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# Analyse rétrospective et reclassification des variants DYSF dans une grande cohorte de patients français

Théo Charnay

► **To cite this version:**

Théo Charnay. Analyse rétrospective et reclassification des variants DYSF dans une grande cohorte de patients français. Sciences du Vivant [q-bio]. 2020. dumas-03100465

**HAL Id: dumas-03100465**

**<https://dumas.ccsd.cnrs.fr/dumas-03100465>**

Submitted on 6 Jan 2021

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**Analyse rétrospective et reclassification des variants *DYSF* dans une grande  
cohorte de patients Français**

**T H E S E**

**Présentée et publiquement soutenue devant**

**LA FACULTÉ DES SCIENCES MEDICALES ET PARAMEDICALES  
DE MARSEILLE**

**Le 7 Décembre 2020**

**Par Monsieur Théo CHARNAY**

**Né le 19 mars 1993 à Aix-En-Provence (13)**

Pour obtenir le grade de Docteur en Médecine

D.E.S. de BIOLOGIE MÉDICALE

**Membres du Jury de la Thèse :**

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**Madame le Professeur BARLIER Anne**

**Assesseur**

**Monsieur le Professeur ATTARIAN Shahram**

**Assesseur**

**Monsieur le Professeur KRAHN Martin**

**Directeur**



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Mme le Professeur	JUHAN-VAGUE Irène	31/08/2011
M. le Professeur	PONCET Michel	31/08/2011
M. le Professeur	KASBARIAN Michel	31/08/2011
M. le Professeur	ROBERTOUX Pierre	31/08/2011

### 2009

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M. le Professeur	VERVLOET Daniel	31/08/2012

### 2010

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M. le Professeur	MARTIN Pierre	31/08/2015
M. le Professeur	METRAS Dominique	31/08/2015

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M. le Professeur	MATTEI Jean-François	31/08/2015
M. le Professeur	OLIVER Charles	31/08/2015
M. le Professeur	VERVLOET Daniel	31/08/2015

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<b>THERAPEUTIQUE; MEDECINE D'URGENCE; ADDICTOLOGIE 4804</b>
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MAJ 01.09.2019



# REMERCIEMENTS

## **Au Professeur Nicolas Lévy,**

Pour avoir accepté de présider ce jury et pour votre accueil au sein du Département de Génétique Médicale du CHU La Timone. Je vous remercie également pour vos observations lorsque je vous ai présenté une partie de mon travail, et la connaissance que vous m'avez transmise. Je n'oublierai pas d'adresser « au petit nico » vos salutations lors de mon futur master. Pour finir je vous remercie pour les différents documents administratifs que vous avez du remplir afin de présenter ce travail me permettant ainsi d'obtenir le titre de Docteur.

## **Au Professeur Martin Krahn,**

Pour avoir accepté de participer à ce jury et pour avoir accepté de me trouver ce projet de thèse. Vous avez écouté mes arguments afin de me trouver le sujet qui réponde le mieux à mes attentes, et en attribuant au Dr. Svetlana Gorokhova la codirection de ce projet vous m'avez donné les clefs de la réussite. Au début vous aviez exprimé vos doutes quant au temps très restreint qu'il me restait avant de devoir partir pour mon stage de master, mais vous avez su m'encourager tout du long, et m'apporter une aide précieuse lorsque la difficulté s'est fait ressentir. Je n'aurais pas pu réaliser ce manuscrit sans vous, je n'aurais pas tenu les dernières semaines sans vous. Vous avez également su défendre mon projet d'année recherche devant le jury quand ils doutaient de celui-ci à cause de la situation sanitaire et ainsi obtenir une bourse.

## **Au Professeur Anne Barlier,**

Pour avoir accepté de participer à ce jury et pour m'avoir aidé à coordonner mon parcours professionnel. Vous avez guidé mon apprentissage et je suis heureux d'avoir débuté dans le domaine de la génétique avec un stage dans votre laboratoire. Vous avez su me faire découvrir les talents nécessaires à la direction d'une unité tout en maintenant une cohésion parfaite et une bonne ambiance entre ses intervenants. Vous avez également su apprécier mon humour et en apercevoir les maladresses afin de mon conseiller au mieux pour mon avenir. Vous avez organisé des réunions pour m'aider quand j'en ai eu besoin.

**Au Professeur Shahram Attarian,**

Pour avoir accepté de participer à ce jury alors que rien ne vous y obligeais. Vos connaissances et votre renommée apportent un poids supplémentaire à ce travail, même si du coup elles apportent également plus de pression. J'espère que vous trouverez dans ce travail des données qui susciteront votre intérêt et je prêterai la plus grande importance aux suggestions que vous pourrez y apporter.

**Au Docteur Svetlana Gorokhova,**

Pour m'avoir guidé tout le long de ce travail et le travail acharné que tu as réalisé afin de pouvoir soumettre l'article qui découle de cette thèse. Tu as malheureusement dû mettre de côté ta famille un certain temps et je n'oublierai pas ce cadeau que tu m'as fait et le sacrifice qu'il t'en a coûté. Tu as également été une cointerne formidable avec énormément de connaissances et de sympathie. Et si nous avons eu quelques différences de point de vue qui m'ont amené à te voir tel un adversaire devant un échiquier, j'ai toujours gardé un profond respect pour toi et tes connaissances. Au final même si j'ai perdu la majorité des parties je serais toujours prêt à jouer avec toi afin de t'égaliser.

**Au Professeur Christophe Bérout,**

Pour avoir pris le temps de me transmettre une partie de vos connaissances dans le domaine de l'épissage et permis d'utiliser HSF sans limitation dans ce projet. Malgré certains désaccords vis-à-vis de certains aspects de ce travail vous avez répondu à toutes mes interrogations et démontré de l'intérêt vis-à-vis du résultat. Je vous remercie aussi pour votre vision du contexte actuel de la génétique au niveau mondial, ce qui contribue aujourd'hui pour certains points à me forger ma vision des choses.

**Au Maître de Conférences Pauline Romanet,**

Pour avoir commencé ma formation dans le domaine du diagnostic en biologie moléculaire. Tu m'as également fait confiance et appris à aiguïser mon sens critique. Tu as toujours pris le temps de répondre à mes questions même quand le sujet ne relevait plus de ton travail. Tu es celle qui m'a adressé au Dr. Marc Bartoli pour mes projets de recherche en thérapie et ainsi permis d'obtenir mon stage de master aux USA. Tu m'as appris à présenter et à persévérer, mais en plus tu m'as apporté l'indépendance car la bourse qui va me nourrir l'année à venir c'est grâce à toi que je l'ai eu. Par-dessus tout tu es devenue une amie et je ne t'oublierai pas.

**Au Docteur Marc Bartoli,**

Pour son expertise et ses conseils au niveau moléculaire concernant la dysferline. Pour son aide inconditionnel dans mes projets de thérapie génique.

**Au Maître de Conférences Alexandru Saveanu,**

Pour m'avoir appris à rédiger un article scientifique mais aussi pour sa vision de la recherche qui m'a permis de développer mon sens critique.

**Au Docteur Florence Riccardi,**

Pour son aide dans la compréhension de ce projet et son aide à la rédaction.

**A mes parents,**

Ils ont su me donner la confiance et la compétence qui m'a mené jusqu'ici. Ils m'ont donné le gout d'aller toujours plus loin et ils me poussent à aller toujours plus loin. Ils se sont investis dans ce travail et m'ont permis de le perfectionner. C'est aussi le gout pour l'effort qu'ils m'ont transmis à travers le VTT qui m'a permis de tenir les délais de ma thèse.

**A mon frère,**

Pour son efficacité à m'aider dans la mise en place de ce travail. On a grandi ensemble et tu continues de me faire grandir.

**A Mamie blanche,**

Toi qui a trouvé dans le passage de ma thèse ta nouvelle joie de vivre après que papy nous ait quitté. Vous m'avez hébergé au tout début de mes études et vous m'avez toujours apporté de la tendresse. Je sais que c'était un rêve pour vous qu'il y ait un médecin dans la famille et je suis heureux de pouvoir l'exhausser.

**Au mes grands-parents Simone et Jean-claude,**

Qui m'ont toujours apporté la sérénité et appris à apprécier les plaisirs simples de la vie.

**A Mélanie Sentis,**

Tu as su tenir bon dans ce moment difficile. Tu as su me supporter et m'encourager tel que je l'avais fait pour ta thèse. Tu sais m'aimer tel que je t'aime.

A mes cousins et cousine Valentin, Dorian, Noa, Lauriane, Florian, Amauri, à mes oncles et tantes Éric, Françoise, Bénédicte, Rénaud, à mes amis d'enfance Maelis, Manon, Yoan, Clément, Robin, Florent, Cyrien, à mes cointernes Camille, Maélie, Camille, Clara, Florent, Rémi, Marceau, Estelle, mais également Patrice, Anna, Arnaud, Daniel, Morgane... et tous les autres que le temps me contraint à ne pas citer mais présents dans mon cœur... A vous tous merci d'avoir fait de moi la personne que je suis aujourd'hui.

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# 1 Mise en contexte

## 1.1 Situation globale

Le gène de la dysferline (*DYSF*), impliqué dans les myopathies appelées dysferlinopathies, est un gène de grande taille (55 exons codants) et a un spectre mutationnel large, ce qui a des implications sur la complexité d'interprétation de variants identifiés lors d'analyses de diagnostic en génétique moléculaire. L'analyse de la séquence nucléotidique du gène *DYSF* est par conséquent majoritairement réalisée dans des centres experts spécialistes dans le domaine des maladies neuromusculaires. Le Département de Génétique Médicale du CHU La Timone est l'un des principaux centres experts dans le diagnostic des dysferlinopathies depuis près de 20 ans sur le plan international, avec à ce jour près de 600 analyses effectuées, avec une expertise notamment dans le domaine de l'interprétation des données mutationnelles pour ce gène. Dans le cadre de la perpétuelle évolution des recommandations concernant la classification des variants de séquence, en particulier depuis l'adoption internationale des recommandations de l'American College of Medical Genetics and Genomics (ACMG)(1), nous avons entrepris une actualisation de la classification de l'ensemble des variants de la cohorte dans l'objectif de mettre en évidence d'éventuelles discordances entre le rendu de résultat initial et une classification actuelle, et d'en évaluer les conséquences et adaptations nécessaires pour le diagnostic génétique des dysferlinopathies.

Afin de comprendre l'intérêt de ce travail et avant d'en présenter le contenu nous allons détailler :

- les capacités actuelles du séquençage
- l'état de l'art concernant le diagnostic en génétique médicale
- les connaissances des pathologies du gène *DYSF*

Nous présenterons alors son contenu suivant la forme d'un article scientifique en précisant :

- la méthodologie utilisée
- les résultats obtenus
- la discussion critique des résultats
- la conclusion

Une partie de ce travail dirigé par le Pr. Martin Krahn et le Dr. Svetlana Gorokhova a été soumise pour publication sous l'intitulé « **Retrospective analysis and reclassification of *DYSF* variants in a large French series of dysferlinopathy patients** » (annexe Charnay et al. à paraître).

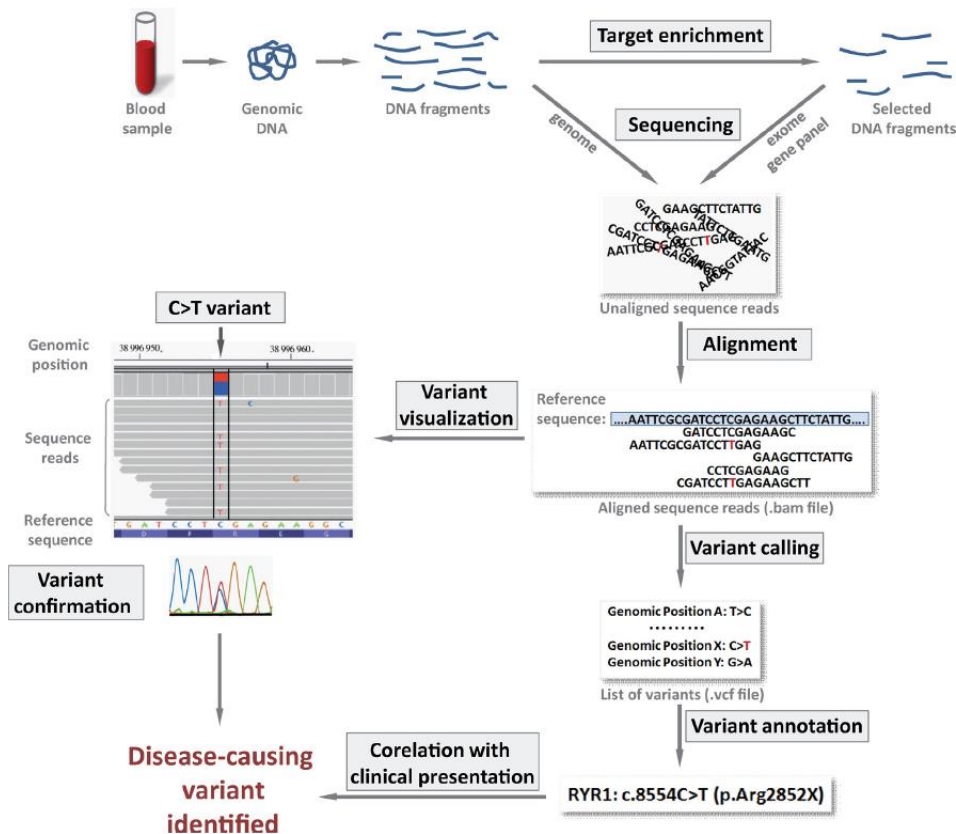
## 1.2 Le séquençage de nouvelle génération (NGS)

L'arrivée depuis moins de 20 ans du NGS aussi appelé séquençage haut débit ou séquençage massivement parallèle a permis de générer une quantité de données phénoménale vis-à-vis de ce que permettaient les méthodes de séquençage Sanger utilisées jusque-là. Le traitement de ces données a été permis par l'évolution concomitante de l'informatique et des logiciels de bio-informatique, ce qui a abouti à l'utilisation du NGS en diagnostic à partir de 2009.

Le principe de cette technologie repose sur la détection de l'incorporation successive de nucléotides complémentaires à la séquence d'intérêt, dans un processus comportant des étapes d'amplification de millions de molécules en parallèle (avec des technologies permettant d'enregistrer l'ensemble de ces détections en parallèle).

A ce jour les deux techniques de séquençage à haut débit les plus utilisées sont: le séquençage avec incorporation de nucléotides modifiés fluorescents d'Illumina, et le séquençage par mesure de variation de pH lors de l'incorporation de nucléotides de Thermo Fisher (Buermans and den Dunnen 2014 (2)). Les grandes étapes du séquençage à haut débit sont communes à ces deux technologies. La Figure 1 ci-dessous décrit les différentes étapes de ce processus (adapté de Gorokhova et al. 2015 (3)).

Figure 1

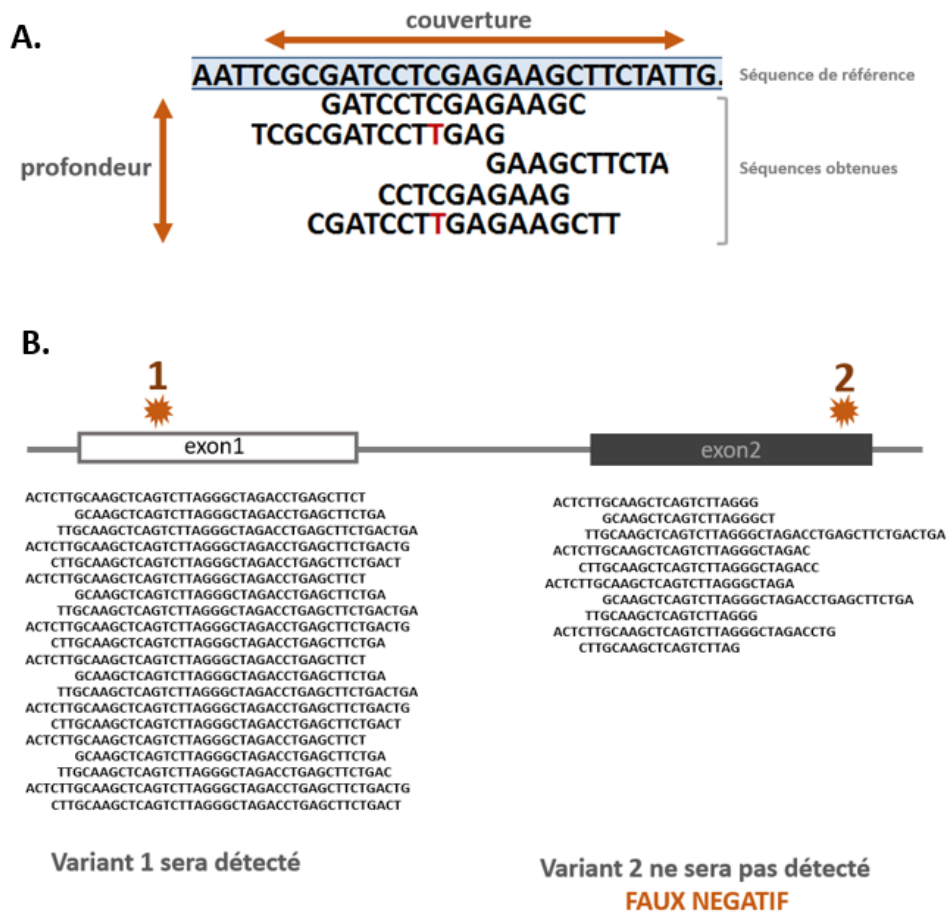




La première étape consiste à extraire l'ADN contenu dans l'échantillon d'intérêt et à le purifier afin d'éliminer les autres composants cellulaires pouvant interférer avec les techniques de détection. Par la suite l'ADN est fragmenté afin d'obtenir des séquences comprenant entre 30 et 250 nucléotides environ. Comme on ne séquence généralement pas l'ensemble du génome, il faut sélectionner les fragments correspondants aux gènes d'intérêt, et cette étape appelée « enrichissement » peut se réaliser soit par capture (via des séquences d'ADN complémentaires couplées à des billes magnétiques) soit par amplification en chaîne par polymérase (PCR : Polymérase Chain Reaction) permettant l'amplification de séquences spécifiques prédéterminées par des couples d'amorces spécifiques. Par conséquent l'enrichissement n'est pas nécessaire lors du séquençage d'un génome entier. Intervient alors l'étape de séquençage où lors d'un cycle de duplication de l'ADN l'appareil détecte et enregistre l'émission de signaux successifs correspondants aux nucléotides s'incorporant afin de former le brin d'ADN complémentaire du brin matrice. Dans le cadre du NGS la détection s'effectue sur des millions de brins d'ADN à chaque instant et l'enregistrement génère un fichier informatique de plusieurs gigaoctet contenant les données de séquence de ces millions de brins d'ADN, appelés « reads » ou « lectures de séquence ».

Il faut alors extraire l'information du fichier généré, et cela passe par l'utilisation successive de différents programmes bio-informatiques, ce que l'on définit comme « un pipeline bio-informatique ». Entre chaque résultat de programme l'intégrité des données est contrôlée, et un score de qualité est attribué à chaque base séquencée. L'ensemble de ces scores qualité sera combiné à la fin de l'analyse pour obtenir un indice global sur la fiabilité de chaque base. La première étape du pipeline consiste à retrouver la position de chaque read sur le génome de référence, et d'obtenir si possible plus de 30 reads pour chaque position (détermination de profondeur de lecture ; voir figure 2) correspondant à l'une des étapes clés de détermination de la qualité du séquençage (génération d'un fichier « .bam »). Les différences entre la séquence de référence et la séquence de l'échantillon d'intérêt, aussi appelées « variants », sont ensuite identifiées par l'étape de « variant calling » (génération d'un fichier « .vcf »). Dans ce fichier sont ensuite ajoutées des informations comme le nom du gène, le type de variant, la position exonique ou intronique, la fréquence dans les bases de données, la prédiction de pathogénicité ou bénignité, et tout autre information dont il serait utile de disposer afin d'établir le diagnostic génétique. Ce recueil d'information pour chaque variant correspond à l'étape « d'annotation ». L'étape finale d'interprétation de la signification clinique des variants sera détaillée plus loin dans l'exemple spécifique aux variants du gène *DYSF*, mais il est à noter l'importance de la

vérification visuelle de la présence avérée des variants extraits pour le compte rendu lors de cette dernière étape (élimination des artefacts résiduels).



**Figure 2 :** Les concepts de la profondeur et la couverture d'un test par séquençage à haut débit.

A) La qualité du séquençage est définie par sa profondeur et sa couverture. La profondeur correspond au nombre de reads alignés sur une région ou combien de fois une position a été « lue » par le séquençage à haut débit. La couverture correspond au pourcentage de la région cible séquencée avec une profondeur donnée. Par exemple, une région peut être couverte à 100% avec une profondeur de 1x (chaque nucléotide a été lu au moins une fois) mais avoir une couverture de 50% à 20x (qu'une moitié des positions ont été lues au moins 20 fois).

B) Un exemple de résultats de séquençage pour deux exons d'un gène donné. L'exon 1 a une très bonne couverture par les reads obtenus lors du séquençage. Le variant 1 sera détecté sans difficulté par des algorithmes bio-informatiques. La couverture de l'exon 2 n'est pas suffisante. Le variant 2, localisé dans la partie très mal couverte n'a été « lu » par le séquençage que deux fois. Ce variant ne sera pas détecté par les algorithmes bio-informatiques, conduisant à un résultat faux négatif.

De nos jours en diagnostic génétique la technologie de NGS se divise principalement en 3 grandes approches :

1. La première en « panel de gène » (ou « liste de gène ») est la plus utilisée en routine. Elle est le ciblage spécifique d'une dizaine voire d'une centaine de gènes connus pour être impliqués dans une pathologie donnée, et ceci par une technique d'enrichissement afin de n'obtenir que l'ensemble des séquences exoniques ainsi que les bornes introniques à proximité de ces gènes-là (Gorokhova et al. 2015 (3)); Krahn et al., 2016 (4)).
2. La deuxième approche appelée séquençage d'exome entier (whole-exome sequencing ou W.E.S.) consiste à séquencer l'ensemble des exons des gènes du génome humain (environ 180000 exons codants pour les environ 20000 gènes du génome), et leurs régions introniques flanquantes. C'est une méthode en plein essor actuellement (the NDD Exome Scoping Review Work Group et al. 2019 (5)).
3. La dernière méthode séquence le génome entier (whole-genome sequencing ou W.G.S.), et génère près de 100 fois plus de données que le W.E.S.

Cette capacité du NGS d'analyser l'ensemble des gènes impliqués dans une maladie, et d'augmenter ainsi le rendement diagnostique en fait une approche incontournable pour explorer les maladies ayant une expression phénotypique proche (présentation clinique similaire). Elle contribue également à réduire l'errance diagnostique par rapport à l'approche classique utilisant le séquençage selon la méthode de Sanger, qui en raison des capacités limitées de génération de données de séquence ne permet qu'une approche dite « gène par gène » (Gorokhova et al. 2015 (3); Krahn and Collège national des enseignants et praticiens de génétique 2016 (6); Krahn et al. 2016 (4)). Le NGS est donc parfaitement indiqué dans le domaine des maladies neuromusculaires (dont font partie les dysferlinopathies) à la vue de leur très grande hétérogénéité clinique et génétique, et en est décrit depuis 2015 comme une des principales méthodes diagnostiques (Gorokhova et al. 2015 (3)).

### 1.3 Classification ACMG

Avec la croissance de plus en plus grande du nombre de variants génétiques identifiés (notamment grâce au NGS), l'interprétation de la signification clinique de ceux-ci dans un délai raisonnable est devenue un enjeu important pour les laboratoires. Ceci est particulièrement le cas lorsque le laboratoire ne possède pas encore d'expertise pour un gène ou une pathologie, ou que le variant n'a jamais été décrit dans la littérature.

Comme l'approche en panel de gène, celle qui génère le moins de variants à analyser en NGS, aboutit généralement à quelque(s) centaine(s) de variants par échantillon, il est nécessaire d'appliquer des filtres standardisés afin de réduire à une dizaine environ le nombre de variants qui nécessitera une analyse plus poussée.

