</i>). There was an absence of Dysferlin on western blot. [PM3 and PP4 moderate were not assigned since this variant is not rare]. This variant was shown to co-segregate with dysferlinopathy in this family with 2 affected individuals [PP1 supporting]. This variant is present in 39 individuals (0 homozygotes) in gnomAD v2.1.1 (POPMAX filtering allele frequency: 0,12% 38/30604 in South Asian population). It is predicted to affect protein function (CADD = 28 (v1.6), REVEL = 0,715) [PP3]. There are no ClinVar or LOVD entries for this variant (submitted LOVD ID: 711848). This variant was returned (before ACMG/AMP classification was established in our laboratory) using terms that were consistent with VUS category.</p> <p><u>Major reasons for not reaching the Likely pathogenic classification:</u> <i>not rare in the general population [no PM2, therefore no PP4, no PM3]</i></p>	<p>Uncertain significance: PP1 supporting + PP3</p>

Supplemental Table S3: ACMG/AMP classification of 17 *DYSF* variants assigned to VUS, Likely benign or Benign categories.

Seventeen variants classified into VUS, Likely benign or Benign are listed. The detailed evidence used to classify each variant according to the modified ACMG/AMP criteria (see the Supplementary Methods for details) is shown in the “Evidence for each variant” column. This column also included information about terms that were used to return each variant to the referring clinician as well as major reasons for not reaching the Likely pathogenic classification. The column “ACMG classification” shows the classification into ACMG pathogenic classes (“Pathogenic”, “Likely Pathogenic”, “VUS”, “Likely Benign” and “Benign”) as well the codes attributed to each variant