Cette analyse exhaustive de variants nécessite une homogénéisation et standardisation afin que pour chaque patient, quel que soit le laboratoire diagnostique dans lequel l'analyse est réalisée, le même résultat soit obtenu en termes de classification. C'est à cet égard que des sociétés savantes comme l'ESHG (European Society of Human Genetics) ou l'ACMG-AMP (American College of Medical Genetics and Genomics & the Association for Molecular Pathology) ont établies des recommandations d'interprétation à l'intention des généticiens moléculaires (Amendola et al. 2016 (7); Matthijs et al. 2016 (8); Richards et al. 2015 (1)), pour la classification des variants en différentes catégories de pathogénicité ou bénignité : Bénin (classe 1), Probablement Bénin (classe 2), De Signification Inconnue (classe 3), Probablement Pathogène (classe 4), et Pathogène (classe 5). En dépit des ressemblances de méthodologie dans ces documents, actuellement les recommandations de l'ACMG-AMP correspondant à la classification de Richards et al. de 2015 ont à ce jour été retenues en tant que standard international.

La méthodologie exposée par Richards et al. 2015 divise en 8 catégories/arguments de force variable chaque critère d'interprétation de variants, permettant par un système d'attribution de score d'établir la classe retenue pour le variant interprété. Il est important d'insister sur la possibilité de faire varier le niveau de preuve associé à chaque argument (figure 3 : ACMG STANDARDS AND GUIDELINES selon Richards et al. 2015, les arguments étant représentés en ligne, et leur variation de force en colonne).

**Figure 3 : ACMG STANDARDS AND GUIDELINES**

	Benign			Pathogenic		
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
<b>Population data</b>	MAF is too high for disorder BA1/BS1 OR observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
<b>Computational and predictive data</b>		Multiple lines of computational evidence suggest no impact on gene /gene product BP4  Missense in gene where only truncating cause disease BP1  Silent variant with non predicted splice impact BP7  In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5  Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
<b>Functional data</b>	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
<b>Segregation data</b>	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
<b>De novo data</b>				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
<b>Allelic data</b>		Observed in <i>trans</i> with a dominant variant BP2  Observed in <i>cis</i> with a pathogenic variant BP2		For recessive disorders, detected in <i>trans</i> with a pathogenic variant PM3		
<b>Other database</b>		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
<b>Other data</b>		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

Le niveau de preuve permet de pondérer la force de chaque argument, soit dans le sens de la bénignité soit dans celui de la pathogénicité :

- Le caractère pathogène se décomposant en 4 niveaux allant de « faible » (PP, Pathogenic Poor), à « moyen » (PM, Pathogenic Moderate), puis « fort » (PS, Pathogenic Strong), et enfin « très fort » (PVS, Pathogenic Very Strong).
- Le caractère bénin se décompose en 3 niveaux allant de « faible » (BP, Benign Poor), à « fort » (BS, Benign Strong), jusqu'à « suffisant seul » (BA, Benign stand Alone).

A noter que les critères BS et BA ne sont pas individualisés sur la figure ci-dessus malgré leur impact différent sur la classification finale.

Il est aussi important de prendre en compte qu'il ne faut pas comptabiliser plusieurs critères dans la même catégorie, mais dans la mesure du possible les additionner pour augmenter la force de l'association, ou alors ne comptabiliser que l'argument qui a le plus de force. Encore une fois il existe des recommandations quant au calcul final de la classe de pathogénicité ou bénignité du variant (les 5 classes sont décrites dans la figure 4 ci-dessous), mais c'est également là que le rôle de l'expertise d'un centre permet de pondérer dans un sens ou dans l'autre la classification finale.

Figure 4

<input type="checkbox"/> PATHOGENE	<input type="checkbox"/> PROBABLEMENT PATHOGENE	<input type="checkbox"/> DE SIGNIFICATION INCONNUE	<input type="checkbox"/> PROBABLEMENT BENIN	<input type="checkbox"/> BENIN
<input type="checkbox"/> PVS1 + PS <input type="checkbox"/> PVS1 + 2PM <input type="checkbox"/> PVS1 + PM + PP <input type="checkbox"/> PVS1 + 2PP <input type="checkbox"/> 2PS <input type="checkbox"/> PS + 3PM <input type="checkbox"/> PS + 2PM + 2PP <input type="checkbox"/> PS + PM + 4PP	<input type="checkbox"/> PVS1 + PM <input type="checkbox"/> PS + PM <input type="checkbox"/> PS + 2PP <input type="checkbox"/> 3PM <input type="checkbox"/> 2PM + 2PP <input type="checkbox"/> 1PM + 4PP	<input type="checkbox"/> Critères insuffisants  <input type="checkbox"/> Critères contradictoires	<input type="checkbox"/> BS + BP <input type="checkbox"/> 2BP	<input type="checkbox"/> BA1 <input type="checkbox"/> 2BS

Critères de classification définis par Richards et al., *Genetics in Medicine*, 2015, Vol 17 (5) pages 405-424.

(A) Critères pour classer les variants pathogènes : PVS1 (Very Strong), PS (Strong), PM (Moderate), PP (Supporting) (B) Critères pour classer les variants bénins : BA1 (Stand Alone), BS (Strong), BP (Supporting)

A noter la correspondance variant de classe 1 = Bénin, classe 2 = probablement bénin, classe 3 = de signification inconnue, classe 4 = probablement pathogène, classe 5 = pathogène utilisée par certains auteurs. Par ailleurs dans la suite de ce document le terme « variant » sera utilisé pour décrire toute variation de séquence alors que le terme « mutation » uniquement pour les variants prouvés pathogènes ou probablement pathogènes.

Cette méthodologie de classification a été intégrée dans les recommandations nationales françaises émises par le Groupe de Travail du Réseau NGS-Diag, en collaboration avec l'ANPGM (Association Nationale des Praticiens de Génétique Moléculaire), l'ACLF (Association des Cytogénéticiens de Langue Française), AchroPuce (Réseau d'Analyse Chromosomique sur Puce à ADN) et le GGC (Groupe Génétique et Cancer), ainsi que plusieurs Filières des Maladies Rares. La première version de ce document a été publiée en juin 2018 avec un objectif de limiter la variabilité inter- et intra-laboratoire dans l'interprétation des variants en génétique moléculaire et d'aboutir à un consensus national. Depuis, ces recommandations ont été adoptées par la grande majorité des laboratoires de diagnostics en France (9).

Toutefois ces recommandations générales datant de 2015 laissent place à l'interprétation pour de nombreux critères (ce qui était nécessaire pour s'appliquer à tous les cas de figure, ou parce que l'on n'avait pas encore les connaissances nécessaires pour définir les bornes de chaque critère). Par conséquent il existe une hétérogénéité résiduelle de résultats entre les laboratoires de diagnostics d'un même domaine (Amendola et al. 2016 (7)), que de nouvelles recommandations essaient de réduire. Par exemple la définition des seuils de fréquence allélique permet de s'affranchir des différentes interprétations de la fréquence attendue chez les porteurs sains (ancienne définition du seuil en dessous duquel on attribuait le critère PM2).

C'est ainsi que des recommandations produites par des groupes de travail d'expert, pouvant être commanditées par des groupes tels que ClinGen (Rehm et al. 2015 (10)), apparaissent depuis 2015 et s'appliquent à l'ensemble des situations (Abou Tayoun et al. 2018 (11); Biesecker et al. 2018 (12); Brnich et al. 2019 (13); Ghosh et al. 2018 (14); SVI\_Recommendation\_for\_PM3\_Criterion, 2019; SVI\_Recommendation\_for\_PS2\_and\_PM6\_criteria, 2018), mais aussi de manière gène-spécifique dont nous citerons que quelques exemples : MYH7, Rasopathies, PAH, Surdités, CDH1, PTEN, MEN1, RUNX1, GAA et TP53 (ClinGen\_Lysosomal\_Storage\_Disorders\_Expert\_Panel, 2019; ClinGen\_TP53\_Expert\_Panel, 2019; Gelb et al. 2018 (15); Kelly et al. 2018 (16); Lee et al. 2018 (17); Luo et al. 2019 (18); Mester et al. 2018 (19); Oza et al. 2018 (20); Romanet et al. 2019 (21); Zastrow et al. 2018 (22)).

Il est donc important de suivre la littérature afin d'incorporer les nouvelles règles au diagnostic biologique dans le cadre de la médecine fondée sur les preuves, et d'être à jour au niveau de l'état de l'art médical. **Nous avons utilisé cette démarche dans le travail présenté ici pour la classification de variants du gène *DYSF*, et nous détaillerons dans la partie matériel et méthode chaque critère utilisé.**

Il est à noter qu'en France l'homogénéisation et la standardisation des résultats passe aussi par la création de listes de gènes de références impliqués dans certaines pathologies, notamment réalisée par des groupes d'experts des Filières Nationales de Santé Maladie Rares (23), telle que la filière FILNEMUS (Filière Nationale des Maladies Rares Neuromusculaires) pour les myopathies (dont font partie les dysferlinopathies). Ces listes s'accompagnent également d'informations sur l'association gène-pathologie pour chaque gène.

## 1.4 Les dysferlinopathies

La protéine dysferline a été identifiée et désignée par son nom pour la première fois en 1998 par J Liu et al. et Bahsir R. et al (24,25) qui rapportaient une mutation d'un même nouveau gène chez 9 familles de patients présentant des dystrophies musculaires distales (dont la Myopathie de Miyoshi (MM), la dystrophie musculaire des ceintures de type 2B (LGMD2B), et la myopathie distale de début tibial antérieur). Par extension les auteurs ont nommé ce gène *dysferline* (Human Genome Organization Gene Nomenclature : *DYSF*) comme la protéine. Les pathologies qui toucheront ce gène seront désignées comme les dysferlinopathies.

La structure de la dysferline a été décrite par Sula et al. (26) par cristallographie en 2014 et a permis de mettre en évidence la présence de 7 domaines de liaison (d'affinité différentes (27)) aux phospholipides calcium dépendants distribués tout le long de la protéine (annoté C2 sur la figure 5 ci-dessous, C2A ayant sa capacité de fixation à différents phospholipides membranaires démontrée), 2 domaines DysF fusionnant partiellement entre eux au niveau central de la protéine, 3 domaines Ferline, ainsi qu'un domaine transmembranaire à son extrémité C terminale. La majeure partie de la protéine (de C2A jusqu'au domaine transmembranaire) est intracytoplasmique.

Figure 5 : répartition des domaines protéiques de la dysferline

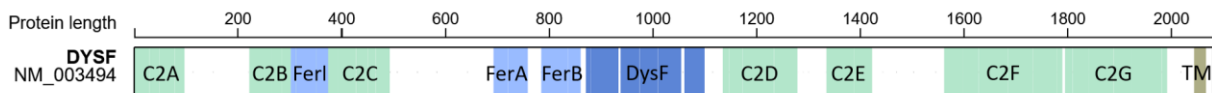
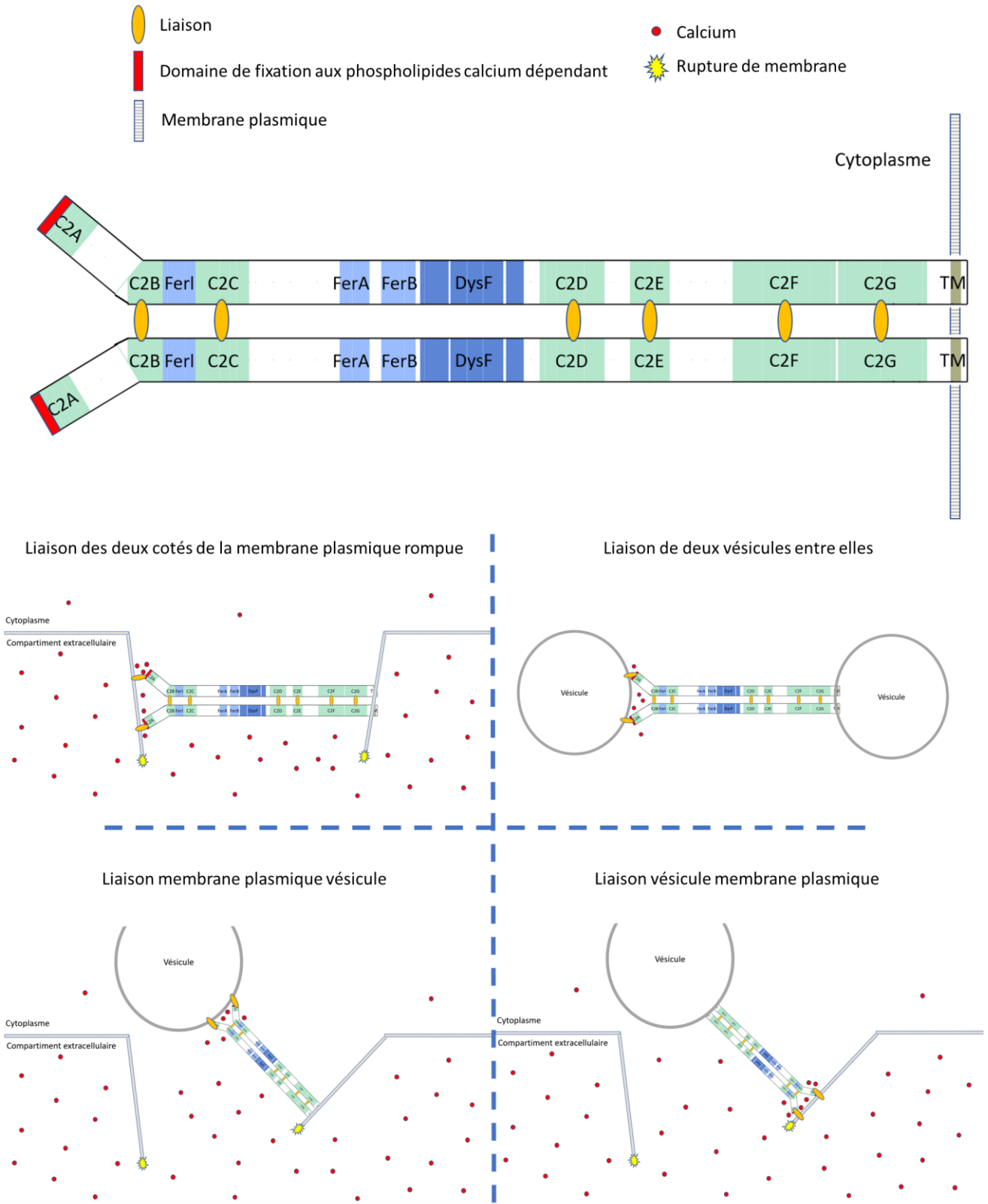


Figure réalisée avec ProteinPaint (<https://proteinpaint.stjude.org/>) et les positions de domaines définies par Sula et al 2014

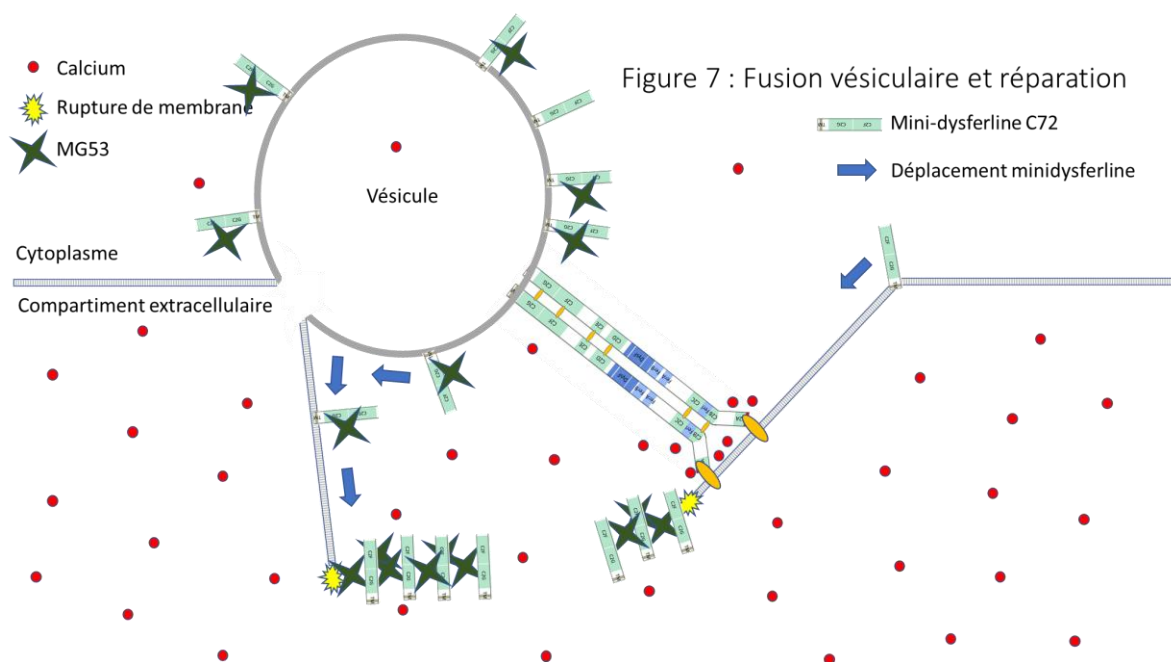
La dysferline a pour rôle d'orienter et de permettre la fusion vésiculaire lors des processus de réparation membranaire, notamment par la capacité de son domaine C2A de se lier de façon calcium dépendant aux phospholipides (28–31), mais également de recruter et de stabiliser des protéines impliquées dans la réparation membranaire (tel que la protéine AHNAK via le domaine C2A (32)). Elle interagit également avec de nombreuses autres protéines comme par exemple avec MG53, qui est impliquée dans l'acheminement et la fusion des vésicules aux sites de lésions de la membrane plasmique (interaction via C2A (33)) ou encore la cavéoline 3, l'alpha-tubuline et les annexines qui jouent un rôle similaire. La fonction de réparation membranaire de la dysferline serait en partie permise par sa capacité à s'homodimériser et ainsi de se lier aux phospholipides des membranes à ses extrémités (34) comme schématisé dans la figure 6 ci-après.



Figure 5 : Homodimère de dysferline et modes de fonction supposés



Les calpaïnes (1 et 2) agissent de manière différente : c'est par leur capacité à cliver la dysferline de manière calcium dépendante entre les domaines C2E et C2F (au niveau de l'exon 40) qu'elles permettent la formation d'une mini-dysferline C72 (contenant les domaines C2F et C2G avec le domaine transmembranaire), ressemblant fortement à un module synaptotagmin-like aux capacités de fusion membranaires. Cette mini-dysferline C72 s'accumulerait également dans certaines vésicules qui viendraient ensuite délivrer leur contenu au niveau de la lésion membranaire. Il y a alors lieu une formation de complexes mini-dysferline C72 et MG53 qui viennent former un maillage colmatant la brèche membranaire (de plus le module ressemblant à la synaptotagmine de ce complexe aurait une aptitude probable à fusionner les vésicules (35,36)).



A la lumière des fonctions de cette protéine il est aisé de comprendre que des variants de séquence dans son gène puissent avoir des effets délétères. Les premières études, mais également les résultats obtenus jusqu'à aujourd'hui, ont mis en évidence la nécessité d'avoir les deux allèles du gène *DYSF* mutés pour voir apparaître des signes phénotypiques. Les dysferlinopathies sont donc des maladies autosomiques récessives. Par ailleurs tout variant de séquence dans le gène *DYSF* n'entraîne pas forcément d'effet délétère protéique, et il faut savoir que la gravité tout comme la présentation clinique peut varier en fonction des mutations.

Il existe par ailleurs un mécanisme majeur de régulation cellulaire appelé Nonsense-Mediated Decay (NMD) qui agit entre la transcription (production d'un ARN messager à partir de l'ADN du gène) et la traduction (production d'une protéine à partir de l'ARN messager). Ce NMD dégrade les ARN messagers mutés présentant un codon stop prématuré (avant les 50 dernières

bases de la dernière jonction entre deux exons (37)) et conduit donc à une absence totale de protéine. Rares sont les exceptions qui échappent à ce phénomène de NMD et l'ARN messager de la dysferline n'en fait pas partie. Les mutations qui entraineraient ce phénomène de NMD représentent plus de 60% des cas de dysferlinopathie diagnostiqués dans la littérature (38–40). Toutefois un autre mécanisme dénommé Endoplasmic Reticulum-Associated Degradation (ERAD) dégraderait lui les protéines mutées et aboutirait à une diminution voir également à une absence de protéine (41). Ces deux phénomènes expliquent l'utilité en diagnostic de la quantification (relative) de la protéine sur des biopsies musculaires par des techniques de Western Blot (WB) ou d'ImmunoHistoChimie (IHC).

A noter qu'il est possible que l'atteinte d'autres gènes, et tout particulièrement ceux codant pour les protéines interagissant avec la dysferline, puissent également faire diminuer la quantité de dysferline détectable à la biopsie musculaire (via divers mécanismes de régulation cellulaire). Dans ces cas-là la diminution n'est généralement pas totale, ce qui explique que lorsque la protéine dysferline est complètement indétectable (WB=0 ou IHC=0) à la biopsie musculaire notre suspicion de mutations dans le gène *DYSF* soit beaucoup plus importante.

Au niveau clinique les dysferlinopathies présentent une très grande hétérogénéité allant d'une simple augmentation isolée de la concentration de créatine kinase (CK) dans le sang, aux maladies neuromusculaires de début plus ou moins précoces selon les formes, et ceci à ce jour sans démonstration d'une corrélation génotype-phénotype. A noter que les patients présentant généralement les premiers symptômes entre 15 et 30 ans pour les deux formes premièrement décrites que sont la Myopathie de Miyoshi et la Dystrophie musculaire des ceintures de type 2B (elles représentent environ 50% des dysferlinopathies (42)).

La Myopathie de Miyoshi se caractérise par une faiblesse du compartiment postérieur de l'extrémité inférieure distale (muscles gastrocnémiens et soléaires) et associée à des difficultés à se tenir sur la pointe des pieds (43).

La dystrophie musculaire des ceintures autosomique récessive type 2B (LGMD2B) se caractérise quant à elle par une faiblesse proximale, lentement progressive et par une atrophie des muscles des ceintures scapulaire et pelvienne (44). L'ensemble des atteintes symptomatiques (dont la myopathie distale de début tibiale antérieur et les atteintes atypiques) présentent une élévation de la concentration sanguine en CK. Bien que l'on sépare la MM et la LGM2B la maladie débute souvent par des formes proximo-distales où les symptômes des deux formes s'entremêlent (jusqu'à 35% des dysferlinopathies).

Aujourd'hui en France l'approche d'analyse par NGS en panel de gène a été définie par la filière FILNEMUS (Filière Nationale de Santé des Maladies Rares Neuromusculaires) en fonction de la présentation clinique de la myopathie du patient (45). Concernant les panels contenant le gène *DYSF*, FILNEMUS a défini une liste principale de 7 gènes et une liste exhaustive de 40 gènes pour les dystrophies musculaires des ceintures, mais également une liste de gènes unique exhaustive de 24 gènes pour les myopathies distales et scapulopéronières. Ces panels permettant d'augmenter grandement le rendement diagnostique vis-à-vis de l'approche monogénique généralement réalisée en séquençage Sanger sans augmenter le temps de manipulation technique nécessaire.

Le gène *DYSF* est un gène de grande taille de plus de 230 kilobases, et possède une isoforme musculaire majoritaire de 55 exons contenant 6240 nucléotides codant pour une protéine de 2080 acides aminés (46). La notion d'isoforme protéique est importante, et explique que l'on puisse obtenir un organisme aussi complexe que le corps humain même si nous ne possédons que 20 000 gènes puisqu'il existe de subtiles variations de l'expression protéique des gènes en fonction des tissus leur permettant d'avoir des fonctions différentes (chaque forme protéique définissant une isoforme). Par conséquent toute notre étude sera réalisée sur cette isoforme musculaire (aussi appelée isoforme 8, [ENST00000258104.3](#), et NM\_003494.3), de par la nature des maladies que sont les myopathies, et ce même si la nomenclature internationale utilise préférentiellement une autre isoforme, pourtant peu représentée dans le muscle ([ENST00000410020.3](#), NM\_001130987.2). La répartition des différentes isoformes peut être visualisée sur GTExPortal (47) dans la section « exon expression for... » en sélectionnant la sous-section « Isoform Expression ».

Il est décrit aujourd'hui plus de 1100 variants de séquence dans le gène *DYSF* dont certaines sont pathogènes (Leiden Open Variation Database (48)). Ces variants se répartissent assez régulièrement tout le long du gène et ce même en limitant la liste aux variants décrits comme pathogènes ou probablement pathogènes dans la littérature. Il n'y a donc à ce jour aucun hotspot mutationnel décrit (Krahn et al. 2008 (49)) pour ce gène. Pour autant une grande partie de ces variants ayant été classée avant l'implémentation des recommandations ACMG, avec des méthodologies apportant souvent trop d'importance aux prédictions in silico, et en s'appuyant sur des données populationnelles incomplètes, il n'est donc pas exclu qu'un ou des hotspots mutationnels puissent exister (39).

La compréhension des différentes recommandations, leur pertinence dans le contexte des dysferlinopathies, la nécessité de rechercher régulièrement de nouvelles recommandations ainsi

que la prise en compte de l'expérience du centre, rendent l'interprétation des variants *dysferline* complexe et nécessitent souvent l'avis d'experts comme par exemple dans le domaine de l'épissage ou de l'interprétation des résultats en minigène.

Le Département de Génétique Médicale du CHU La Timone est un centre expert dans le domaine des dysferlinopathies et possède l'une des plus grandes cohortes internationales de diagnostic, avec presque 600 échantillons recueillis et analysés depuis près de 20 ans. Les patients inclus dans cette cohorte ont été sélectionnés sur des critères diagnostiques forts permettant d'atteindre un rendement diagnostique de plus de 50%, malgré le fait que la majorité des analyses ont été effectuées avant le NGS. Ces critères de sélection ont été permis par l'expertise clinique des centres de diagnostic génétique français (Centres de Référence de Maladies Neuromusculaires), ainsi que des laboratoires anatomopathologiques.

La première étude de Nguyen et al. en 2005 (50) présentait les premiers résultats avec une cohorte alors constituée de 34 cas indexes, identifiant ainsi 54 variants différents et amenant à la confirmation diagnostique pour 23 patients avec au moins deux variants probablement pathogènes.

Une seconde étude dirigée par la même auteure en 2007 (42) s'intéressait à l'expression phénotypique des dysferlinopathies en s'appuyant sur l'observation de 40 patients français (au diagnostic confirmé par la présence de deux variants pathogènes au séquençage associée à un WB fortement diminué ou nul). Ils mettront en évidence la forme « proximo-distale » de la maladie, une forme caractérisée par la combinaison de la clinique de la Myopathie de Miyoshi et de la dystrophie musculaire des ceintures de type 2B, qui représente près de 35% des patients dans cette étude. Une élévation de la créatine kinase sans atteinte neuromusculaire visible chez deux sujets asymptomatiques sera également décrite mettant en évidence une hétérogénéité clinique importante dans les dysferlinopathies. Bien d'autres détails clinico-biologiques seront décrits et servent de base clinique dans le diagnostic de la pathologie.

En 2008 ce sera Krahn et al. (49) qui décriront les résultats de diagnostic génétique de la plus grande cohorte du moment avec près de 134 patients dont 89 avec deux variants pathogènes identifiés, avec notamment l'identification de 30 nouveaux variants pathogènes non décrits au préalable. La cohorte avec ses 64 variants pathogènes rassemblait alors 25% des variants pathogènes connus mondialement. Ces résultats ont été complétés en 2009 (51) avec le diagnostic pour la première fois de délétions/duplication d'exons au sein du gène *DYSF* par la

technique d'amplification multiplex de sondes dépendant d'une ligation (MLPA), technique confirmée par PCR quantitative en temps réel.

En 2011 avec la création de la base UMD-DYSF (pour Universal Mutation Database) qui rassemblait l'ensemble des variants du gène *DYSF* décrits dans le monde et leur interprétation (52) le Département de Génétique Médicale du CHU la Timone devenait le référent mondial du diagnostic moléculaire des dysferlinopathies.

D'autres études ont été réalisées par la suite, avec la mise en place d'un panel de gènes pour les dystrophies musculaires autosomiques récessives pour les cas non résolus en 2014 (53), ou la mise en place d'une technique de « mini-gène » afin d'explorer les impacts sur l'épissage de certains variants (54).

Dans le cadre de l'étude présentée ici, basée sur l'expertise du Département de Génétique Médicale de Marseille dans le domaine de l'interprétation des données mutationnelles du gène *DYSF*, nous avons entrepris une évaluation rétrospective de reclassification des variants identifiés comme pathogènes ou probablement pathogènes chez les patients français ayant bénéficiés d'analyses mutationnelles pour ce gène dans notre laboratoire. Ce travail s'inscrit dans un contexte majeur d'évolution récente des recommandations concernant la classification des variants de séquence, en particulier depuis l'adoption internationale des recommandations de l'American College of Medical Genetics and Genomics en 2015 (1). Ce travail avait pour objectif principal de mettre en évidence d'éventuelles discordances entre le rendu de résultat initial et une classification actuelle, et d'en évaluer les conséquences et adaptations nécessaires pour le diagnostic génétique des dysferlinopathie, et de manière plus large de disposer d'une base de données actualisée permettant de sélectionner les variants les plus pertinents à explorer en recherche, comme par exemple pour définir des hotspot mutationnels.

L'actualisation de la classification des variants a été réalisée grâce à un outil tableur Excel de référence prédéfinissant l'ensemble des critères attribuables, personnalisable pour chaque variant en saisissant un minimum d'information, afin de répondre au défi de l'harmonisation et de la systématisation pour de futures applications en diagnostic génétique.

## 2 MATERIELS ET METHODES

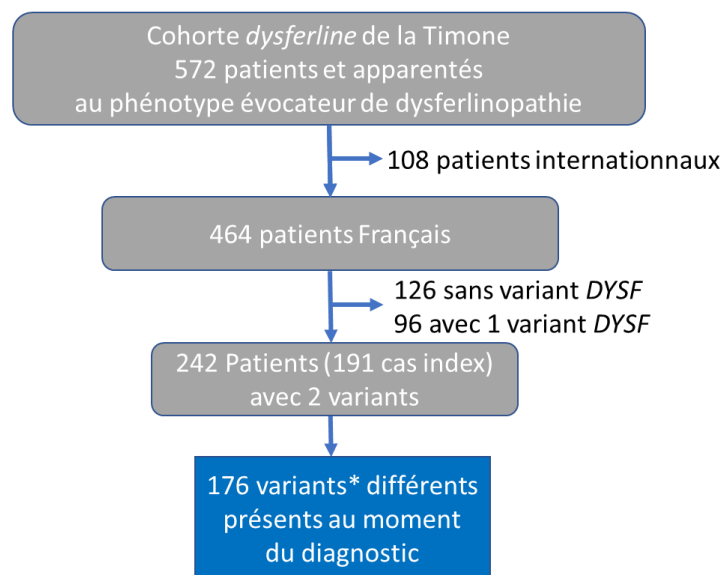
### 2.1 Description de la cohorte analysée:

La cohorte *dysferline* de la Timone a depuis 2001 inclus 572 échantillons de patients différents présentant à minima soit une forme clinique de dysferlinopathie primaire, soit une déficience en protéine dysferline à la biopsie musculaire. L'association de la clinique et du déficit en protéine était préférentiellement requise car beaucoup plus spécifique d'une mutation dans le gène de la *dysferline*.

La majorité des échantillons ayant été traitée avant la mise en place du NGS, ce sont les techniques de polymorphisme de conformation des acides nucléiques simple brin (SSCP) et de chromatographie liquide à haute performance sur gel dénaturant (DHPLC) qui ont été utilisées afin de sélectionner les brins d'intérêt pour le séquençage Sanger (qui est plus chronophage). Les techniques de SSCP et de DHPLC s'appuient sur le principe qu'une variation d'une base nucléotidique dans une séquence amplifiée par PCR va posséder des propriétés de migration différentes dans certaines conditions. Par conséquent la comparaison des résultats par rapport à ceux d'un patient de référence permet de définir les séquences où la séquence nucléotidique varie, et on détermine ensuite dans un deuxième temps la ou les bases ayant changées par le séquençage Sanger.

La sélection des variants à réanalyser a été effectuée comme décrit dans l'organigramme ci-dessous, les variants retenus finalement étant ceux rendus dans le dossier du patient au moment du diagnostic (figure 8).

Figure 8 : Diagramme de flux de la sélection des variants pour la reclassification.



\* variants retenus initialement comme pathogène au moment du rendu de résultat

## 2.2 Classification des variants

Nous avons utilisé le transcrit ENST00000258104.7 correspondant au NM\_003494.4 pour l'ensemble de notre étude. La visualisation des variants sur la structure du gène *DYSF* dans la figure 1 a été réalisée avec ProteinPaint (55,56), en utilisant les positions de domaine décrites par Sula et al. 2014.

La nomenclature HGVS (Human Genome Variation Society) de chaque variant a été vérifiée et corrigée si besoin à l'aide de l'outil ClinGen Allele Registry (57).

La pathogénicité des 176 variants de la cohorte de la Timone a été réévaluée selon les critères ACMG, tels que définis par Richards et al. 2015, avec les modifications suivantes :

- Nous avons appliqué les critères PVS1 (variant nul dans un gène où la perte de fonction est le mécanisme pathogène connu) selon les recommandations de ClinGen Sequence Variant Interpretation (SVI) pour l'interprétation de la perte de fonction PVS1 ACMG/AMP Variant Criteria (11). Lors de l'attribution de PVS1 à des variants d'épissage, la présence de sites d'épissage cryptiques autour du variant a été explorée à l'aide de Human Splicing Finder (58) <https://www.genomnis.com/access-hsf>. Notre interprétation du PVS1 a été confrontée aux résultats obtenus par le prédicteur AutoPVS1 (59) <http://autopvs1.genetics.bgi.com/> et nous avons conservé le critère obtenu le moins fort pour la classification finale.
- Le score PS3 (Des études fonctionnelles in vitro ou in vivo bien établies montrent un effet affectant le gène ou son "produit") a été attribué aux variants avec un résultat en analyse de modélisation par minigène "Impact sur l'épissage" dans Kergoulay et al. 2014, et diminué en PM4 si le variant n'entraînait ni décalage du cadre de lecture ni de variation de plus de 10% de la longueur de la protéine.
- Le score PM2 (Absent des contrôles (ou très faible fréquence si récessif) dans les bases de données) a été attribué grâce à la fréquence allélique de filtrage obtenue par GnomAD (60) avec les seuils suivants : PM2 si inférieur à 0.02%, BS1 si supérieur à 0.5% et inférieur à 5%, BA1 si supérieur à 5%.
- Le score PM3 (présence de variants en *trans* avec un variant pathogène) a été attribué conformément à la recommandation SVI pour le critère en *trans* PM3 (version 1.0,



[https://www.clinicalgenome.org/site/assets/files/3717/svi\\_proposal\\_for\\_pm3\\_critereion\\_-\\_version\\_1.pdf](https://www.clinicalgenome.org/site/assets/files/3717/svi_proposal_for_pm3_critereion_-_version_1.pdf)).

- Le score PP1 (données de ségrégation) a été attribué conformément aux recommandations du groupe de travail Hearing Loss ClinGen qui s'est concentré en particulier sur les maladies récessives (20).
- Nous avons appliqué le score PP3 (prédiction in silico de la pathogénicité) si le score CADD ou REVEL était supérieur à 25 et 0,7 respectivement. Les scores REVEL (61) ont été obtenus à partir de leur site de téléchargement (62) et les scores CADD (63) à partir de <https://cadd.gs.washington.edu/snv> en utilisant le modèle GRCh37-V1.6. Nous avons utilisé HSF (Human Splicing Finder, REF), qui combine et permet de visualiser les scores de l'algorithme d'HSF et ceux de MaxENT (64), pour attribuer le score PP3 aux variants non canoniques d'épissage avec leurs seuils prédéfinis (pour HSF un  $\Delta CV$  de -10% pour toutes les positions sauf pour la position +4 où il est de -7%, et pour MaxENT un score au-dessus de 3 ou supérieur à 70% du score du site canonique).
- Comme la présentation clinique de tous les patients de notre cohorte était évocatrice d'une dysferlinopathie (faiblesse musculaire progressive avec preuve d'une atteinte musculaire proximale ou distale confirmée par biopsie ou EMG), un score d'intensité PP4 supporting a été attribué à tous les variants, tandis qu'un score d'intensité modéré (PP4\_mod) a été attribué lorsque la protéine dysferline était complètement absente des tissus musculaires (analyse par Immuno-Histo-Chimie ou Western Blot).
- Le critère PM1 n'a pas été utilisé car les domaines fonctionnels de la dysferline ne sont pas dépourvus de variant bénins.
- Les critères BP1/PP2 n'ont pas été utilisés car des variants bénins et pathogènes sont présents avec une fréquence élevée dans le gène DYSF.
- Les critères PP5 et BP6 n'ont pas été utilisés conformément aux recommandations du groupe de travail ClinGen SVI (12).

Afin d'attribuer l'ensemble de ces critères nous avons utilisé l'intégralité des données présentes dans la cohorte de la Timone, ainsi que chaque observation de chaque variant, soit dans une publication soit dans la base de donnée LOVD, jusqu'à obtenir suffisamment de critères pour le classer en classe 5. Pour cela nous avons utilisé et vérifié les références citées dans Clinvar, LOVD, VarSome et UCSC, mais également recherché chaque variant dans Pubmed ainsi que Litvar. Pour les variants n'ayant pas obtenu suffisamment de critères avec cette méthodologie,

nous avons recherché manuellement la présence d'informations dans l'ensemble des publications téléchargées au fur et à mesure de l'étude.

Nous avons par ailleurs extrait manuellement la classification automatisée de VarSome (65) afin d'en évaluer les avantages et inconvénients en diagnostic clinique.

## 2.1 Standardisation et homogénéisation du traitement des données.

Les données nécessaires à la classification ont ensuite été saisies pour chaque variant dans un fichier Excel différent (Figure 9 en page suivante). La présentation initiale de ce tableau de données a été réalisée par le Dr. Svetlana Gorokhova. Par la suite le tableur Excel a été spécifiquement modifié pour la classification des variants de la dysferline, contenant l'ensemble des critères ACMG modifiés attribuables. Nous avons intégré plus d'une centaine de formules conditionnelles contenant du texte et reliées aux différentes cases à remplir dans le tableur, afin de réduire au minimum la quantité d'informations nécessaire à saisir pour justifier chaque critère tout en conservant une justification spécifique pour chaque critère.

Exemple du code de trois cases différentes :

```
=SI(SOMME(B26:B28)>0,9;S5&" ";"")&SI((SOMME(D26:D28)+SOMME(G26:G28))>0,9;S6&" ";"")&SI(A18>0,9;S4&" ";"")&SI(AJ8>0,9;S3&" ";"")&SI(K9<>"";K9;"")&SI(K13<>"";K13;"")&SI(K19<>"";K19;"")&SI(K28<>"";K28;"")&SI(K14<>"";" "&K14;"")&SI(K23<>"";" "&K23;"")&SI(K27<>"";" "&K27;"")&"]. "
```

```
= "We have identified "&F2&" in "&(SOMME(D26:D28)+SOMME(G26:G28))&" patient"&SI((SOMME(D26:D28)+SOMME(G26:G28))>1;"s";"")&" ("&AP38&" index) in heterozygous state "&SI(SOMME(AP34:AQ36)>0;"(associated with pathogenic or likely pathogenic variant"&SI(SOMME(AP34:AQ36)>1;"s";"")&":"&AT34&"");"")&" from the Timone Dysferlinopathy cohort."&SI(SOMME(AP26:AP31)>0;AG3;"")&SI(SOMME(AQ26:AQ31)>0;AG4;"")
```

```
=SI(OU(AW18=AW19;AW17=AW19;AW16=AW19;AW15=AW19;AW14=AW19;AW13=AW19;AW12=AW19;AW11=AW19;AW10=AW19);"";AW19)
```

Pour la synthèse finale des informations concernant la classification finale de chaque variant une macro en utilisant le code Visual Basic for Application (VBA) a été réalisée (comme par exemple pour agréger les phrases ensemble et supprimer les répétitions d'espace) (annexe code 1). Une autre macro a servi à l'extraction de la synthèse finale sous forme de tableau (annexe code 2). De même, d'autres macros ont été utilisées afin d'appliquer les mises au point s'avérant nécessaires lors de l'avancement de ce travail, et ce à l'ensemble des 176 fichiers Excel sans avoir à les modifier un à un (annexe code 3).

L'analyse et la visualisation d'une partie des données ainsi que l'intégration des résultats des bases de données de Clinvar et de LOVD ont été effectuées par le Dr. Svetlana Gorokhova avec R 4.0.3 en utilisant la collection de paquets tidyverse (v.1.3.0, <https://www.tidyverse.org/>) et le paquet ggforce (v.0.3.2).

Figure 9: présentation de la mise en forme des données

Cases remplies manuellement

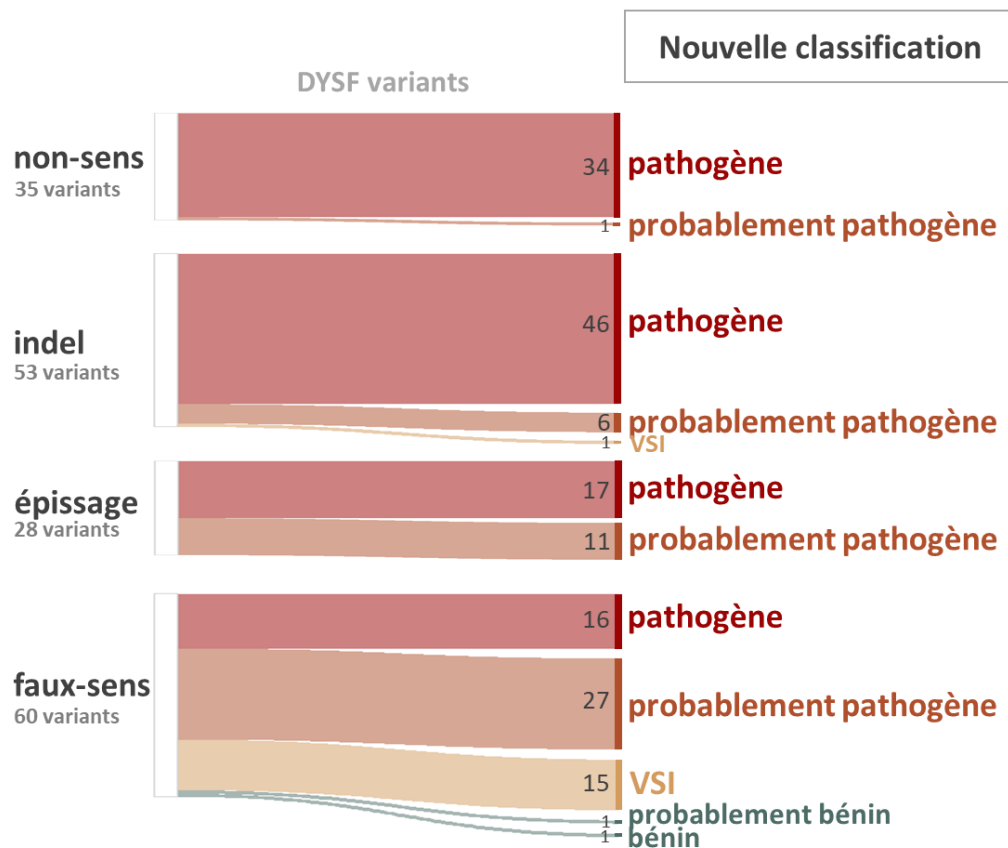
A	B	C	D	E	F	G	H	I	J	K	L	M	N			
Chr	Start	End	Ref	Alt	c.nom	p.non	Exon		CADD	p REVEL	SpliceAI					
2	71778761	7,2E+07	C	T	c.1663C>T	p.(Arg	19		30	0,94						
NM_003494.3:c.1663C>T p.(Arg555)			CA222133	2:71778761:C:T		2:71778761-C-1 2:71778761 C:T		2,71778761,C,T								
NM_001130987.2:c.1717C>T																
ACMG: Pathogenic				Allele count	allele number	nb of homozygote	Population	Allele count pop	allele number pop	freq pop						
				9	271416	0	South Asian	3	28874		0,002738					
				AutopVSI score:				score		Even though additional evidence of pathoger						
patient count	cases	Clinical Significance	Case Info	PMID heterozygote	PMID homozygote	other variant	Expression proteique (0 à 100%)	other.variant	PVS1	PM3 very	We have identified this variant in 8 patients in homozygous state from the associated in 3 cases with no change of Dsuffetin or Western Blot / no associated in 4 cases (PMID: 24522482, ) with a decrease of Dsuffetin					
LOVD									PM3 very		VARIANT =>					
				25591676		5	0	c.3220_3221del	PS1		PMID =>					
				24522482		5	5	c.2494C>T	PS2		PMID =>					
				27602406		3	0	c.737T>G	PS3		PMID =>					
					27602406		0		PM3 strong		NB Affected patient - NB Unaffected					
				32400077		5	NA	c.2997G>T	PP4 strong		PP1 strong					
	6 homozygotes LOVD			32400077		5	NA	c.4497del	PP1 strong		C2A doma C2G doma D					
ClinVar									PS2-moderate		PM1					
									PM2	PM2	It is present in only 9 individuals in gnomADv2					
									PM3		VARIANT =>					
									PM4		PMID =>					
PubMed									PM5		VARIANT =>					
									PM6		PMID =>					
									PP4 moderate		PP1 mode					
APHM	NB Homozygote	WB	NB Hetero	Expression proteique + other variant	score + other variant	NB Hetero	Expression proteique + other variant	score + other variant	PP1 supp	PP1 supp	This variant was shown to co-segregate w					
		1 <= Non rec		1	0	5	c.610C>T	1	NA	4	c.1157_1168del	PP3		PP3	It is predicted to affect protein function (CAD	
		2 <= 0		1	NA	5	c.3932C>T	1	0	2	c.509C>A	PP4 supp		PP4 supporting		
		<= diminué		1	0	3	c.342+5G>A	1	NA	4	c.4005+1G>A	PP3 supp				
Total:											PM3 very strong PM2 PP1 supporting PP3 PP4 support Pathogenic			BA1		
classer											4 PM3 very strong PM2 PP1 supporting PP3 PP4 supporting			BS1		
https://genome.ucsc.edu/cgi-bin/hgTracks?db=hg19&lastVirtModeType=default&lastVirtModeExtraState=&virtModeType=default&virtMode=0&nonVirtPosition=&positi												BS2	attention pénétrance e			

# 3 RESULTATS

## 3.1 Résultats généraux

Nous avons résumé les résultats de la reclassification dans la figure 10 (partie A et B) ci-dessous. A noter que les variants appartenant à la fois à la catégorie insertion/délétion et à la catégorie épissage ont été comptés comme des variants appartenant uniquement à la catégorie insertion/délétion.

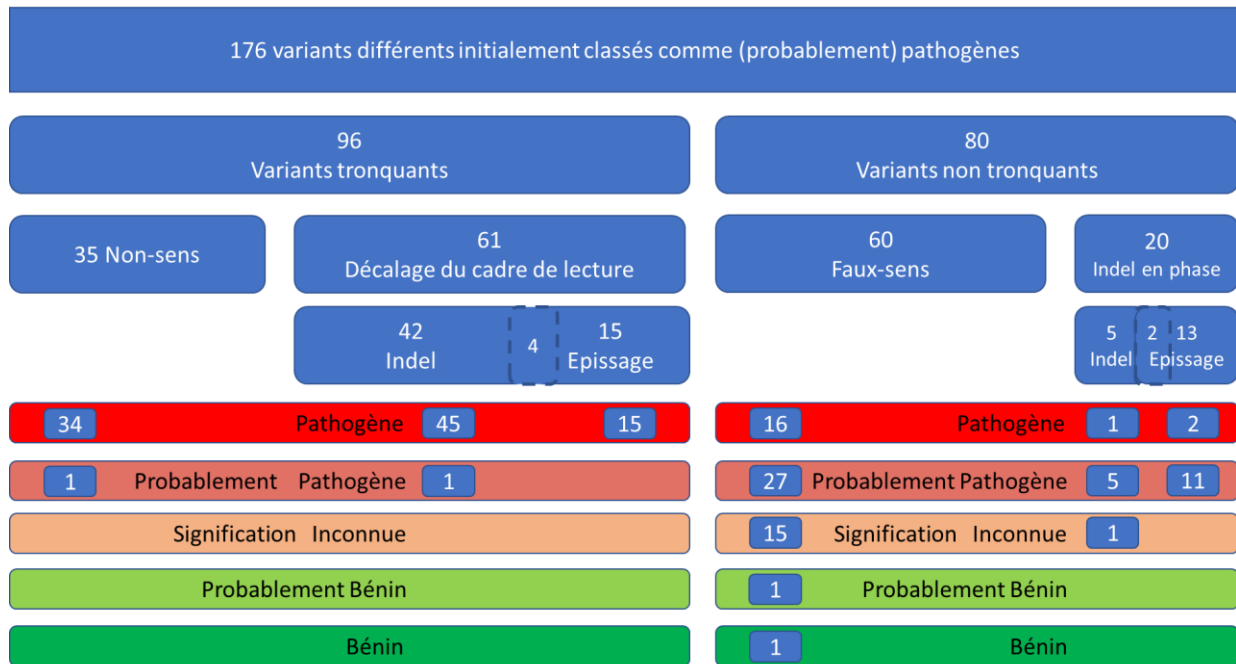
Figure 10 A : Nature des 176 variants reclassifiés



La grande majorité des variants a été classée avant que la classification en 5 classes de l'ACMG ne soit adoptée dans notre laboratoire (2016). La figure 10 A montre le nombre de variants reclassés en cinq classes de pathogénicité, regroupés par type de variant (35 variants non-sens, 53 indels, 28 variants affectant l'épissage et 60 variants faux-sens). Les variants non-sens et indels sont proportionnellement les plus nombreux à avoir été classés dans le groupe "pathogène" (respectivement 34/35 et 46/53). Sur 176 variants, la classification de la pathogénicité de 18 variants a été déclassée en "VUS" (Variants de signification inconnue), "probablement bénigne" ou "bénigne" (concordance globale avec la classification originale de

90 %). Le groupe de variants "Missense" (faux-sens) présentait la plus grande proportion de variants dont la pathogénicité a été déclassée (17 sur 60).

**Figure 10 B : Détail de la nature des 176 variants reclassifiés en fonction de l'effet tronquant ou non sur la protéine**



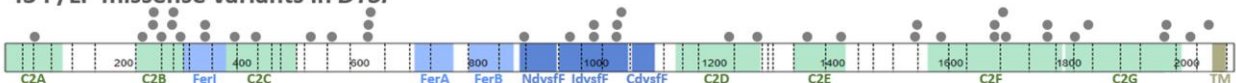
La figure 10 B met en évidence le fait que l'ensemble des 18 variants déclassés sont des variants sans effet tronquant sur la protéine (n'induisent pas de codon stop prématuré).

Dans notre cohorte 35 des 176 variants (dont 11 faux-sens) ne sont ni décrits dans Clinvar ni dans LOVD, ils seront donc soumis avec nos résultats dans LOVD.

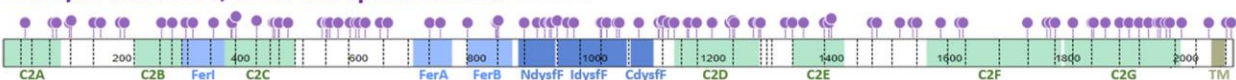
### 3.2 Distribution des variants au niveau de la protéine

**Figure 11: Distribution des variants sur la protéine dysferline (isoforme ENST00000258104.7)**

43 P/LP missense variants in *DYSF*



115 P/LP nonsense, indel and splice variants in *DYSF*



Quarante-trois variants faux-sens (missense) cliniquement pertinents (pathogènes et probablement pathogènes) identifiés dans la cohorte de Marseille sont présentés dans le panneau supérieur de la figure 11 (en gris). Cent quinze variants indel, non-sens, et d'épissage

cliniquement pertinents identifiés dans la cohorte de dysferlinopathies de Marseille sont présentés dans le panneau inférieur de la figure 11 (violet).

Les variants de perte de fonction (en violet) sont répartis de manière homogène sur la longueur de la protéine, tandis que les variants faux-sens (en gris) semblent avoir une répartition plus importante dans les deux domaines que sont C2B et DysF, avec chacun 7 des 43 variants faux-sens pathogènes ou probablement pathogènes de la cohorte.

### 3.3 Résultat de l'homogénéisation et de la standardisation des résultats

L'utilisation de macro VBA sur les tableurs Excel a permis à partir d'environ 10 cases renseignées pour chacun des 176 variants la génération du tableau de données contenant près de 23 000 mots justifiant l'attribution de chaque critère et sa classification finale (voir annexe supplementary data supplemental table 2). La figure 12 ci-dessous est un exemple du texte généré à partir du tableau d'un seul variant rempli dans la figure 9 et la standardisation de la mise en forme y est clairement visible.

Figure 12 : Synthèse extraite après utilisation de la macro sur les tableurs utilisés

<p>DYSF NM_003494.3:c.1663C&gt;T :p.(Arg555Trp) exon 19 (hg19)chr2:71778761 Canonical Allele Identifier: CA222133</p>	<p>We have identified this variant in 3 patients in homozygous state from the Timone Dysferlinopathy cohort, associated in 2 cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. We have identified c.1663C&gt;T in 6 patients (6 index) in heterozygous state (associated with pathogenic or likely pathogenic variants: c.610C&gt;T, c.3832C&gt;T (trans), c.1157_1168del, c.4005+1G&gt;A) from the Timone Dysferlinopathy cohort, associated in 3 cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. This variant has previously been reported in heterozygous state in 5 cases in the literature (associated with c.3220_3221del, c.2494C&gt;T, c.797T&gt;G, c.2997G&gt;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 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 gnomADv2.1.1 (maximum population 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 (Timone cohort) [PP1 supporting]. It is predicted to affect protein function (CADD = 30 (GRCh37 v1.6), REVEL = 0,94) [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.</p>	<p>ACMG: Pathogenic: PM3 very strong + PM2 + PP1 supporting + PP3 + PP4 supporting</p>
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## 3.4 Comparaison des résultats obtenus avec les nouveaux outils d'aide au diagnostic

### 3.4.1 *VarSome et attribution automatique des critères*

La comparaison de la classification globale avec VarSome met en évidence 25 variants avec des conclusions discordantes. Dans 3 cas la classification VarSome rend des variants que nous avons classés variants de signification inconnue comme bénins ou probablement bénins.

Dans les 22 autres cas le diagnostic en génétique moléculaire est grandement affecté, car pour 7 de ces cas nous n'avons pas réussi à obtenir suffisamment d'informations pour les classer autrement qu'en variants de signification indéterminée, et VarSome les classe en probablement pathogène. Ces 7 variants reçoivent un critère PP2 (mal attribué car il existe en réalité un grand nombre de variants faux-sens bénins et pathologiques décrits tout le long du gène), ainsi qu'un critère PM1, lui aussi écarté de notre classification (voir matériel et méthode).

Parmi ces 22 variants les 15 restants sont classés pathogènes ou probablement pathogènes dans ce travail, alors que VarSome les classe en variants de signification inconnue, malgré le fait qu'il continue d'attribuer un critère PP2 qui ne devrait pas l'être. La différence se fait principalement sur le critère PM3 qui est décisif en faveur de la pathogénicité.

### 3.4.2 *PVS1 et AutoPVS1*

AutoPVS1 a permis d'obtenir facilement la prédiction du critère PVS1 sans aucune erreur pour les 35 variants non-sens, et avec seulement 1 erreur sur 42 variants de type insertions/délétions entraînant un décalage du cadre de lecture. Concernant les variants d'épissage, en l'absence de méthode de référence nous pouvons juste observer que dans 6 cas (c.4639-2A>G, c.1284+1G>A, c.1284+2T>C, c.3349-1G>T, c.5341-2A>C, c.5430-15\_5431del) les prédictions étaient différentes de celles obtenues grâce à HSF (les résultats de HSF ayant été interprétés avec l'aide du Pr. Christophe Bérout).

## 4 DISCUSSION

### 4.1 Utilité de la reclassification

Depuis 2015 et la mise en place de la classification avec les critères ACMG, un certain nombre d'études ont décrit la réévaluation rétrospective des variants de diverses maladies génétiques. La réinterprétation des variants peut être abordée sous deux angles différents : celui des variants et celui des patients. Dans la première approche, les variants sont réinterprétés sur la base de données accessibles au public, telles que les informations sur les fréquences populationnelles ou les publications récentes. Des études à grande échelle de ce type peuvent être entreprises par n'importe quel groupe sans avoir besoin d'accéder aux informations relatives aux patients. Parmi les exemples récents de ces grandes études, on peut citer la reclassification des variants dans les gènes responsables d'épilepsies (66). En comparaison la reclassification basée sur le patient prend en compte les données du patient et des apparentés, telles que la ségrégation ou les données phénotypiques, et les combine avec les fréquences populationnelles des variants et les informations de la littérature mis à jour. Pour être bien réalisées ce genre d'études doit être idéalement conduites par le même groupe qui a publié les résultats de la cohorte originelle, ce qui explique qu'elles soient rarement réalisées. Dans le cadre des maladies rares ces études sont encore moins fréquentes (67–69). Dans notre étude nous avons réanalysé rétrospectivement les variants d'une grande cohorte de patients atteints de dysferlinopathies, permettant la reclassification de 18 d'entre eux.

Sur les 176 variants analysés lors de cette étude, 158 ont été retenus comme classés pathogènes ou probablement pathogènes après la réévaluation. Plusieurs aspects de cette étude peuvent expliquer cette concordance relativement faible (90%). Premièrement nous avons inclus dans l'analyse des variants identifiés comme pathogènes au tout début du diagnostic par séquençage du gène *DYSF* quand les connaissances au sujet de ce gène et ses variants étaient encore limitées. Même si certains de ces variants ont par la suite été réinterprétés comme bénins ou de signification indéterminée, nous pensons qu'il est important de reclasser formellement ces variants et de corriger la soumission de leur caractère pathogénique dans les bases de données publiques telle que LOVD. Deuxièmement les dysferlinopathies sont plus rares que beaucoup d'autres maladies héréditaires, ce qui complique l'interprétation des variants par leur très faible fréquence (étant présents chez seulement 1 à quelques patients dans la majorité des cas). En effet, une étude récente réinterprétant des variants de cancer héréditaire dans des gènes aussi fréquemment mutés que *BRCA1* avait 99,3% de concordance avec les variants pathogènes (70).

Troisièmement l'interprétation des variants entraînant une perte de fonction est plus simple que celle des variant faux-sens. Comme ceci est montré dans la figure 10, la plupart des discordances proviennent des variants faux-sens et aucun des 96 variants perte de fonction ne perd son caractère pathogène. Puisque le gène *DYSF* n'est pas un gène où seuls les variants perte de fonction sont connus pour être pathogènes, il a fallu les comptabiliser.

A noter que malgré le fait que certains variants tronquants ne soient pas soumis au phénomène de NMD, ils ont tous pu être classés comme pathogènes. Toutefois il convient de rester prudent dans ce genre de situations et la réévaluation de tels variants nous semble tout de même importante.

En étudiant la manière dont les résultats étaient rendus en 2008 il parait évident que les prédictions *in silico* avaient une place prépondérante dans le diagnostic final (100% de concordance avec les résultats obtenus de UMD Predictor), et que les données populationnelles étaient largement incomplètes. De nos jours la prédiction informatique permet uniquement d'attribuer PP3 comme critère de pathogénicité, soit la plus basse des classes de critères, ce qui rend minime son impact dans la classification finale.

Il y a beaucoup d'exemples de variants et de gènes faussement classés pathogènes dans la littérature et différentes bases de données (71–73). Et c'est uniquement en gardant un processus dynamique de réanalyse permanente qu'il est possible de s'assurer des résultats que l'on rend. Pourtant cette réévaluation des anciens variants publiés avant l'avènement des bases populationnelles exhaustives (Exac/GnomAD (60)) et de la classification ACMG est rarement réalisée. C'est pourquoi des processus de curation des bases de données, ainsi que l'établissement de critères gènes-spécifiques par des consortiums tels que ClinGen sont en cours. Toutefois ils ne pourront pas réaliser la curation de l'ensemble des bases de données à eux seuls, ce qui détermine l'importance de travaux tels que celui effectué dans cette thèse en collaboration avec les curateurs de bases de donnée pour maintenir l'ensemble des connaissances à jour. En résumé, notre nouvelle étude met en évidence plusieurs défis communs dans les diagnostics génomiques et attire l'attention sur le besoin critique de réévaluer l'interprétation des variants des cohortes anciennement publiées.

## **4.2 Critères de classification discriminants**

Le critère pour lequel la revue de la littérature a le plus d'intérêt est le critère PM3. C'est également le critère avec le plus de poids dans notre étude après PVS1. Toutefois son application se fait selon des règles strictes, dont l'une stipule que les deux variants que l'on

cherche à classer doivent être suffisamment rares (et donc satisfaire aux exigences du critère PM2) avant que le moindre point ne puisse être attribué. Ensuite le compte des points en fonction de la phase du second variant s'effectue facilement et permet de faire basculer les variants vers la pathologie et ce même avec assez peu de cas présentant le même variant. Toutefois dans plusieurs cas il aurait été intéressant de disposer de la phase de ces deux variants entre eux, en particulier dans les situations où l'on dispose de la seule occurrence rapportée dans la littérature, afin de pouvoir appliquer le critère PM3 sous sa forme modérée. Dans ce cadre là l'analyse des prélèvements parentaux prend toute son importance.

Le troisième critère le plus discriminant est le critère PS1, mais c'est également l'un des moins attribués. La technique disponible pour les variants d'épissage de minigène mise en place dans l'équipe Neuromyologie Translationnelle de Marseille Medical Genetic a permis de résoudre un certain nombre de cas où les critères n'étaient pas suffisants. Malgré l'apport de cette technique seule une cinquantaine de variants du gène *DYSF* ont été investigués (54). Pourtant avec les nouvelles recommandations du critère PVS1 l'exploration de l'impact de l'épissage prend tout son sens, et seul le séquençage de l'ARNm du patient sera plus informatif que l'analyse de modélisation par minigène (la construction du minigène pouvant ne pas être soumise aux mêmes mécanismes de régulation de l'épissage que *in vivo*).

### **4.3 Automatisation de l'information**

L'automatisation de ce travail sous différentes formes (macro VBA Excel, alignement sur les bases de données avec le logiciel R) a permis la réalisation de cette étude en un temps très restreint, et représente l'avenir du traitement de l'information. D'autre part l'adaptation de nos résultats à de futures recommandations sera grandement facilitée par l'utilisation de ces outils. Malgré tout une légère adaptation des textes de synthèse finale a été nécessaire (pour l'obtention de l'annexe supplementary data supplemental table S2), l'adaptation manuelle des cas particuliers étant moins chronophage que la création d'un code spécifique.

## **4.4 Interprétation des nouveaux outils d'aide au diagnostic**

### **4.4.1 VarSome : forces et limites**

VarSome est un outil à fort potentiel permettant d'agrèger de multiples informations pour l'attribution systématique et harmonisée de beaucoup de critères de la classification ACMG. Toutefois il faut en connaître ses limites afin d'obtenir rapidement uniquement les informations

fiables qu'il contient. En effet dans cette étude nous avons pu observer l'attribution, en particulier, du critère PM1 et du critère PP2 de manière erronée menant pour 7 variants à une surévaluation du caractère pathogène des variants pouvant mener à de faux diagnostics pour toute personne pas assez renseignée sur les caractéristiques du gène qu'elle étudie. De même la compétence du généticien moléculaire est primordiale puisqu'elle permet ici pour 15 variants de faire le diagnostic. Ces 22 variants représentent 12,5% des variants de notre cohorte, démontrant l'incapacité seule de cet outil à mener au diagnostic dans un domaine où les résultats peuvent avoir une implication capitale dans la prise en charge des patients.

#### ***4.4.2 AutoPVS1 : une approche intéressante***

AutoPVS1 est un outil intéressant, son interface permettant d'accéder facilement à la position du variant au sein des exons de la protéine et aux recommandations de Clingen spécifiques pour certains gènes, faisant varier l'attribution du critère PVS1. Toutefois il nécessite encore quelques mises au point avant de pouvoir être exploité sans arrière-pensée pour les variants entraînant un décalage du cadre de lecture, étant donné que pour c.6233\_6240del il prédit PVS1 alors que cette délétion concerne les 7 dernières bases codantes pour la protéine. Il faut prendre en compte que cela induit l'inclusion de 137 acides aminés supplémentaires, ce qui a très probablement un effet pathogène par un phénomène différent appelé Non Stop Decay (NSD). Toutefois les connaissances actuelles ne nous permettent pas de prédire avec certitude si un variant est soumis au NSD, et d'autre part les créateurs de AutoPVS1 ne font aucune mention de la prise en compte de ce phénomène. La prédiction manuelle reste donc plus fiable et donc nécessaire pour un critère aussi important dans la classification que PVS1. L'outil possède également un intérêt pour les variants d'épissage, mais nous pouvons lui reprocher de ne pas détailler à chaque fois sur quel argument la classification est effectuée. Cela ne permet pas de tester l'outil et d'obtenir un avis critique sur son mode de fonctionnement. Par exemple il nous est impossible de savoir s'il prend en compte la position vis-à-vis du point de branchement, ce qui fournit pourtant un argument fort concernant l'utilisation potentielle d'un site cryptique ou non canonique d'épissage. Un autre problème de cet algorithme est une signalisation d'erreur quand des variants trop longs, ou des variants de duplication en nomenclature HGVS, sont soumis (ce qui empêche de sélectionner une autre isoforme que celle de référence).

## 4.5 Limites et biais

Cette étude étant une étude rétrospective elle est soumise aux biais rétrospectifs, et pour certains patients des fractions de données (Western blot, liens de parenté...) n'ont pas pu être retrouvées. Afin de limiter au maximum ce biais, au moindre doute nous avons appliqué la situation apportant le moins de poids aux critères concernés.

L'étude de l'ensemble des variants a été effectuée sans connaître la fonctionnalité et la position des différents domaines afin d'éviter toute part de subjectivité lors de l'attribution des critères.

Nous acceptons la possibilité que les patients de la cohorte soient porteurs de pathologies liées à l'implication d'autres gènes. Toutefois, l'association des critères cliniques à une expression protéique de la dysferline grandement diminuée voire nulle permet de diminuer au maximum ce risque.

La classification des variants de la littérature utilisée pour classer nos variants a nécessité un temps important, et au vu du temps limité pour la réalisation de l'étude seuls les critères facilement obtenables ont été attribués, sous estimant souvent leur pouvoir pathogène en les classant en probablement pathogène au lieu de pathogène. Toutefois l'ensemble des variants de notre cohorte qui n'ont pas atteint la classe 5 (pathogène) ont vu l'ensemble des éléments pris en compte pour leur classification être approfondis de manière exhaustive (les variants de la littérature associés ayant tous alors été analysés dans leur intégrité afin de leur attribuer leur classe de pathogénicité maximale).

Les dernières recommandations de ClinGen concernant le critère PM2 (SVI Recommendation for Absence/Rarity (PM2) - Version 1.0) déclassent la force de ce critère en supporting au lieu de moderate. Puisque notre étude a débuté avant la parution de ces recommandations, et que leurs auteurs acceptent le fait que ce changement de poids du critère PM2 nécessite des ajustements complémentaires quand à l'interprétation globale de la pathogénicité des variants, nous avons décidé de ne pas appliquer ces recommandations dans le cadre de notre travail. Nous adapterons facilement le résultat de notre analyse quand l'ensemble des ajustements nécessaires aura été réalisé.

En définissant notre méthode de classification nous avons diminué le nombre de critères, ce qui implique dans de rares cas le manque d'un ou deux critères supporting pour passer d'un variant de signification inconnue à un variant probablement pathogène. Nous avons accepté ce risque mesuré de faux négatifs car il permet en contrepartie d'obtenir un risque de faux positifs proche de zéro.



## 4.6 Variants particuliers

Malgré le fait que pour appliquer le critère PM3 les variants aient du être définis comme suffisamment rares, nous avons défini une exception concernant le variant c.2643+1G>A (avec une Popmax Filtering AF d'environ 1/1000) pour les raisons suivantes : il était décrit chez 13 patients différents de notre cohorte, dont 8 avec une forme homozygote ; en l'absence du critère PM3 il aurait tout de même été classé comme probablement pathogène ; il est décrit de multiples occurrences de ce variant dans la littérature ; et il est associé à plus de 14 variants sous formes hétérozygotes composite ce qui représente près de 8% de notre cohorte. L'exception a été présentée puis discutée en staff avant d'être acceptée.

Les autres variants ayant une fréquence au-delà du critère PM2 ne possédant les mêmes arguments diagnostiques, ils ont tous été soumis à un comité d'experts afin de juger du cas de chaque variant.

Comme décrit ci-dessus, l'association en *trans* à un variant est l'une des informations les plus importantes. Toutefois nous allons décrire l'importance de pouvoir distinguer l'association en *cis* de deux variants, de par la description de 3 paires de variants suspectées d'être en déséquilibre de liaison :

- Association des variants c.2643+1G>A et c.4577A>C : ces variants ont été décrits 7 fois avec preuve de ségrégation en *cis* (dont 4 fois sous forme homozygote). Le variant c.4577A>C n'a jamais été décrit sans la présence simultanée du variant c.2643+1G>A dans notre cohorte. Les données populationnelles de ces deux variants sont relativement proches. Dans la littérature c.4577A>C a été décrit 2 fois comme pathogène, une fois homozygote par Krahn et al. 2008 et l'autre fois hétérozygote associé à c.2643+1G>A par Rosales et al. 2010 (74). Au vu des résultats en notre possession aujourd'hui il n'y a pas de doute sur l'association en déséquilibre de liaison de ces deux variants et le critère BP2 alors donc attribué à c.4577A>C (variant observé en *cis* avec un variant pathologique dans le cadre d'une maladie récessive).
- Association des variants c.1877T>C et c.896G>A : ces variants ont été décrits chez deux cas index en *cis* dans notre cohorte et une fois à l'état hétérozygote par Harris et al. 2016 (75). En l'absence d'informations sur la phase dans la littérature et

comme les fréquences populationnelles des deux variants sont différentes nous ne pouvons pas affirmer notre suspicion de coségrégation pour ces deux variants.

- Association des variants c.1168G>A et c.5302C>T : ces variants ont été décrits chez 3 patients (dont deux cas index), avec des fréquences populationnelles basses pour tous les deux mais différentes l'une de l'autre. Le variant c.1168G>A est décrit comme hétérozygote composite avec d'autres variants sans association avec c.5302C>T, de même c.5302C>T est décrit comme hétérozygote composite avec d'autres variants sans association avec c.1168G>A. Nous ne pouvons alors ni affirmer ni infirmer la présence d'une coségrégation.

Aujourd'hui pour ces deux derniers cas, un doute existe sur la possibilité qu'ils soient associés en cis, et donc que le diagnostic soit faussement établi chez ces 5 patients.

# CONCLUSION

En conclusion notre étude a consisté en l'évaluation de l'impact des recommandations actuelles pour la reclassification de variants de séquence du gène *DYSF* considérés comme pathogènes au moment du rendu de résultat initial, et constitue l'une des premières études de ce genre pour les maladies autosomiques récessives, et la première dans le domaine des maladies neuromusculaires.

Nous avons pu mettre en valeur l'utilité de la reclassification des variants sans perte de fonction, et tout particulièrement des variants faux-sens. L'analyse des variants perte de fonction non soumis au phénomène de NMD (situé après les 50 bases en amont de la dernière jonction exon-exon) nous semble également prioritaire, bien que nous n'ayons reclassé aucun variant de ce type comme non pathogène.

La phase des variants entre eux est primordiale pour la classification, autant dans le but de faire basculer du côté pathogène le classement du variant lors d'occurrences en trans, que de faire basculer du côté bénin le classement lors d'occurrences en cis. Les tests fonctionnels peuvent permettre de pallier au manque de critères des variants rares sans perte de fonction.

Malgré que le nombre de variants faux-sens n'ait pas été suffisant pour la détermination de hotspots mutationnels, le domaine C2B de la protéine dysferline est le seul à présenter 7 variants faux-sens pathogènes répartis régulièrement sur l'ensemble de sa séquence, ce qui pourrait être lié à son rôle dans la dimérisation de la protéine. Avec une répartition plus hétérogène, les 7 variants faux-sens pathogènes du domaine DysF semblent compléter les observations de Izumi et al. 2020 (39) dans le sens d'un hotspot mutationnel de ce domaine.

Les outils automatisés d'aide au diagnostic que sont VarSome et AutoPVS1, et les résultats qu'ils fournissent, doivent absolument être interprétés avec une grande prudence. En aucun cas ils ne peuvent se substituer au recueil d'informations concernant les règles d'interprétation des variants pour chaque gène. Malgré l'aide importante que peut apporter le développement récent d'outils automatisés intégrant les recommandations internationales de classification de variants, notamment pour réduire le temps de classification en situation de nombre élevé de variants générés par les techniques de NGS, l'expertise thématique de génétique moléculaire reste essentielle pour assurer la qualité du rendu de résultat.

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# Annexes

## Preuve de soumission

En remerciant le Dr. Svetlana Gorokhova pour ce travail de soumission

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**Envoyé :** mercredi 25 novembre 2020 00:02

**À :** GOROKHOVA Svetlana

**Objet :** GIM Submission Confirmation for Retrospective analysis and reclassification of DYSF variants in a large French series of dysferlinopathy patients.

24 Nov 2020

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## Charnay et al. à paraître

### Retrospective analysis and reclassification of *DYSF* variants in a large French series of dysferlinopathy patients.

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## **ABSTRACT**

Recent evolution of sequencing technologies and the development of international standards in variant interpretation have profoundly changed the diagnostic approaches in clinical genetics. As a consequence, many variants that were initially claimed to be disease-causing have been reclassified in light of the new data available. Unfortunately, the misclassified variants are still present in the scientific literature and variant databases, greatly interfering with interpretation of diagnostic sequencing results. Despite the urgent need, large-scale efforts to update the classifications of these variants are still not sufficient. We have retrospectively analyzed 176 *DYSF* gene variants that were identified in dysferlinopathy patients referred to the Marseille Medical Genetics Department for diagnostic sequencing since 2001. Since the majority of these variants were returned before implementation of ACMG classifications, we re-classified them into five-tier pathogenicity classes, leading to downgraded pathogenicity for 18 variants. We then updated the information for the variants that have been previously published<sup>4-6</sup> and submitted to variant databases. We also submitted 40 novel *DYSF* variants to a variant database (LOVD). Besides direct benefit for dysferlinopathy diagnostics, our study contributes to the much needed effort to re-analyze variants from previously published cohorts and to work with curators of variant databases to update the entries for erroneously classified variants.

**Key Words:** *DYSF*, dysferlin, LGMDR2, LGMD2B, Miyoshi muscular dystrophy, neuromuscular disease,

variant classification, ACMG criteria, re-classification



## INTRODUCTION

Dysferlinopathies are a group of autosomal recessive muscular dystrophies caused by mutations in the gene encoding dysferlin (*DYSF*; MIM# 603009, 2p13, NM\_003494.4)<sup>1,2</sup>. The two most common clinical presentations of dysferlinopathies are Autosomal Recessive Limb-Girdle Muscular Dystrophy 2 (LGMDR2; previously LGMD2B, MIM# 253601) and Miyoshi muscular dystrophy 1 (MM; MIM# 254130)<sup>3</sup>. Since description of the first pathogenic *DYSF* variants in 1998<sup>1,2</sup>, more than 600 unique pathogenic or likely pathogenic variants have been reported (664 in LOVD (accessed November 20, 2020) and 318 variants in ClinVar (version July 7, 2020)). A significant number of these variants were identified in our diagnostic center in the Medical Genetics Department at the Timone Hospital (Marseille, France), since our laboratory was one of the first centers in Europe to offer diagnostic sequencing of all 55 exons of the *DYSF* gene<sup>4-6</sup>.

Recent evolution of sequencing technologies and bioinformatic tools has allowed accumulation of unprecedented amount of genetic data, leading to development of large databases with variants from the general population as well as rapid expansion of databases with clinically relevant variants identified in patients with genetic diseases. These advances profoundly changed the ways variant pathogenicity is established and led to reclassification of multiple variants identified during prior diagnostic sequencing. For example, a number of variants that were previously considered pathogenic were subsequently identified in the general population at frequencies that were not compatible with pathogenicity, leading to reclassification of these variants as benign<sup>7-9</sup>. The dramatic expansion of clinical sequencing also created a critical need for standardization of variant pathogenicity interpretation in diagnostic settings, leading to the development of a new variant classification system by the ACMG (American College of Medical Genetics)<sup>10</sup>. The evolution of the variant interpretation standards led to many discrepancies between the pathogenicity attributed to newly identified variants and the pathogenicity classifications of “legacy” variants identified in the earlier days of genetic sequencing. Presence of these misclassified variants in the literature and the variant databases greatly interferes with variant interpretation in the current diagnostic setting. In order to solve this problem for the *DYSF* gene, we have retrospectively evaluated the pathogenicity of 176 unique variants identified in dysferlinopathy patients referred to our center for diagnostic sequencing and updated the classification of these variants in public database. Our results highlight the importance of variant re-classification and join the first steps of the international effort to review and reanalyze the pathogenicity classifications of variants present in the literature and public databases.

## MATERIALS AND METHODS

### Selection of *DYSF* variants for re-classification.

The variants described in this study were identified in patients that were referred for sequencing of the *DYSF* gene in our diagnostic laboratory at the Timone Children’s Hospital (Marseille, France) since 2001. The referred patients had clinical presentations highly suggestive of a primary dysferlinopathy, often with dysferlin protein deficiency identified on muscle biopsy samples (by immunohistochemistry and/or Western-blotting). The diagnostic approaches used during these past 20 years included SSCP (Single-Strand Conformation Polymorphism) and DHPLC (Denaturing High Performance Liquid Chromatography) with subsequent direct sequencing of identified abnormal fragments, MLPA (Multiplex Ligation-Dependent Probe Amplification), Q-PCR (Quantitative Polymerase Chain Reaction) and recently a gene panel. The variants identified in dysferlinopathy patients diagnosed at our center were described in several reports<sup>4-6</sup> and were the basis for the UMD-DYSF database<sup>11</sup>. Since 2001, 572 patients

and their family members underwent diagnostic sequencing of *DYSF* gene in our laboratory (Figure 1). Out of 464 suspected dysferlinopathy patients and family members referred by French neurologists, 242 patients (191 probands) carried two *DYSF* variants that were considered pathogenic at the moment of diagnosis. As more information about pathogenicity of *DYSF* variants became available over the years, classification of certain variants has been modified. In this study, we analyzed all 176 unique *DYSF* variants, including those that were at one point returned as pathogenic but were then considered as benign in subsequent diagnostic returns.

#### 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 criteria<sup>10</sup> 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. Forty novel variants were submitted to DYSF-LOVD. We also corrected the outdated classifications of variants in our set.

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 ProteinPaint<sup>12</sup> (<https://pecan.stjude.cloud/proteinpaint>), using the domain positions described by Sula *et al.*<sup>13</sup>.

## RESULTS

In order to address the critical issue of outdated pathogenicity classifications in public databases, we performed a retrospective analysis of 176 *DYSF* variants identified in the large number of patients referred to our center for diagnostic sequencing of the *DYSF* gene since 2001. Our laboratory was one of the first diagnostic centers in Europe to offer analysis of all 55 exons of the *DYSF* gene, providing this diagnostic test to 572 suspected dysferlinopathy patients and their family members over the last 20 years. We focused on families referred for sequencing by French neurologists and curated 176 unique *DYSF* variants identified in 244 patients (191 index cases) carrying two *DYSF* variants that were considered pathogenic at the moment of diagnosis (Figure 1A). It is important to note that certain variants were initially reported pathogenic, but later were re-considered as benign when more information about population frequencies or *in trans/cis* occurrences became available. We decided to include this type of variants in our analysis, since certain of them are still listed as pathogenic in public databases and could potentially influence clinical interpretation of these variants in the future. The variants curated in this study are listed in the Supplementary Table S1, showing each variant's location, type and the final pathogenicity group as well as the concordance of our classification with classifications in ClinVar and LOVD databases. Supplementary Table S2 provides the detailed information about the lines of evidence and the ACMG codes used to classify each variant.

The vast majority of variants in our study were returned before the five-tier ACMG classification was adopted in our laboratory (2016). Figure 1B shows the number of variants reclassified in five ACMG pathogenicity classes, grouped by type of the variant (35 nonsense variants, 53 indels, 28 variants affecting splicing and 60 missense variants). Nonsense and indel variants had the largest proportion of variants assigned to the “pathogenic” group (34/35 and

46/53 respectively). Out of 176 variants, pathogenicity classification of 18 variants was downgraded to “VUS” (Variant of Uncertain Significance), “likely benign” or “benign” (overall concordance with original classification of 90%). The missense variant group had the largest proportion of variants with downgraded pathogenicity (17 out of 60). LOVD entries for all variants with change in classification were updated.

Variant locations along the *DYSF* protein and its functional domains are shown in Figure 2. Forty-three clinically relevant (pathogenic and likely pathogenic) missense variants identified in patients referred to the Marseille center for *DYSF* sequencing are shown in the top panel (gray). One hundred and fifteen clinically relevant indel, nonsense, splice variants are shown in the lower panel (purple). Loss of function variants (purple) are distributed homogeneously along the protein length, while missense variants (gray) are more often found in certain domains, such as C2B.

Forty variants identified in dysferlinopathy patients in our center were novel, as they were not present neither in LOVD nor in ClinVar. We submitted these variants in LOVD (submission IDs pending).

## DISCUSSION

Since the ACMG classification system became widely accepted, a certain number of studies have described retrospective re-analysis of variants in various genetic conditions. Reinterpretation of variants can be approached from two perspectives – variant-based and patient-based. In the first approach, variants are re-interpreted based on publicly available data, such as population frequency or recent publications. Large-scale studies of this type can be undertaken by any group without need to access patient-related information<sup>14,15</sup>. In comparison, patient-based variant reclassification takes into account patient and family related data, such as segregation and phenotypic data, in addition to the updated variant frequency and literature information. This reclassification has to be done by the same group that published the original cohort, thus making this type of studies more difficult to perform. Studies undertaking patient-based re-interpretation of variants identified in rare disease patient cohorts are still not frequent<sup>16-18</sup>. In our study we retrospectively analyzed variants from a large series of dysferlinopathy patients, allowing reclassification of 18 variants.

Out of 176 variants analyzed in this study, 158 variants retained “pathogenic” or “likely pathogenic” classification after the re-evaluation. Several aspects of our study explain this relatively low concordance (90%). First, we included in the analysis certain variants that were identified as pathogenic at the very beginning of diagnostic sequencing of *DYSF* when the knowledge about this gene and its variants was still very limited. Even though these variants were later re-interpreted as benign or uncertain and were no longer returned to patients as pathogenic, we thought that it was important to formally reclassify these variants and to correct the “pathogenic” classification in public databases such as LOVD. Second, earlier pathogenicity classifications were attributing much higher weight to predictions by *in silico* algorithms, in particular for the interpretation of missense variants. In the current ACMG classifications, pathogenicity predictions are used as only a supporting line of evidence (evidence code PP3)<sup>10</sup>. Third, dysferlinopathies are much more rare than many other inherited disorders, making it difficult to interpret pathogenicity of a variant found in only one or several patients. Indeed, a recent study reinterpreting hereditary cancer variants in such frequently mutated genes as *BRCA1* had a higher concordance for pathogenic variants (99,3%)<sup>19</sup>. Fourth, interpretation of loss-of-function variants is much more straightforward than that of missense variants. As seen from the Figures 1B and S1, most of discordance came from missense variants. Indeed, no protein truncating variants had downgraded classifications, while 17 missense variant classifications were downgraded to “VUS”, “likely benign” or “benign”. Since

missense variants in the *DYSF* gene are a common cause of dysferlinopathy, the concordance between reinterpretations of *DYSF* variant pathogenicity is expected to be lower comparing to the concordance between reinterpretations of pathogenic variants in a gene where only loss-of-function variants cause disease.

Despite recent advances aimed to standardize the classification of variants identified by diagnostic sequencing, there are still many examples of erroneous pathogenicity classification in scientific literature and public databases of pathogenic variants<sup>7-9</sup>. International curation efforts, such as ClinGen, are currently underway to establish gene-specific interpretation guidelines and to curate a certain number of variants<sup>20</sup>. However, these efforts are not likely to correct all of the outdated variant classifications that are currently found in the variant databases and the literature. Our study retrospectively analyzes *DYSF* variants identified, published and submitted to variant databases during 20 years of diagnostic sequencing at the Medical Genetics Department in Marseille, France<sup>4-6</sup>. Besides direct benefit for diagnostics of dysferlinopathies, our study is an example of much needed effort to re-evaluate variant interpretations in the previously published cohorts and to work with curators of variant databases to update the information for the erroneously classified variants.

#### Data Availability Statement

The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files. Forty novel variants were submitted to LOVD-DYSF database ([www.lovd.nl/DYSF](http://www.lovd.nl/DYSF)): Individual IDs pending.

#### Acknowledgements

We thank the neurologists in France and beyond for referring patients to our diagnostic center. We also thank the entire team of the Laboratory of Molecular Genetics at the Timone Hospital, Marseille, for their assistance in establishing the diagnostic sequencing of the *DYSF* gene as well as for their help with this analysis over the years. We thank Christophe Bérout for help with analysis of variants with a putative impact on splicing. This study was supported by GIPTIS (Genetics Institute for Patients, Therapies Innovation & Science), AFM-TELETHON and AP-HM (Assistance Publique - Hôpitaux de Marseille).

#### Conflict of Interest Statement

The authors declare that they have no competing financial or non-financial interests.

#### Ethics Statement

The data on *DYSF* variants do not include any identifying information. This data corresponds to retrospective analysis that does not require ethics committee approval at our institution. The appropriate consent was collected from each patient at the moment of variant identification as part of the standard procedure during a diagnostic genetic testing in our laboratory.

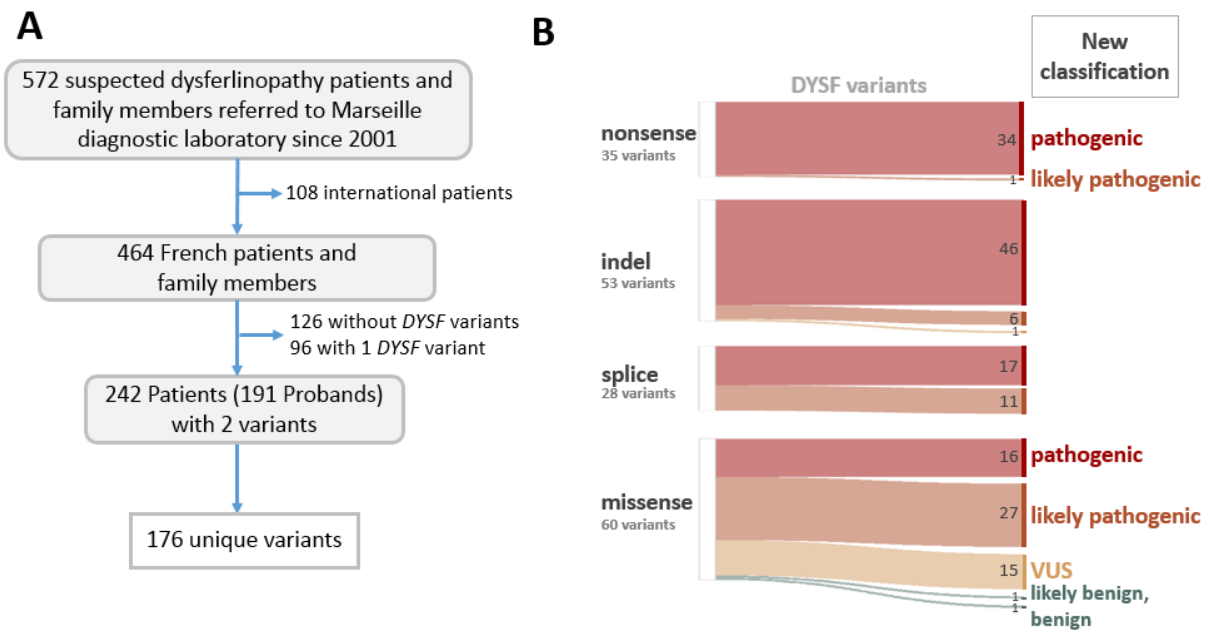
#### Author contributions

T.C. had a major role in the analysis of data and participated in writing of the manuscript; V.B. and C.P. participated in data acquisition; M.B., M.C., F.R., N.B.P., K.N., and N.L. revised the manuscript for content; M.K. and S.G. had major roles in the conception of the study, data interpretation and manuscript writing.

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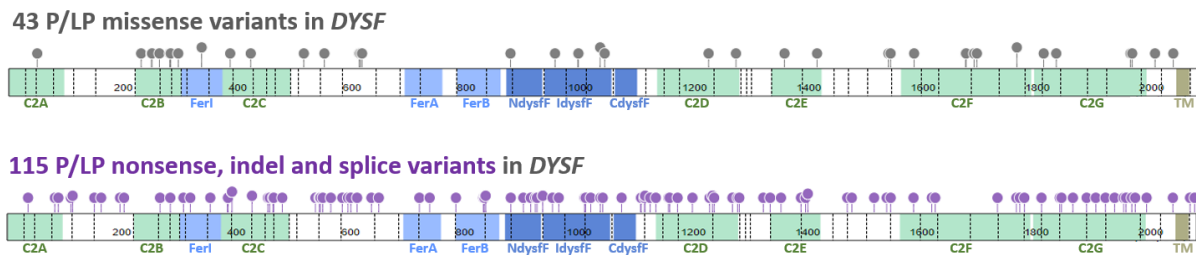
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**Figure 1: Selection and re-classification of 176 unique *DYSF* variants identified in dysferlinopathy patients referred for sequencing to the Medical Genetics Department, Marseille.**

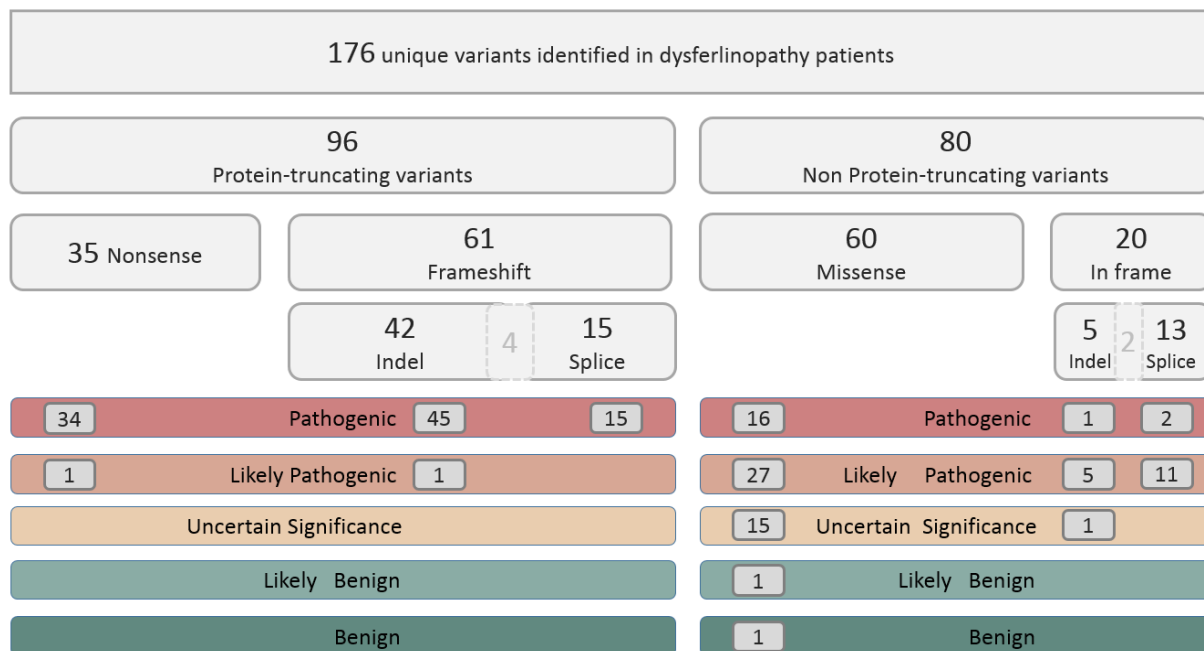
**A.** The variants described in this study were identified in patients that were referred for sequencing of the *DYSF* gene in our diagnostic laboratory at the Timone Children’s Hospital (Marseille, France) since 2001. The patients had clinical presentations highly suggestive of a primary dysferlinopathy, often with dysferlin protein deficiency identified on muscle biopsy samples (by immunohistochemistry and/or Western-blotting). The variants identified in our center were described in several reports<sup>4–6</sup> and were the basis for the UMD-*DYSF* database<sup>11</sup>. Since 2011, 572 patients and their family members underwent diagnostic sequencing of *DYSF* gene in our laboratory. Out of 464 suspected dysferlinopathy patients and family members referred by French neurologists, 242 patients (191 probands) carried two *DYSF* variants that were considered pathogenic at the moment of diagnosis. In this study, we analyzed all 176 unique *DYSF* variants, including those that were at one point returned as pathogenic but were then considered as benign in subsequent diagnostic returns. **B.** The vast majority of 176 variants were returned before the five-tier ACMG classification system was adopted in our laboratory. Left side of the panel shows the four types of variants analyzed - 35 nonsense variants, 53 indels, 28 variants affecting splicing and 60 missense variants. The variants shown in this figure include those that were at one point returned as pathogenic but were then considered as benign in subsequent diagnostic returns. The right side of the panel shows the numbers of variants re-classified into five ACMG pathogenic classes (“Pathogenic”, “Likely Pathogenic”, “VUS”, “Likely Benign” and “Benign”), grouped by variant type. Nonsense and indel variants had the largest proportion of variants assigned to “pathogenic” group (34/35 and 46/53 respectively). Out of 176 variants, pathogenicity classification of 18 variants was downgraded to “VUS” (Variant of Uncertain Significance), “likely benign” or “benign” (overall concordance with original classification of 90%). Missense variant group had the largest proportion of variants with downgraded pathogenicity (17 out of 60). 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.





**Figure 2: Distribution of Pathogenic and Likely pathogenic variants along the dysferlin protein.**

Forty-three clinically relevant (pathogenic and likely pathogenic) missense variants identified in dysferlinopathy patients referred for sequencing to the Department of Medical Genetics in Marseille are shown in the top panel (gray). One hundred and fifteen clinically relevant indel, nonsense, splice variants identified in this set of samples are shown in the lower panel (purple). Exon-intron junctions of the *DYSF* gene as well as the functional domains of dysferlin are visualized. The dysferlin domain nomenclature is shown according to Sula *et al.*<sup>13</sup>. 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.



**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.**

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 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 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 criteria (see the Supplementary Methods for details) is shown in the “Evidence for each variant” column. 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.

## Charnay et al. Supplementary data

### Supplementary methods:

We applied the PVS1 criteria according to ClinGen Sequence Variant Interpretation (SVI) recommendations for Interpreting the Loss of Function PVS1 ACMG/AMP Variant Criteria<sup>1</sup>. When attributing PVS1 to splice variants, presence of cryptic splice sites around the variant was explored using Human Splicing Finder<sup>2</sup> (<https://www.genomnis.com/access-hsf>). PS3 score was assigned for variants with a minigene result “Impact on splicing” in Kergoulay *et al.*<sup>3</sup>. We applied PP3 score (*in silico* prediction of pathogenicity) if CADD or REVEL score were above 25 and 0,7 respectively. REVEL scores<sup>4</sup> were obtained from <https://sites.google.com/site/revelgenomics/downloads> and CADD scores<sup>5</sup> from <https://cadd.gs.washington.edu/snv>, using GRCh37-V1.6 model. HSF<sup>2</sup> and MaxENT<sup>6</sup> were used to assign PP3 for non-canonical splicing variants using the default thresholds. 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](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 disorders<sup>7</sup>. The thresholds for the allele frequency criteria were as following: PM2 - 0.02%, BS1 - 0.5%, BA1 - 5%. 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 variants while moderate strength code (PP4\_mod) was assigned when Dysferlin protein was completely absent in muscle tissues (as detected by Western Blot or Immuno-Histo-Chemistry). 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 Groups<sup>8</sup>.

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**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	Protein	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.(Lys36SerfsTer12)	ex2	indel	-	P/LP	pathogenic
chr2:71709018	c.154T>C	p.(Trp52Arg)	ex3	missense	-	P/LP	likely pathogenic
chr2:71730354	c.247del	p.(Glu83LysfsTer)	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	-	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.(Phe267LeufsTer5)	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.(Ile308MetfsTer22)	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.(Lys355ArgfsTer4)	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	-	-	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	-	-	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	VUS
chr2:71762412	c.1368C>A	p.(Cys456Ter)	ex15	nonsense	P/LP	P/LP	pathogenic
chr2:71762419	c.1375dup	p.(Met459AsnfsTer15)	ex15	indel	P/LP	P/LP	pathogenic
chr2:71762436	c.1392dup	p.(Asp465ArgfsTer9)	ex15	indel	P/LP	P/LP	pathogenic
chr2:71762442	c.1397+1G>C	-	int15	splice	-	-	pathogenic
chr2:71766329_71766332	c.1440_1443del	p.(Ser481CysfsTer11)	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.(Glu548GlyfsTer22)	ex19	indel	P/LP	P/LP	pathogenic
chr2:71778753_71778766	c.1655_1668del	p.(Tyr552SerfsTer13)	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.(Ala601ThrfsTer28)	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.(Leu650TyrfsTer6)	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.(Thr833SerfsTer3)	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.(Lys903GlnfsTer4)	ex26	indel	P/LP	P/LP	pathogenic
chr2:71795405_71795425	c.2747_2767delinsTCCA	p.(Asp916ValfsTer20)	ex26	indel	-	-	pathogenic
chr2:71795430	c.2772G>A	p.(Trp924Ter)	ex26	nonsense	P/LP	P/LP	pathogenic
chr2:71795437	c.2779del	p.(Ala927LeufsTer21)	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.(Phe954ValfsTer2)	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.(Glu1021GlyfsTer11)	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	-	pathogenic
chr2:71801378	c.3225del	p.(Phe1075LeufsTer45)	ex30	indel	-	P/LP	pathogenic
chr2:71801439_71801444	c.3286_3291dup	p.(Arg1097_Met1098insArgArg)	ex30	indel	-	-	VUS

chr2:71801474_71801475	c.3321_3322delinsC	p.(Ala1108LeufsTer12)	ex30	indel	-	-	pathogenic
chr2:71801474_71801477	c.3321_3324dup	p.(Val1109SerfsTer6)	ex30	indel	-	P/LP	pathogenic
chr2:71801496	c.3343dup	p.(Ala1115GlyfsTer8)	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.(Glu1125LysfsTer9)	ex31	indel	-	P/LP	pathogenic
chr2:71816763_71816773	c.3389_3399dup	p.(Phe1135ProfsTer3)	ex31	indel	-	P/LP	pathogenic
chr2:71817369	c.3471C>A	p.(Tyr1157Ter)	ex32	nonsense	-	-	pathogenic
chr2:71817375	c.3477C>A	p.(Tyr1159Ter)	ex32	nonsense	-	-	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.(Asp1237ThrfsTer24)	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.(Ser1354ProfsTer6)	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.(Ile1401SerfsTer47)	ex39	indel	-	P/LP	pathogenic
chr2:71839803	c.4200dup	p.(Ile1401HisfsTer8)	ex39	indel	P/LP	P/LP	pathogenic
chr2:71839804	c.4201dup	p.(Ile1401AsnfsTer8)	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.(Gly1517AlafsTer25)	ex42	indel	-	-	pathogenic
chr2:71883359	c.4577A>C	p.(Lys1526Thr)	ex42	missense	Uncertain/conflicting	Uncertain/conflicting	VUS
chr2:71883398_71883401	c.4616_4619del	p.(Pro1539LeufsTer2)	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.(Glu1624AspfsTer10)	ex44	indel	P/LP	-	pathogenic
chr2:71891526	c.5015C>T	p.(Ser1672Phe)	ex45	missense	-	-	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.(Ser1772ProfsTer54)	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	-	-	likely pathogenic
chr2:71896742	c.5533del	p.(Ile1845LeufsTer121)	ex50	indel	P/LP	-	pathogenic
chr2:71896803	c.5594del	p.(Gly1865AlafsTer101)	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.(Ser1958ProfsTer3)	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_5921insCCCTGTGTGGCACTGTGTAGGAG	p.(Glu1974AlafsTer7)	ex52	indel	-	-	pathogenic
chr2:71908163	c.5979dup	p.(Glu1994ArgfsTer3)	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.(Pro2078LeufsTer?)	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 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 classification of *DYSF* variants identified in dysferlinopathy patients referred to the Medical Genetics Department of Marseille.**

Variant	Evidence for each variant	ACMG 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 1 patient in a homozygous state from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [PM2] (CADD = 22,7 (v1.6), REVEL = 0,479).	Uncertain significance: PM2 + PP4 supporting + PM3 supporting
DYSF NM_003494.3:c.107_108del p.(Lys36SerfsTer12) exon 2 (hg19)chr2:71708031_71708032del Canonical Allele Identifier: CA222121	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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.1758C>G, c.3389_3399dup (trans)) from the Marseille Dysferlinopathy cohort, associated in 1 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomADv2.1.1 [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.154T>C p.(Trp52Arg) exon 3 (hg19)chr2:71709018 Canonical Allele Identifier: CA347216252	We have identified c.154T>C in 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.1655_1668del) from the Marseille Dysferlinopathy cohort. 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 gnomADv2.1.1 [PM2]. It is predicted to affect protein function (CADD = 28 (v1.6), REVEL = 0,736) [PP3].	Likely pathogenic: PM2 + PP4 moderate + PP3 + PM3 supporting
DYSF NM_003494.3:c.247del p.(Glu83LysfsTer) exon 4 (hg19)chr2:71730354del Canonical Allele Identifier: CA1139532912	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 index) in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.6124C>T) from the Marseille Dysferlinopathy cohort. [PP4 supporting]. It is absent from gnomADv2.1.1 [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
DYSF NM_003494.3:c.251C>T p.(Ala84Val) exon 4 (hg19)chr2:71730358 Canonical Allele Identifier: CA1705297	We have identified c.251C>T in 2 patients (2 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.5594del and c.3113G>A) from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is present in only 5 individuals in gnomADv2.1.1 (maximum population frequency: 0,0011% 1/24932 in African population) [PM2] (CADD = 23,1 (v1.6), REVEL = 0,186).	Uncertain significance: PM2 + PP4 supporting + PM3 supporting
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 from the Marseille Dysferlinopathy cohort. We have identified c.265C>T in 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.3126G>A) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is present in only 1 individual in gnomADv2.1.1 (maximum population 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 from the Marseille Dysferlinopathy cohort, 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 or likely pathogenic variant: c.2643+1G>A) from the Marseille Dysferlinopathy cohort [PM3, PP4 moderate]. It is absent from gnomADv2.1.1 [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 index) in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.3516-3517del) from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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 or likely pathogenic variant: c.1663C>T (trans)) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomADv2.1.1 [PM2]. It is predicted to affect protein function (Alteration of the wild type donor site, most probably affecting splicing HSF MaxEnt) [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 from the Marseille Dysferlinopathy cohort, associated in 1 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.3703-1G>A ) from the Marseille Dysferlinopathy cohort, associated in these 2 cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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.565C>G p.(Leu189Val) exon 6 (hg19)chr2:71740953 Canonical Allele Identifier: CA147767	We have identified c.565C>G in 3 patients (3 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.5296G>T homozygous, c.2643+1G>A and c.6029A>G, c.2643+1G>A and c.6017G>A) from the Marseille Dysferlinopathy cohort, associated in these 3 cases with an absence of	Benign: PP4 supporting + BA1

	Dysferlin on Western Blot / ImmunoHistoChemistry. This variant has previously been reported in a heterozygous state in 2 cases in the literature (associated with c.855+4T>C, c.3516_3517del, PMID: 26088049, 17562833). [PP4 supporting]. A minigene assay demonstrating a variant has abnormal dysferlin function (Creation of a cryptic 5' splice site, resulting in 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) [PS3] (CADD = 19,04 (v1.6), REVEL = 0,182). Maximal population frequency in gnomAD v2.1.1 is 6,5643% (1712/24936 in African population, and 66 homozygotes [BA1].	
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 or likely pathogenic variant: c.1155_c.1180+2del) from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.855+1del, c.1663C>T) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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 1 patient in a heterozygous state (not associated with a pathogenic or likely pathogenic variant) from the Marseille Dysferlinopathy cohort. It is absent from gnomADv2.1.1 [PM2]. It is predicted to affect protein function (CADD = 27 (v1.6), REVEL = 0,93) [PP3].	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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.5903G>A, c.5533del) from the Marseille Dysferlinopathy cohort. 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: 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 gnomADv2.1.1 (maximum population 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].	Likely pathogenic: PM3 strong + PM2 + PP4 moderate + 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 from the Marseille Dysferlinopathy cohort. We have identified c.755C>T in 1 patient in a heterozygous state (associated with a variant of unknown significance) from the Marseille Dysferlinopathy cohort, 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	Pathogenic: PM3 strong + PM2 + PP4 moderate + PP1 supporting + PP3

	<p>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 gnomADv2.1.1 (maximum population 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.</p>	
<p>DYSF NM_003494.3:c.757C&gt;T p.(Arg253Trp) exon 7 (hg19)chr2:71742846 Canonical Allele Identifier: CA1705479</p>	<p>We have identified this variant in 1 patient in a homozygous state from the Marseille Dysferlinopathy cohort. We have identified c.757C&gt;T in 5 patients (4 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.1758C&gt;G, c.5813_5821del, c.5871_5872del, c.5979dupA) from the Marseille Dysferlinopathy cohort, 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&gt;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 gnomADv2.1.1 (maximum population 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].</p>	<p>Pathogenic: PM3 very strong + PM2 + PP4 moderate + PP1 supporting + PP3</p>
<p>DYSF NM_003494.3:c.797T&gt;C p.(Leu266Pro) exon 8 (hg19)chr2:71743314 Canonical Allele Identifier: CA347209277</p>	<p>We have identified c.797T&gt;C in 2 patients (1 index) in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.4872_4876delinsCCCC) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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].</p>	<p>Likely pathogenic: PM2 + PP4 moderate + PP1 supporting + PP3 + PM3 supporting</p>
<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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.3687C&gt;A) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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&gt;C p.(Ile284Thr) exon 8 (hg19)chr2:71743368 Canonical Allele Identifier: CA347209709</p>	<p>We have identified c.851T&gt;C in 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.1617C&gt;G) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent</p>	<p>Likely pathogenic: PM2 + PP4 moderate + PP3 + PM3 supporting</p>

	from gnomADv2.1.1 [PM2]. It is predicted to affect protein function (CADD = 25,1 (v1.6), REVEL = 0,906) [PP3].	
DYSF NM_003494.3:c.855+1del: intron 8 (hg19)chr2:71743373del Canonical Allele Identifier: CA253921	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 index) 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) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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.	Pathogenic: PVS1 + PM3 strong + PM2 + PP4 moderate + PP1 supporting
DYSF NM_003494.3:c.857T>A p.(Val286Glu) exon 9 (hg19)chr2:71744120 Canonical Allele Identifier: CA222220	We have identified c.857T>A in 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.5860G>T (trans)) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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.	Pathogenic: PM3 strong + PM2 + PP4 moderate + PP1 supporting + PP3
DYSF NM_003494.3:c.895G>A p.(Gly299Arg) exon 9 (hg19)chr2:71744158 Canonical Allele Identifier: CA253916	We have identified c.895G>A in 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.490G>T (trans)) from the Marseille Dysferlinopathy cohort, 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 (trans), 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 gnomADv2.1.1 (maximum population 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.	Pathogenic: PM3 strong + PM2 + PP4 moderate + PP1 supporting + PP3

<p>DYSF NM_003494.3:c.896G&gt;A p.(Gly299Glu) exon 9 (hg19)chr2:71744159 Canonical Allele Identifier: CA347210280</p>	<p>We have identified c.896G&gt;A in 2 patients (2 index) in a heterozygous state (not associated with pathogenic or likely pathogenic variants) from the Marseille Dysferlinopathy cohort. This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.1877T&gt;C, PMID: 27602406), associated in 1 case (PMID: 27602406) with a decrease of Dysferlin on Western Blot / ImmunoHistoChemistry. It is present in only 1 individual in gnomADv2.1.1 (maximum population frequency: 0,0065% 1/15424 in European (non-Finnish) population) [PM2]. Novel missense variant at amino acid with different pathogenic (c.895G&gt;A) missense change (PM5) (CADD = 25,2 (v1.6), REVEL = 0,947).</p>	<p>Uncertain significance: PM2 + PM5</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 or likely pathogenic variant: c.4410+1G&gt;A) from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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&gt;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&gt;A in 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.855+1del (trans)) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomADv2.1.1 [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>
<p>DYSF NM_003494.3:c.1020C&gt;A p.(Ser340Arg) exon 11 (hg19)chr2:71748001 Canonical Allele Identifier: CA347212168</p>	<p>We have identified c.1020C&gt;A in 2 patients (2 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.5078G&gt;A, c.5594del) from the Marseille Dysferlinopathy cohort, 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&gt;T, c.1168G&gt;A (trans) and c.3181del (trans), 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 gnomADv2.1.1 (maximum population frequency: 0,0033% 1/30616 in South Asian population) [PM2] (CADD = 23,4 (v1.6), REVEL = 0,695).</p>	<p>Likely pathogenic: PM3 strong + PM2 + PP4 moderate</p>
<p>DYSF NM_003494.3:c.1020C&gt;G p.(Ser340Arg) exon 11 (hg19)chr2:71748001 Canonical Allele Identifier: CA10605994</p>	<p>We have identified this variant in 1 patient in a homozygous state from the Marseille Dysferlinopathy cohort, 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&gt;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&gt;A), regardless of nucleotide change [PS1]. It is present in only 1 individual in gnomADv2.1.1 (maximum population frequency: 0,0009% 1/113756 in European (non-Finnish) population) [PM2] (CADD = 23,6 (v1.6), REVEL = 0,695).</p>	<p>Likely pathogenic: PS1 + PM2 + PP4 moderate + PM3 supporting</p>

<p>DYSF NM_003494.3:c.1064_1065del p.(Lys355ArgfsTer4) exon 12 (hg19)chr2:71753360_71753361del Canonical Allele Identifier: CA1139532907</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 from the Marseille Dysferlinopathy cohort. We have identified c.1064_1065del in 2 patients (1 index) in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.2217C&gt;A) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomADv2.1.1 [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.1155_1180+2del: exon 12 (hg19)chr2:71753451_71753478del Canonical Allele Identifier: CA1139532908</p>	<p>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 or likely pathogenic variant: c.591C&gt;G) from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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.1157_1168del p.(Phe386_Asp390delinsTyr) exon 12 (hg19)chr2:71753453_71753464del Canonical Allele Identifier: CA1139532900</p>	<p>We have identified c.1157_1168del in 3 patients (2 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.5078G&gt;A (trans), c.1663C&gt;T) from the Marseille Dysferlinopathy cohort, associated in 1 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomADv2.1.1 [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].</p>	<p>Likely pathogenic: PM2 + PM3 + PM4 + PP4 moderate + PP1 supporting</p>
<p>DYSF NM_003494.3:c.1168G&gt;A p.(Asp390Asn) exon 12 (hg19)chr2:71753464 Canonical Allele Identifier: CA10604166</p>	<p>We have identified c.1168G&gt;A in 5 patients (4 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.5302C&gt;T, c.5302C&gt;T, c.1177C&gt;T) from the Marseille Dysferlinopathy cohort, 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&gt;A (cis) and c.3181del (trans), PMID: LOVD ID 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 gnomADv2.1.1 (maximum population 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.</p>	<p>Pathogenic: PM3 strong + PM2 + PP4 moderate + PP1 supporting + PP3</p>
<p>DYSF NM_003494.3:c.1177C&gt;T p.(Gln393Ter) exon 12 (hg19)chr2:71753473 Canonical Allele Identifier: CA347213670</p>	<p>This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.1177C&gt;T in 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.1168G&gt;A) from the Marseille Dysferlinopathy cohort. This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.1180+5G&gt;A, PMID: not found, LOVD ID 00215246) [PM3 supporting, PP4 supporting]. It is present in only 1 individual in gnomADv2.1.1 (maximum population 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.</p>	<p>Pathogenic: PVS1 + PM2 + PP4 supporting + PM3 supporting</p>



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 from the Marseille Dysferlinopathy cohort. We have identified c.1180_1180+7del in 1 patient in a heterozygous state (associated with a variant of unknown significance) from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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) from the Marseille Dysferlinopathy cohort, 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 (trans), c.4194del (trans), 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 gnomADv2.1.1 (maximum population frequency: 0,0009% 1/111678 in European (non-Finnish) population) [PM2]. It is predicted to affect protein function (alteration of the wild type donor site predicted by HSF and SpliceAI) [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 or likely pathogenic variant: c.2858dup) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 [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) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort. This variant has previously been reported in a heterozygous state in 7 cases in the literature (associated with c.2997G>T, c.4756C>T (trans), c.2494C>T, c.4795-2A>G, c.663+1G>C, c.5078G>A, c.823del, PMID: 32400077,	Pathogenic: PM3 very strong + PM2 + PVS1

	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 gnomADv2.1.1 (maximum population 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.	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 or likely pathogenic variant: c.5713C>T) from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [PM2]. It is predicted to affect protein function (CADD = 25,6 (v1.6), REVEL = 0,66) [PP3].	Uncertain significance: PM2 + PP3 + PP4 supporting + 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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.5920_5921insCCCTGTGTGGACTGTGTAGGAG, c.1555G>A) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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 or likely pathogenic variant: c.1638+1G>C) from the Marseille Dysferlinopathy cohort, 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.5197A>G, c.2030-?_2439+?del, c.3036G>C, c.3137G>A, c.1464delT, PMID: 28403181, 27647186) [PM3, PP4 moderate]. It is absent from gnomADv2.1.1 [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.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 from the Marseille Dysferlinopathy cohort. 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 gnomADv2.1.1 (maximum population 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	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 pathogenic or likely	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate

Canonical Allele Identifier: CA347215842	pathogenic variant: c.5533del (trans)) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomADv2.1.1 [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.	
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 or likely pathogenic variant: c.3059dup) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 or likely pathogenic variant: c.1368C>A) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 [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) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. [PP4 moderate]. It is absent from gnomADv2.1.1 [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.	Pathogenic: PVS1 + PM2 + PP4 moderate
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 or likely pathogenic variant: c.1375dup) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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

<p>DYSF NM_003494.3:c.1642dup p.(Glu548GlyfsTer22) exon 19 (hg19)chr2:71778740dup Canonical Allele Identifier: CA10606691</p>	<p>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 or likely pathogenic variant: c.5594del) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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.</p>	<p>Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting</p>
<p>DYSF NM_003494.3:c.1655_1668del p.(Tyr552SerfsTer13) exon 19 (hg19)chr2:71778753_71778766del Canonical Allele Identifier: CA1139532910</p>	<p>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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.5979dup (trans)) from the Marseille Dysferlinopathy cohort, associated in 1 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomADv2.1.1 [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 + PM3 + PP4 moderate</p>
<p>DYSF NM_003494.3:c.1663C&gt;T p.(Arg555Trp) exon 19 (hg19)chr2:71778761 Canonical Allele Identifier: CA222133</p>	<p>We have identified this variant in 3 patients in a homozygous state from the Marseille Dysferlinopathy cohort, associated in 2 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. We have identified c.1663C&gt;T in 6 patients (6 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.610C&gt;T, c.3832C&gt;T (trans), c.1157_1168del, c.4005+1G&gt;A) from the Marseille Dysferlinopathy cohort, 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&gt;T, c.797T&gt;G, c.2997G&gt;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 gnomADv2.1.1 (maximum population 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 available in the literature for this variant, the evidence listed here allows the maximal classification (Class5/pathogenic) for this variant.</p>	<p>Pathogenic: PM3 very strong + PM2 + PP1 supporting + PP3 + PP4 supporting</p>
<p>DYSF NM_003494.3:c.1699_1719del p.(His567_Glu573del) exon 19 (hg19)chr2:71778797_71778817del Canonical Allele Identifier: CA1139532901</p>	<p>We have identified c.1699_1719del in 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.3597G&gt;A) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [PM2]. Protein-length-changing variant (deletion without frameshift) [PM4].</p>	<p>Likely pathogenic: PM2 + PM4 + PP4 moderate + PM3 supporting</p>
<p>DYSF NM_003494.3:c.1758C&gt;G p.(Tyr586Ter) exon 20 (hg19)chr2:71780146 Canonical Allele Identifier: CA347218306</p>	<p>This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.1758C&gt;G in 4 patients (4 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.107_108del, c.3321_3324dup, c.757C&gt;T, c.937+1G&gt;A) from the Marseille Dysferlinopathy cohort, associated in 1 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. It is absent from gnomADv2.1.1 [PM2] (CADD = 40</p>	<p>Pathogenic: PVS1 + PM3 strong + PM2 + PP4 moderate</p>

	(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.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 from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort, associated in these 2 cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort, 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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.3967C>T, c.4872_4876delinsCCCC) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.4854C>G (trans), c.4550del and c.1351A>G) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 [PM2]. It is predicted to affect protein function (CADD = 26,6 (v1.6), REVEL = 0,914) [PP3].	Likely pathogenic: PM2 + PM3 + PP4 moderate + PP3
DYSF NM_003494.3:c.1852G>A p.(Gly618Arg) exon 40 (hg19)chr2:71780240 Canonical Allele Identifier: CA241938	We have identified this variant in 2 patients in a homozygous state from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population frequency: 0,0046% 1/21646 in European	Likely pathogenic: PM2 + PM3 + PP4 moderate + PP1 supporting + PP3

	(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].	
DYSF NM_003494.3:c.1861G>A p.(Gly621Arg) exon 20 (hg19)chr2:71780249 Canonical Allele Identifier: CA10606091	We have identified this variant in 1 patient in a homozygous state from the Marseille Dysferlinopathy cohort, 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 (trans), 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 gnomADv2.1.1 (maximum population 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.	Pathogenic: PM3 very strong + PM2 + PP4 moderate + PP3
DYSF NM_003494.3:c.1877T>C p.(Met626Thr) exon 20 (hg19)chr2:71780265 Canonical Allele Identifier: CA1705983	We have identified c.1877T>C in 2 patients (2 index) in a heterozygous state (not associated with pathogenic or likely pathogenic variants) from the Marseille Dysferlinopathy cohort. This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.896G>A, PMID: 27602406), associated in 1 case (PMID: 27602406) with a decrease of Dysferlin on Western Blot / ImmunoHistoChemistry. CADD = 8,856 (v1.6), REVEL = 0,122. This variant is not predicted to affect protein function (REVEL score <0,15) [BP4].	Uncertain significance: BP4
DYSF NM_003494.3:c.1911C>A p.(Tyr637Ter) exon 20 (hg19)chr2:71780299 Canonical Allele Identifier: CA347218736	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) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 [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 + PP4 moderate + PM3 supporting
DYSF NM_003494.3:c.1948del p.(Leu650TyrfTer6) 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 from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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 or likely pathogenic variant: c.5668-7G>A) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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

<p>DYSF NM_003494.3:c.2217C&gt;A p.(Tyr739Ter)          exon 23 (hg19)chr2:71788936          Canonical Allele Identifier: CA347209063</p>	<p>This variant is a null variant in a gene where LOF is a known mechanism of Dysferlinopathy [PVS1]. We have identified c.2217C&gt;A in 3 patients (2 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.4005+1G&gt;A, c.1064_1065del) from the Marseille Dysferlinopathy cohort, associated in these 2 cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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.</p>	<p>Pathogenic: PVS1 + PM2 + PP4 moderate + PP1 supporting + PM3 supporting</p>
<p>DYSF NM_003494.3:c.2355+1G&gt;A: intron 23          (hg19)chr2:71789075          Canonical Allele Identifier: CA10604289</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 2 patients in a homozygous state from the Marseille Dysferlinopathy cohort, associated in 1 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. We have identified c.2355+1G&gt;A in 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.5399_5400dup) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is present in only 1 individual in gnomADv2.1.1 (maximum population 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.</p>	<p>Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate + PP1 supporting</p>
<p>DYSF NM_003494.3:c.2456G&gt;A p.(Arg819Gln)          exon 24 (hg19)chr2:71791288          Canonical Allele Identifier: CA1706192</p>	<p>We have identified c.2456G&gt;A in 1 patient in a heterozygous state (associated with a variant of unknown significance) from the Marseille Dysferlinopathy cohort. This variant has previously been reported in a heterozygous state in 1 case in the literature (associated with c.1980G&gt;A, PMID: 30564623). It is present in only 11 individuals in gnomADv2.1.1 (maximum population frequency: 0,0029% 3/30616 in South Asian population) [PM2] (CADD = 22,6 (v1.6), REVEL = 0,078). This variant is not predicted to affect protein function (REVEL score &lt;0,15) [BP4].</p>	<p>Uncertain significance: PM2 + BP4</p>
<p>DYSF NM_003494.3:c.2496_2499del          p.(Thr833SerfsTer3) exon 24          (hg19)chr2:71791328_71791331del Canonical          Allele Identifier: CA1706197</p>	<p>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 index) in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.5036G&gt;A (trans)) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is present in only 1 individual in gnomADv2.1.1 (maximum population 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.</p>	<p>Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate + PP1 supporting</p>
<p>DYSF NM_003494.3:c.2504_2507del          p.(Phe835Ter) exon 24          (hg19)chr2:71791336_71791339del Canonical          Allele Identifier: CA892695933</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 from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 moderate + PM3 supporting</p>

<p>DYSF NM_003494.3:c.2511+1G&gt;A: intron 24 (hg19)chr2:71791344 Canonical Allele Identifier: CA347211915</p>	<p>We have identified c.2511+1G&gt;A in 2 patients (1 index) in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.3137G&gt;A) from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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)).</p>	<p>Likely pathogenic: PM2 + PVS1 moderate + PP1 supporting + PP4 supporting + PM3 supporting</p>
<p>DYSF NM_003494.3:c.2512-1512_3148del: exon 25_29del (hg19)chr2:71793569_71797845del Canonical Allele Identifier: CA1139532913</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 from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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.</p>	<p>Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting</p>
<p>DYSF NM_003494.3:c.2641A&gt;C p.(Thr881Pro) exon 25 (hg19)chr2:71795210 Canonical Allele Identifier: CA347214045</p>	<p>We have identified c.2641A&gt;C in 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.1911C&gt;A) from the Marseille Dysferlinopathy cohort, 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&gt;A, c.4756C&gt;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 gnomADv2.1.1 [PM2]. Protein-length-changing variant (Exon 25 skipping without frameshift PMID: 25312915) [PM4] (CADD = 29,9 (v1.6), REVEL = 0,736).</p>	<p>Likely pathogenic: PM2 + PM3 + PM4 + PP4 moderate</p>
<p>DYSF NM_003494.3:c.2643+1G&gt;A: intron 25 (hg19)chr2:71795213 Canonical Allele Identifier: CA222141</p>	<p>We have identified this variant in 8 patients in a homozygous state from the Marseille Dysferlinopathy cohort, associated in 4 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. We have identified c.2643+1G&gt;A in 5 patients (4 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.4167+1G&gt;C, c.3349-1G&gt;T, c.331C&gt;T) from the Marseille Dysferlinopathy cohort, 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&gt;A, c.827del, c.3112C&gt;T, c.2790G&gt;C, c.937+1G&gt;A, c.4497del, c.2997G&gt;T, c.3959delinsGA, c.1566C&gt;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 Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 very strong, PP4 moderate]. 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.</p>	<p>Pathogenic: PM3 very strong + PVS1 moderate + PP4 moderate + PP1 moderate</p>
<p>DYSF NM_003494.3:c.2706dup p.(Lys903GlnfsTer4) exon 26 (hg19)chr2:71795364dup Canonical Allele Identifier: CA913189537</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 from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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>



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 from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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 or likely pathogenic variant: c.4872_4876delinsCCCC) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort. We have identified c.2779del in 5 patients (3 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.5713C>T, c.4390G>T and c.3624C>G, c.5979dupA) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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 from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is present in only 33 individuals (1 homozygote) in gnomADv2.1.1 (maximum population 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].	Uncertain significance: PM2 + PP3 + PP4 supporting + PM3 supporting
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 or likely pathogenic variant: c.2875C>T (trans)) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population frequency: 0,0009% 1/112156 in European (non-Finnish) population) [PM2]. It is predicted to affect protein function (CADD = 28,1 (v1.6), alteration of the wild-type donor site predicted by HSF and SpliceAI) [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 from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [PM2]. This variant was shown to co-segregate with dysferlinopathy in one affected family, with 3 affected individuals, and 0 unaffected individual	Pathogenic: PVS1 + PM2 + PP1 moderate + PP4 supporting + PM3 supporting

	(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.	
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 from the Marseille Dysferlinopathy cohort. We have identified c.2858dup in 2 patients (2 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.526C>T) from the Marseille Dysferlinopathy cohort [PM3, PP4 supporting]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. We have identified c.2875C>T in 4 patients (3 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.3112C>G (trans), c.2810+5G>A (trans)) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.6233_6240del, c.4200dup) from the Marseille Dysferlinopathy cohort, 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 individual in gnomADv2.1.1 (maximum population 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.	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting
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 index) in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.3373del) from the Marseille Dysferlinopathy cohort, 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	Pathogenic: PM3 very strong + PM2 + PP4 moderate + PP1 supporting + PP3

	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 gnomADv2.1.1 (maximum population 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.	
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 from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort, 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 index) in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.855+1del) from the Marseille Dysferlinopathy cohort [PM3, PP4 moderate]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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 Canonical Allele Identifier: CA1706427	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 or likely pathogenic variant: c.1440_1443del) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is present in only 2 individuals in gnomADv2.1.1 (maximum population 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.	Pathogenic: PVS1 + PM2 + PP4 moderate + PM3 supporting
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 index) in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.2875C>T (trans)) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is	Likely pathogenic: PM2 + PM3 + PM5 + PP4

	absent from gnomADv2.1.1 [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).	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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.5594del, c.3832C>T, c.3805G>T) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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 from the Marseille Dysferlinopathy cohort, 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 or likely pathogenic variant: c.5594del) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.265C>T, c.855+1del) from the Marseille Dysferlinopathy cohort, associated in 1 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomADv2.1.1 [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.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 from the Marseille Dysferlinopathy cohort, 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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.3477C>A, c.2511+1G>A) from the Marseille Dysferlinopathy cohort, associated	Pathogenic: PM3 strong + PM2 + PP4 moderate + PP1 supporting + PP3

	<p>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&gt;T, c.965T&gt;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 gnomADv2.1.1 (maximum population 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.</p>	
<p>DYSF NM_003494.3:c.3225del p.(Phe1075LeufsTer45) exon 30 (hg19)chr2:71801378del Canonical Allele Identifier: CA1139532915</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 from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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.3286_3291dup p.(Arg1097_Met1098insArgArg) exon 30 (hg19)chr2:71801439_71801444dup Canonical Allele Identifier: CA1139532899</p>	<p>We have identified c.3286_3291dup in 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.755C&gt;T) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [PM2].</p>	<p>Uncertain significance: PM2 + PP4 moderate + PM3 supporting</p>
<p>DYSF NM_003494.3:c.3321_3322delinsC p.(Ala1108LeufsTer12) exon 30 (hg19)chr2:71801474_71801475delinsC Canonical Allele Identifier: CA1139532916</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 2 patients in a homozygous state from the Marseille Dysferlinopathy cohort, associated in 1 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 + PP4 moderate + PP1 supporting + PM3 supporting</p>
<p>DYSF NM_003494.3:c.3321_3324dup p.(Val1109SerfsTer6) exon 30 (hg19)chr2:71801474_71801477dup Canonical Allele Identifier: CA533655769</p>	<p>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 or likely pathogenic variant: c.1758C&gt;G) from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is present in only 1 individual in gnomADv2.1.1 (maximum population frequency: 0,0009% 1/113572 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.</p>	<p>Pathogenic: PVS1 + PM2 + PP4 supporting + PM3 supporting</p>
<p>DYSF NM_003494.3:c.3343dup p.(Ala1115GlyfsTer8) exon 30 (hg19)chr2:71801496dup Canonical Allele Identifier: CA1139532917</p>	<p>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) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. [PP4 moderate]. It is absent from gnomADv2.1.1 [PM2]. Even though</p>	<p>Pathogenic: PVS1 + PM2 + PP4 moderate</p>

	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.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 or likely pathogenic variant: c.5314_5318del (trans)) from the Marseille Dysferlinopathy cohort [PM3, PP4 supporting]. It is absent from gnomADv2.1.1 [PM2]. It is predicted to affect protein function (alteration of the wild-type donor site predicted by HSF and SpliceAI) [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 or likely pathogenic variant: c.2643+1G>A) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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 index) in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.2997G>T) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 index) in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.107_108del (trans)) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort, associated in 1 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.3137G>A, c.5979dup (trans)) from the Marseille Dysferlinopathy cohort, associated in 1 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate

	moderate]. It is absent from gnomADv2.1.1 [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.	
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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.342+1G>A) from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is present in only 7 individuals in gnomADv2.1.1 (maximum population 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 pathogenic or likely pathogenic variant: c.1699_1719del) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. [PP4 moderate]. It is absent from gnomADv2.1.1 [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) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 or likely pathogenic variant: c.799_800del) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 or likely pathogenic variant: c.5509G>A) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 index) in a heterozygous state (associated with a pathogenic or likely pathogenic	Pathogenic: PVS1 + PM2 + PP4 moderate + PP1 supporting + PM3 supporting

	variant: c.490G>T) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort, associated in 1 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomADv2.1.1 [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 index) in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.3112C>T) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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 index) in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.3843+1G>A) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. [PP4 moderate]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort. We have identified c.3832C>T in 4 patients (4 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.5979dup, c.1663C>T (trans), c.3112C>T) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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 index) in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.3826C>G) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. [PP4 moderate]. It is absent from	Likely pathogenic: PM2 + PM4 + PP4 moderate + PP1 supporting



	gnomADv2.1.1 [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)).	
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 or likely pathogenic variant: c.1834C>T) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.1663C>T, c.2217C>A, c.855+1del) from the Marseille Dysferlinopathy cohort, associated in 2 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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 or likely pathogenic variant: c.2643+1G>A) from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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 pathogenic or likely pathogenic variant: c.5899G>A) from the Marseille Dysferlinopathy cohort. [PP4 supporting]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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 from the Marseille Dysferlinopathy cohort, associated in 2 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. We have identified c.4200dup in 3 patients (3 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.2894G>A, c.4433G>A) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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 supporting
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 from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort, associated in 1 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.917_923dup and c.4410+1G>A) from the Marseille Dysferlinopathy cohort. [PP4 supporting]. It is predicted to affect protein function (CADD = 26,9 (v1.6), REVEL = 0,586) [PP3]. Maximal population frequency in gnomAD v2.1.1 is 2,2974% (607/24780 in African population, and 4 homozygotes) [BS1].	Uncertain significance: PP3 + PP4 supporting + 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 or likely pathogenic variant: c.917_923dup) from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [PM2] (CADD = 34 (v1.6)). Even though additional evidence of	Pathogenic: PVS1 + PM2 + PP4 supporting + PM3 supporting

	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.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 or likely pathogenic variant: c.4200dup) from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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 Canonical Allele Identifier: CA1139532921	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 pathogenic or likely pathogenic variant: c.1351A>G and c.1848C>A) from the Marseille Dysferlinopathy cohort. [PP4 supporting]. It is absent from gnomADv2.1.1 [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
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 6 patients (6 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.4577A>C (cis) and c.2643+1G>A homozygous, c.4577A>C (cis) and c.2643+1G>A homozygous, c.4577A>C (cis) and c.2643+1G>A homozygous, c.4577A>C (cis) and c.2643+1G>A homozygous, c.2643+1G>A and c.1088A>T, c.2643+1G>A and c.565C>G and c.6029A>G) from the Marseille Dysferlinopathy cohort, associated in 5 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. It is predicted to affect protein function (CADD = 25,1 (v1.6), REVEL = 0,712) [PP3]. In our cohort this variant is observed in cis with a pathogenic variant (4 patients with c.2643+1G>A homozygous) in a recessive inheritance pattern [BP2].	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 from the Marseille Dysferlinopathy cohort, associated in 1 case with a decrease of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort, 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 or likely pathogenic variant: c.1180+5G>A) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort, associated in 1 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population frequency: 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.	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate
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 or likely pathogenic variant: c.3343dup) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 pathogenic or likely pathogenic variant: c.1848C>A (trans)) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is present in only 1 individual in gnomADv2.1.1 (maximum population 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 from the Marseille Dysferlinopathy cohort, 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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.2772G>A, c.1834C>T (trans)) from the Marseille Dysferlinopathy cohort, associated in 3 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 strong, PP4 moderate]. It is absent from gnomADv2.1.1 [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 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.799_800del) from the Marseille Dysferlinopathy cohort, associated in 1 case with a decrease of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [PM2]. It is predicted to affect protein function (CADD = 29,8 (v1.6), REVEL = 0,592) [PP3].	Uncertain significance: PM2 + PP3 + PP4 supporting + PM3 supporting

<p>DYSF NM_003494.3:c.5033G&gt;C p.(Cys1678Ser)          exon 45 (hg19)chr2:71891544          Canonical Allele Identifier: CA1707259</p>	<p>We have identified c.5033G&gt;C in 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.3137G&gt;A) from the Marseille Dysferlinopathy cohort, 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&gt;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 gnomADv2.1.1 (maximum population 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].</p>	<p>Likely pathogenic: PM2 + PP4 moderate + PP3 + PM3 supporting</p>
<p>DYSF NM_003494.3:c.5036G&gt;A p.(Gly1679Glu)          exon 45 (hg19)chr2:71891547          Canonical Allele Identifier: CA347220615</p>	<p>We have identified c.5036G&gt;A in 2 patients (1 index) in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.2496_2499del (trans)) from the Marseille Dysferlinopathy cohort, 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&gt;T, PMID: 32400077) [PM3, PP4 moderate]. It is absent from gnomADv2.1.1 [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 = 26,2 (v1.6), REVEL = 0,853) [PP3].</p>	<p>Likely pathogenic: PM2 + PM3 + PP4 moderate + PP1 supporting + PP3</p>
<p>DYSF NM_003494.3:c.5078G&gt;A p.(Arg1693Gln)          exon 46 (hg19)chr2:71892312          Canonical Allele Identifier: CA351294</p>	<p>We have identified c.5078G&gt;A in 3 patients (2 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.1157_1168del (trans)) from the Marseille Dysferlinopathy cohort, 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&gt;C, c.1175del, c.2997G&gt;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 gnomADv2.1.1 (maximum population 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.</p>	<p>Pathogenic: PM3 strong + PM2 + PP4 moderate + PP1 supporting + PP3</p>
<p>DYSF NM_003494.3:c.5093C&gt;T p.(Pro1698Leu)          exon 46 (hg19)chr2:71892327          Canonical Allele Identifier: CA347220971</p>	<p>We have identified c.5093C&gt;T in 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.2875C&gt;T) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [PM2]. It is predicted to affect protein function (CADD = 29,7 (v1.6), REVEL = 0,874) [PP3].</p>	<p>Likely pathogenic: PM2 + PP4 moderate + PP3 + PM3 supporting</p>
<p>DYSF NM_003494.3:c.5200+1G&gt;A: intron 46          (hg19)chr2:71892435          Canonical Allele Identifier: CA1707318</p>	<p>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 from the Marseille Dysferlinopathy cohort. We have identified c.5200+1G&gt;A in 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.5314_5318del) from the Marseille Dysferlinopathy cohort. 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</p>	<p>Pathogenic: PVS1 + PM2 + PM3 + PP4 supporting</p>

	Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 supporting]. It is present in only 1 individual in gnomADv2.1.1 (maximum population 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.	
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 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.5296G>T homozygous and c.565C>G homozygous) from the Marseille Dysferlinopathy cohort, 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: 27671536), associated in 1 case (PMID: 27671536) with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [PM2]. It is predicted to affect protein function (CADD = 28,9 (v1.6), REVEL = 0,921) [PP3]. In our cohort this variant is observed in cis with a pathogenic variant (c.5296G>T p.(Glu1766Ter) homozygous) in a recessive inheritance pattern [BP2].	Uncertain significance: PM2 + PP4 moderate + PM3 supporting + PP3 + 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 from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.1168G>A, c.5594delG, c.5979dupA, c.1168G>A) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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 from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population	Likely pathogenic: PM2 + PM5 + PP4 moderate + PM3 supporting

	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).	
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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.5200+1G>A) from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is present in only 1 individual in gnomADv2.1.1 (maximum population 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 from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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 from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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 from the Marseille Dysferlinopathy cohort [PM3 supporting, PP4 supporting]. It is absent from gnomADv2.1.1 [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 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.1284+1G>A) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. [PP4 moderate]. It is absent from gnomADv2.1.1 [PM2]. It is predicted to affect protein function (CADD = 32 (v1.6), REVEL = 0,885) [PP3].	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 from the Marseille Dysferlinopathy cohort. We have identified c.5509G>A in 2 patients (2 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.3702+1G>A, c.855+1del) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population frequency: 0,0056% 1/17910 in East Asian population) [PM2]. It is predicted to affect protein function (CADD = 32 (v1.6),	Pathogenic: PM3 strong + PP1 strong + PM2 + PP4 moderate + PP3

	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.	
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 or likely pathogenic variant: c.1663C>T) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 [PM2]. It is predicted to affect protein function (HSF and SpliceAI: activation of a cryptic Acceptor site 13bp before natural splice site) [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 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.1397+1G>C (trans)) from the Marseille Dysferlinopathy cohort, associated in 1 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomADv2.1.1 [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.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 from the Marseille Dysferlinopathy cohort, associated in 2 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. We have identified c.5594del in 7 patients (6 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.3112C>T, c.855+1del, c.3113G>A, c.1642dup, c.5302C>T) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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 (associated with a variant of unknown significance) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. CADD = 24,1 (v1.6), REVEL = 0,377. Maximal population frequency in gnomAD v2.1.1 is 0,7757% (265/30616 in South Asian population, and 11 homozygotes) [BS1]. Observed in cis with a pathogenic variant (c.796_797del p.(Leu266PhefsTer6) PMID:17897828) in a recessive inheritance pattern [BP2].	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 from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. We have identified c.5668-7G>A in 2 patients (2 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.2163-2A>G, c.5908C>T (trans)) from the Marseille Dysferlinopathy cohort, 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	Pathogenic: PS3 + PM3 strong + PM2 + PP4 moderate



	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: PMID: 25312915) [PS3]. It is present in only 3 individuals in gnomADv2.1.1 (maximum population 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.	
DYSF NM_003494.3:c.5713C>T p.(Arg1905Ter) exon 51 (hg19)chr2:71901372 Canonical Allele Identifier: CA118412	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 from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. We have identified c.5713C>T in 3 patients (3 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.2779del, c.3517dup (trans)) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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.	Pathogenic: PVS1 + PM3 strong + PM2 + PP4 moderate
DYSF NM_003494.3:c.5767+1G>A: intron 51 (hg19)chr2:71901427 Canonical Allele Identifier: CA49771597	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 from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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.5813_5821del p.(Thr1938_Lys1940del) exon 52 (hg19)chr2:71906232_71906240del Canonical Allele Identifier: CA1139532924	We have identified c.5813_5821del in 2 patients (2 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.855+1del, c.757C>T) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 [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.	Pathogenic: PM3 strong + PM2 + PM4 + PP4 moderate + PP1 supporting
DYSF NM_003494.3:c.5860G>T p.(Glu1954Ter) exon 52 (hg19)chr2:71906279 Canonical Allele Identifier: CA347226407	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 pathogenic or likely pathogenic variant: c.857T>A (trans)) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3, PP4 moderate]. It is absent from gnomADv2.1.1	Pathogenic: PVS1 + PM2 + PM3 + PP4 moderate

	[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.	
DYSF NM_003494.3:c.5871_5872del p.(Ser1958ProfsTer3) exon 52 (hg19)chr2:71906290_71906291del Canonical Allele Identifier: CA1139532925	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 index) in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.757C>T) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. [PP4 moderate]. It is absent from gnomADv2.1.1 [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
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 from the Marseille Dysferlinopathy cohort, 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 or likely pathogenic variant: c.4191C>G) from the Marseille Dysferlinopathy cohort [PM3, PP4 moderate]. It is absent from gnomADv2.1.1 [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) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 [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 or likely pathogenic variant: c.5668-7G>A (trans)) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 [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 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 or likely pathogenic variant: c.1368C>A) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [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

<p>DYSF NM_003494.3:c.5979dup p.(Glu1994ArgfsTer3) exon 53 (hg19)chr2:71908163dup Canonical Allele Identifier: CA222200</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 4 patients in a homozygous state from the Marseille Dysferlinopathy cohort, associated in 1 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. We have identified c.5979dup in 7 patients (6 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.2779del, c.1655_1668del (trans), c.757C&gt;T, c.3477C&gt;A (trans), c.3832C&gt;T) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 (maximum population 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 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 very strong + PM2 + PP4 moderate + PP1 supporting</p>
<p>DYSF NM_003494.3:c.5999G&gt;A p.(Arg2000Gln) exon 53 (hg19)chr2:71908183 Canonical Allele Identifier: CA1707599</p>	<p>We have identified c.5999G&gt;A in 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.857T&gt;A (trans) and c.5860G&gt;T (cis)) from the Marseille Dysferlinopathy cohort, 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.5194G&gt;T, PMID: 17070050), associated in 1 case (PMID: 17070050) with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. It is predicted to affect protein function (CADD = 32 (v1.6), REVEL = 0,734) [PP3]. In our cohort this variant is observed in cis with a pathogenic variant (c.5860G&gt;T p.(Glu1954Ter)) in a recessive inheritance pattern [BP2].</p>	<p>Uncertain significance: PP3 + BP2</p>
<p>DYSF NM_003494.3:c.6017G&gt;A p.(Arg2006Gln) exon 53 (hg19)chr2:71908201 Canonical Allele Identifier: CA1707602</p>	<p>We have identified c.6017G&gt;A in 2 patients (1 index) in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.2643+1G&gt;A (trans)) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. [PP4 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]. It is predicted to affect protein function (CADD = 28 (v1.6), REVEL = 0,715) [PP3].</p>	<p>Uncertain significance: PP4 moderate + PP1 supporting + PP3</p>
<p>DYSF NM_003494.3:c.6029A&gt;G p.(Asn2010Ser) exon 53 (hg19)chr2:71908213 Canonical Allele Identifier: CA347226863</p>	<p>We have identified c.6029A&gt;G in 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.2643+1G&gt;A and c.4577A&gt;C) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry [PM3 supporting, PP4 moderate]. It is absent from gnomADv2.1.1 [PM2]. Novel missense variant at amino acid with different pathogenic (c.6029A&gt;C p.(Asn2010Thr)) missense change (PM5). It is predicted to affect protein function (CADD = 26,7 (v1.6), REVEL = 0,609).</p>	<p>Likely pathogenic: PM2 + PM5 + PP4 moderate + PM3 supporting</p>
<p>DYSF NM_003494.3:c.6119G&gt;A p.(Trp2040Ter) exon 54 (hg19)chr2:71909722 Canonical Allele Identifier: CA347227156</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 2 patients in a homozygous state from the Marseille Dysferlinopathy cohort [PM3, PP4 supporting]. It is absent from gnomADv2.1.1 [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.</p>	<p>Pathogenic: PVS1 + PM2 + PM3 + PP4 supporting</p>
<p>DYSF NM_003494.3:c.6124C&gt;T p.(Arg2042Cys) exon 54 (hg19)chr2:71909727 Canonical Allele Identifier: CA222203</p>	<p>We have identified c.6124C&gt;T in 4 patients (3 index) in a heterozygous state (associated with pathogenic or likely pathogenic variants: c.247del, c.5077C&gt;T) from the Marseille Dysferlinopathy cohort, associated in 2 of these cases with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. This variant has</p>	<p>Pathogenic: PM3 very strong + PM2 + PP4</p>

	<p>previously been reported in a heterozygous state in 6 cases in the literature (associated with c.1180+5G&gt;A, c.4872_4876delinsCC CC, c.3444_3445delinsAA, c.5979dup, c.4408C&gt;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 in gnomADv2.1.1 (maximum population 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.</p>	<p>moderate + PP1 supporting + PP3</p>
<p>DYSF NM_003494.3:c.6210T&gt;G p.(Tyr2070Ter) exon 55 (hg19)chr2:71913589 Canonical Allele Identifier: CA347227857</p>	<p>We have identified c.6210T&gt;G in 1 patient in a heterozygous state (associated with a variant of unknown significance) from the Marseille Dysferlinopathy cohort, associated in this case with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. [PP4 moderate]. It is absent from gnomADv2.1.1 [PM2]. Since this protein truncating variant is present in the last exon (NMD not predicted), [PVS1 moderate] was assigned.</p>	<p>Likely pathogenic: PM2 + PM4 + PVS1 moderate</p>
<p>DYSF NM_003494.3:c.6233_6240del p.(Pro2078LeufsTer?) exon 55 (hg19)chr2:71913612_71913619del Canonical Allele Identifier: CA1139532906</p>	<p>We have identified c.6233_6240del in 1 patient in a heterozygous state (associated with a pathogenic or likely pathogenic variant: c.2894G&gt;A) from the Marseille Dysferlinopathy cohort, 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 gnomADv2.1.1 [PM2]. Since this protein truncating variant is present in the last exon (NMD not predicted), [PVS1 moderate] was assigned.</p>	<p>Likely pathogenic: PM2 + PM3 + PVS1 moderate + PP4 moderate</p>

**Supplemental Table S2: The detailed evidence used for ACMG 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 criteria (see the Supplementary Methods for details) is shown in the “Evidence for each variant” column. 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.

## Annexe code 1: Macro de mise en forme de la synthèse de chaque variant

```
Sub actionFileIn()
ChDir ("C:\Users\cham\Desktop\V8A9")

fs = Dir("*.xlsm")

Do While Len(fs) > 0
Workbooks.Open (fs)
  Sheets("Feuil2").Select
  Range("N6").Select
  ActiveCell.FormulaR1C1 = "="&Feuil1!R[24]C[9]&"&"&"&"
  Range("N6").Select
  Application.CutCopyMode = False
  Selection.Copy
  Range("J5").Select
  Selection.PasteSpecial Paste:=xlPasteValues, Operation:=xlNone, SkipBlanks _
    :=False, Transpose:=False
  Range("N8").Select
  ActiveCell.FormulaR1C1 = "=Feuil1!R[18]C[10]"
  Range("N9").Select
  Range("N8").Select
  Selection.Copy
  Range("B5").Select
  Selection.PasteSpecial Paste:=xlPasteValues, Operation:=xlNone, SkipBlanks _
    :=False, Transpose:=False
  Cells.Replace What:=" + + + + + ", Replacement:=" + ", LookAt:= _
    xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, _
    ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2
  Cells.Replace What:=" + + + + + ", Replacement:=" + ", LookAt:= _
    xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, _
    ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2
  Cells.Replace What:=" + + + + ", Replacement:=" + ", LookAt:= _
    xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, _
    ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2
  Cells.Replace What:=" + + + + ", Replacement:=" + ", LookAt:= _
    xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, _
    ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2
  Cells.Replace What:=" + + + ", Replacement:=" + ", LookAt:= _
```

xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, \_  
 ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2  
 Cells.Replace What:=" + + ", Replacement:=" + ", LookAt:= \_  
 xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, \_  
 ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2  
 Cells.Replace What:=".,", Replacement:=".", LookAt:= \_  
 xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, \_  
 ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2  
 Cells.Replace What:=". ", Replacement:=" and", LookAt:= \_  
 xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, \_  
 ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2  
 Cells.Replace What:=":", Replacement:=":", LookAt:= \_  
 xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, \_  
 ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2  
 Cells.Replace What:=".", Replacement:="(", LookAt:= \_  
 xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, \_  
 ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2  
 Cells.Replace What:="(", Replacement:="(", LookAt:= \_  
 xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, \_  
 ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2  
 Cells.Replace What:=")", Replacement:=")", LookAt:= \_  
 xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, \_  
 ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2  
 Cells.Replace What:="{ + ", Replacement:="", LookAt:= \_  
 xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, \_  
 ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2  
 Cells.Replace What:=" + }", Replacement:="", LookAt:= \_  
 xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, \_  
 ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2  
 Cells.Replace What:="{", Replacement:="", LookAt:= \_  
 xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, \_  
 ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2  
 Cells.Replace What:="}", Replacement:="", LookAt:= \_  
 xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, \_  
 ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2  
 Cells.Replace What:="(", Replacement:="(", LookAt:= \_  
 xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, \_  
 ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2  
 Cells.Replace What:="[", Replacement:="[", LookAt:= \_  
 xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, \_

```

ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2
Cells.Replace What:=" ", Replacement:=" ", LookAt:= _
xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, _
ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2
Cells.Replace What:=" ", Replacement:=" ", LookAt:= _
xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, _
ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2
Cells.Replace What:=":", Replacement:=":", LookAt:= _
xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, _
ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2
Cells.Replace What:="()", Replacement:="", LookAt:= _
xlPart, SearchOrder:=xlByRows, MatchCase:=False, SearchFormat:=False, _
ReplaceFormat:=False, FormulaVersion:=xlReplaceFormula2
Application.CutCopyMode = False
Range("B4").Select
Selection.Copy
Range("A25").Select
Selection.PasteSpecial Paste:=xlPasteValues, Operation:=xlNone, SkipBlanks _
:=False, Transpose:=False
Range("B5").Select
Selection.Copy
Range("B25").Select
Selection.PasteSpecial Paste:=xlPasteValues, Operation:=xlNone, SkipBlanks _
:=False, Transpose:=False
Range("J5").Select
Selection.Copy
Range("C25").Select
Selection.PasteSpecial Paste:=xlPasteValues, Operation:=xlNone, SkipBlanks _
:=False, Transpose:=False
ActiveWorkbook.Save
Workbooks(fs).Close False

fs = Dir
Loop

End Sub

```

## Annexe code 2: Macro d'extraction des résultats

```
Sub Macroahah()
Dim CC As Workbook 'déclare la variable CC (Classeur Cible)
Dim OC As Worksheet 'déclare la variable OC (Onglet Cible)
Dim F As String 'déclare la variable F (Fichiers)
Dim CS As Workbook 'déclare la variable CS (Classeur Source)
Dim OS As Worksheet 'déclare la variable OS (Onglet Source)
Dim PL As Range 'déclare la variable PL (PLage)
Dim DEST As Range 'déclare la variable DEST (cellule de DESTination)

Set CC = ThisWorkbook 'définit le classeur cible CC
Set OC = CC.Sheets("extraction") 'définit l'onglet cible OC (à adapter)
F = Dir("C:\Users\charn\Desktop\V8A9\*.xlsm") 'définit le fichier F (premier fichier Excel du dossier contenant ce classeur)
Do While F <> "" 'boucle tant qu'il existe des fichiers
    If Not F = CC.Name Then 'condition : si F n'est pas ce classeur
        Workbooks.Open (F) 'ouvre le fichier F
        Set CS = ActiveWorkbook 'définit le classeur source CS
        Set OS = CS.Sheets("Feuil2") 'définit l'onglet source OS (à adapter)
        Set PL = OS.Range("A25:K25") 'définit la plage PL (à adapter, peut aussi être PL=OS.Rows(1))
        'définit la cellule de destination DEST (A1 si A1 est vide,
        'sinon la première cellule vide de la colonne 1 (=A) de l'onglet cible OC (à adapter)
        Set DEST = If(OC.Range("A1").Value = "", OC.Range("A1"), OC.Cells(Application.Rows.Count, 1).End(xlUp).Offset(1, 0))
        PL.Copy DEST 'copy la plage PL dans DEST
        CS.Close 'ferme le classeur source
    End If 'fin de la condition
    F = Dir 'redéfinit le fichier F (prochain fichier Excel du dossier contenant ce classeur)
Loop 'boucle
End Sub
```



### Annexe code 3: patch correctif mise à jour de l'ensemble des tableaux.

```
Sub actionFileInPatchV10()
```

```
ChDir ("C:\Users\cham\Desktop\V8A9")
```

```
fs = Dir("*.xlsm")
```

```
Do While Len(fs) > 0
```

```
Workbooks.Open (fs)
```

```
Sheets("Feuil1").Select
```

```
Range("K26").Select
```

```
ActiveCell.FormulaR1C1 = _
```

```
"=IF(AND(R[-5]C[3]="",R[-6]C="",R[-6]C[1]="",R[-16]C[3]="",R[-18]C[1]=""),IF(OR(R[-24]C[-1]>24.999,R[-24]C>0.6999),RC[-1],""),"")"
```

```
Range("L26").Select
```

```
ActiveCell.FormulaR1C1 = "=RC[4]"
```

```
Range("P26").Select
```

```
ActiveCell.FormulaR1C1 = _
```

```
"=IF(RC[-5]<>"",""It is predicted to affect protein function ""&IF(R[-24]C[-6]="",""(CADD = ""&R[-24]C[-6]&"" (GRCh37 v1.6), ""&IF(R[-24]C[-5]="",""REVEL = ""&R[-24]C[-5])&IF(RC[-5]<>"","" [PP3]. ""&"". ""))"
```

```
Range("P7").Select
```

```
ActiveCell.FormulaR1C1 = _
```

```
"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."
```

```
Range("S6").Select
```

```
ActiveCell.FormulaR1C1 = _
```

```
"=""We have identified ""&R[-4]C[-13]&"" in ""&(SUM(R[20]C[-15]:R[22]C[-15])+SUM(R[20]C[-12]:R[22]C[-12]))&"" patient""&IF((SUM(R[20]C[-15]:R[22]C[-15])+SUM(R[20]C[-12]:R[22]C[-12]))>1,""s""&"" in heterozygous state ""&IF(SUM(R[28]C[23]:R[30]C[24])>0,""(associated with pathogenic or likely pathogenic variant""&IF(SUM(R[28]C[23]:R[30]C[24])>1,""s""&""&""&R" & _
```

```
"[28]C[27]&""&"" from the Timone Dysferlinopathy cohort.""&IF(SUM(R[20]C[23]:R[25]C[23])>0,R[-3]C[14],"")&IF(SUM(R[20]C[24]:R[25]C[24])>0,R[-2]C[14],"") & _
```

```
""
```

```
Range("P29").Select
```

```
ActiveCell.FormulaR1C1 = _
```

```
"=""Maximal population frequency in gnomAD v2.1.1 is ""&ROUND(R[-23]C[-4],4)&""% ("&R[-23]C[-7]&""&R[-23]C[-6]&"" in ""&R[-23]C[-8]&"" population""&IF(R[-23]C[-10]<>"",", and ""&R[-23]C[-10]&"" homozygote""&IF(R[-23]C[-10]>1,""s""&""&""&"" [BA1]. ""
```

```
Range("P30").Select
```

```
ActiveCell.FormulaR1C1 = _
```

```
"=""Maximal population frequency in gnomAD v2.1.1 is ""&ROUND(R[-24]C[-4],4)&""% ("&R[-24]C[-7]&""&R[-24]C[-6]&"" in ""&R[-24]C[-8]&"" population""&IF(R[-24]C[-10]<>"",", and ""&R[-24]C[-10]&"" homozygote""&IF(R[-24]C[-10]>1,""s""&""&""&"" [BS1]. ""
```

Range("P18").Select

ActiveCell.FormulaR1C1 = \_

"="It is present in only ""&(R[-12]C[-12]-R[-12]C[-10])&"" individual""&IF((R[-12]C[-12]-R[-12]C[-10])>1, ""s"", """"")&IF(R[-12]C[-10]>0, "" ("&R[-12]C[-10]&"" homozygote""&IF(R[-12]C[-10]>1, ""s"", """"")&""""&"" in gnomADv2.1.1 (maximum population frequency: ""&ROUND(R[-12]C[-4],4)&""% ""&R[-12]C[-7]&""/""&R[-12]C[-6]&"" in ""&R[-12]C[-8]&"" population) [PM2]. """"

Range("W30").Select

ActiveCell.FormulaR1C1 = \_

"=R[-24]C[-22]&"": ""&R[-22]C[-12]&"" + ""&R[-21]C[-12]&"" + ""&R[-20]C[-12]&"" + ""&R[-19]C[-12]&"" + ""&R[-18]C[-12]&"" + ""&R[-17]C[-12]&"" + ""&R[-16]C[-12]&"" + ""&R[-15]C[-12]&"" + ""&R[-14]C[-12]&"" + ""&R[-13]C[-12]&"" + ""&R[-12]C[-12]&"" + ""&R[-11]C[-12]&"" + ""&R[-10]C[-12]&"" + ""&R[-9]C[-12]&"" + ""&R[-8]C[-12]&"" + ""&R[-7]C[-12]&"" + ""&R[-6]C[-12]&"" & \_

+ ""&R[-5]C[-12]&"" + ""&R[-4]C[-12]&"" + ""&R[-3]C[-12]&"" + ""&R[-2]C[-12]&"" + ""&R[-1]C[-12]&"" + ""&RC[-12]&"" + ""&R[1]C[-12]&"" + ""&R[2]C[-12]&"" + ""&R[3]C[-12]&"" + ""&R[4]C[-12]&"" + ""&R[5]C[-12]&"" + ""&R[6]C[-12]&"" + ""&R[7]C[-12]&"" + ""&R[8]C[-12]&"" + ""&R[9]C[-12]&"" + ""&R[10]C[-12]&"" & \_

""

Range("W31").Select

Range("AU8:BD9").Select

Range("BD9").Activate

Selection.Copy

Range("BL8").Select

ActiveSheet.Paste

Range("BL10").Select

ActiveCell.FormulaR1C1 = "=RC[-17]"

Range("BL11").Select

Range("BL10").Select

Selection.AutoFill Destination:=Range("BL10:BU10"), Type:=xlFillDefault

Range("BL10:BU10").Select

Range("BL24").Select

ActiveCell.FormulaR1C1 = \_

"=IF(OR(R[-1]C[-17]=RC[-17],R[-2]C[-17]=RC[-17],R[-3]C[-17]=RC[-17],R[-4]C[-17]=RC[-17],R[-5]C[-17]=RC[-17],R[-6]C[-17]=RC[-17],R[-7]C[-17]=RC[-17],R[-8]C[-17]=RC[-17],R[-9]C[-17]=RC[-17],R[-10]C[-17]=RC[-17],R[-11]C[-17]=RC[-17],R[-12]C[-17]=RC[-17],R[-13]C[-17]=RC[-17],R[-14]C[-17]=RC[-17]), """"", RC[-17])"

Range("BL24").Select

Selection.AutoFill Destination:=Range("BL24:BU24"), Type:=xlFillDefault

Range("BL24:BU24").Select

Range("BL24").Select

ActiveCell.FormulaR1C1 = \_

"=IF(OR(R[-1]C[-17]=RC[-17],R[-2]C[-17]=RC[-17],R[-3]C[-17]=RC[-17],R[-4]C[-17]=RC[-17],R[-5]C[-17]=RC[-17],R[-6]C[-17]=RC[-17],R[-7]C[-17]=RC[-17],R[-8]C[-17]=RC[-17],R[-9]C[-17]=RC[-17],R[-10]C[-17]=RC[-17],R[-11]C[-17]=RC[-17],R[-12]C[-17]=RC[-17],R[-13]C[-17]=RC[-17],R[-14]C[-17]=RC[-17]), """"", RC[-17])"

Range("BL23").Select

ActiveCell.FormulaR1C1 = \_

"=IF(OR(RC[-17]=R[1]C[-17],R[-1]C[-17]=R[1]C[-17],R[-2]C[-17]=R[1]C[-17],R[-3]C[-17]=R[1]C[-17],R[-4]C[-17]=R[1]C[-17],R[-5]C[-17]=R[1]C[-17],R[-6]C[-17]=R[1]C[-17],R[-7]C[-17]=R[1]C[-17],R[-8]C[-17]=R[1]C[-17],R[-9]C[-17]=R[1]C[-17],R[-10]C[-17]=R[1]C[-17],R[-11]C[-17]=R[1]C[-17],R[-12]C[-17]=R[1]C[-17],R[-13]C[-17]=R[1]C[-17]),"",R[1]C[-17])"

Range("BL24").Select

Selection.AutoFill Destination:=Range("BL23:BL24"), Type:=xlFillDefault

Range("BL23:BL24").Select

Range("BL23").Select

Selection.AutoFill Destination:=Range("BL11:BL23"), Type:=xlFillDefault

Range("BL11:BL23").Select

Range("BL23").Select

ActiveCell.FormulaR1C1 = \_

"=IF(OR(R[-1]C[-17]=RC[-17],R[-2]C[-17]=RC[-17],R[-3]C[-17]=RC[-17],R[-4]C[-17]=RC[-17],R[-5]C[-17]=RC[-17],R[-6]C[-17]=RC[-17],R[-7]C[-17]=RC[-17],R[-8]C[-17]=RC[-17],R[-9]C[-17]=RC[-17],R[-10]C[-17]=RC[-17],R[-11]C[-17]=RC[-17],R[-12]C[-17]=RC[-17],R[-13]C[-17]=RC[-17]),"",RC[-17])"

Range("BL22").Select

ActiveCell.FormulaR1C1 = \_

"=IF(OR(R[-1]C[-17]=RC[-17],R[-2]C[-17]=RC[-17],R[-3]C[-17]=RC[-17],R[-4]C[-17]=RC[-17],R[-5]C[-17]=RC[-17],R[-6]C[-17]=RC[-17],R[-7]C[-17]=RC[-17],R[-8]C[-17]=RC[-17],R[-9]C[-17]=RC[-17],R[-10]C[-17]=RC[-17],R[-11]C[-17]=RC[-17],R[-12]C[-17]=RC[-17]),"",RC[-17])"

Range("BL21").Select

ActiveCell.FormulaR1C1 = \_

"=IF(OR(R[-1]C[-17]=RC[-17],R[-2]C[-17]=RC[-17],R[-3]C[-17]=RC[-17],R[-4]C[-17]=RC[-17],R[-5]C[-17]=RC[-17],R[-6]C[-17]=RC[-17],R[-7]C[-17]=RC[-17],R[-8]C[-17]=RC[-17],R[-9]C[-17]=RC[-17],R[-10]C[-17]=RC[-17],R[-11]C[-17]=RC[-17]),"",RC[-17])"

Range("BL11").Select

ActiveCell.FormulaR1C1 = "=IF(OR(R[-1]C[-17]=RC[-17]),"",RC[-17])"

Range("BL12").Select

ActiveCell.FormulaR1C1 = \_

"=IF(OR(R[-1]C[-17]=RC[-17],R[-2]C[-17]=RC[-17]),"",RC[-17])"

Range("BL13").Select

ActiveCell.FormulaR1C1 = \_

"=IF(OR(R[-1]C[-17]=RC[-17],R[-2]C[-17]=RC[-17],R[-3]C[-17]=RC[-17]),"",RC[-17])"

Range("BL14").Select

ActiveCell.FormulaR1C1 = \_

"=IF(OR(R[-1]C[-17]=RC[-17],R[-2]C[-17]=RC[-17],R[-3]C[-17]=RC[-17],R[-4]C[-17]=RC[-17]),"",RC[-17])"

Range("BL15").Select

ActiveCell.FormulaR1C1 = \_

"=IF(OR(R[-1]C[-17]=RC[-17],R[-2]C[-17]=RC[-17],R[-3]C[-17]=RC[-17],R[-4]C[-17]=RC[-17],R[-5]C[-17]=RC[-17]),"",RC[-17])"

Range("BL16").Select

ActiveCell.FormulaR1C1 = \_

"=IF(OR(R[-1]C[-17]=RC[-17],R[-2]C[-17]=RC[-17],R[-3]C[-17]=RC[-17],R[-4]C[-17]=RC[-17],R[-5]C[-17]=RC[-17],R[-6]C[-17]=RC[-17]),"",RC[-17])"

Range("BL17").Select

ActiveCell.FormulaR1C1 = \_

"=IF(OR(R[-1]C[-17]=RC[-17],R[-2]C[-17]=RC[-17],R[-3]C[-17]=RC[-17],R[-4]C[-17]=RC[-17],R[-5]C[-17]=RC[-17],R[-6]C[-17]=RC[-17],R[-7]C[-17]=RC[-17]),"",RC[-17])"

Range("BL18").Select

ActiveCell.FormulaR1C1 = \_

"=IF(OR(R[-1]C[-17]=RC[-17],R[-2]C[-17]=RC[-17],R[-3]C[-17]=RC[-17],R[-4]C[-17]=RC[-17],R[-5]C[-17]=RC[-17],R[-6]C[-17]=RC[-17],R[-7]C[-17]=RC[-17],R[-8]C[-17]=RC[-17]),"",RC[-17])"

Range("BL19").Select

ActiveCell.FormulaR1C1 = \_

"=IF(OR(R[-1]C[-17]=RC[-17],R[-2]C[-17]=RC[-17],R[-3]C[-17]=RC[-17],R[-4]C[-17]=RC[-17],R[-5]C[-17]=RC[-17],R[-6]C[-17]=RC[-17],R[-7]C[-17]=RC[-17],R[-8]C[-17]=RC[-17],R[-9]C[-17]=RC[-17]),"",RC[-17])"

Range("BL20").Select

ActiveCell.FormulaR1C1 = \_

"=IF(OR(R[-1]C[-17]=RC[-17],R[-2]C[-17]=RC[-17],R[-3]C[-17]=RC[-17],R[-4]C[-17]=RC[-17],R[-5]C[-17]=RC[-17],R[-6]C[-17]=RC[-17],R[-7]C[-17]=RC[-17],R[-8]C[-17]=RC[-17],R[-9]C[-17]=RC[-17],R[-10]C[-17]=RC[-17]),"",RC[-17])"

Range("BL11").Select

Selection.AutoFill Destination:=Range("BL11:BU11"), Type:=xlFillDefault

Range("BL11:BU11").Select

Range("BL12").Select

Selection.AutoFill Destination:=Range("BL12:BU12"), Type:=xlFillDefault

Range("BL12:BU12").Select

Range("BL13").Select

Selection.AutoFill Destination:=Range("BL13:BU13"), Type:=xlFillDefault

Range("BL13:BU13").Select

Range("BL14").Select

Selection.AutoFill Destination:=Range("BL14:BU14"), Type:=xlFillDefault

Range("BL14:BU14").Select

Range("BL15").Select

Selection.AutoFill Destination:=Range("BL15:BU15"), Type:=xlFillDefault

Range("BL15:BU15").Select

Range("BL16").Select

Selection.AutoFill Destination:=Range("BL16:BU16"), Type:=xlFillDefault

Range("BL16:BU16").Select

Range("BL17").Select

Selection.AutoFill Destination:=Range("BL17:BU17"), Type:=xlFillDefault

Range("BL17:BU17").Select

Range("BL18").Select

Selection.AutoFill Destination:=Range("BL18:BU18"), Type:=xlFillDefault  
 Range("BL18:BU18").Select  
 Range("BL19").Select  
 Selection.AutoFill Destination:=Range("BL19:BU19"), Type:=xlFillDefault  
 Range("BL19:BU19").Select  
 Range("BL20").Select  
 Selection.AutoFill Destination:=Range("BL20:BU20"), Type:=xlFillDefault  
 Range("BL20:BU20").Select  
 Range("BL21").Select  
 Selection.AutoFill Destination:=Range("BL21:BU21"), Type:=xlFillDefault  
 Range("BL21:BU21").Select  
 Range("BL22").Select  
 Selection.AutoFill Destination:=Range("BL22:BU22"), Type:=xlFillDefault  
 Range("BL22:BU22").Select  
 Range("BL23").Select  
 Selection.AutoFill Destination:=Range("BL23:BU23"), Type:=xlFillDefault  
 Range("BL23:BU23").Select  
 Range("AP37").Select  
 ActiveCell.FormulaR1C1 = "SOMME score aphm case rempli hétérozygote"  
 Range("AP38").Select  
 ActiveCell.FormulaR1C1 = "=R[-4]C+R[-3]C+R[-2]C+R[-4]C[1]+R[-3]C[1]+R[-2]C[1]"  
 Range("S6").Select  
 ActiveCell.FormulaR1C1 = \_  
 ""="We have identified ""&R[-4]C[-13]&"" in ""(SUM(R[20]C[-15]:R[22]C[-15])+SUM(R[20]C[-12]:R[22]C[-12]))&""  
 patient""&IF((SUM(R[20]C[-15]:R[22]C[-15])+SUM(R[20]C[-12]:R[22]C[-12]))>1, ""s"", """"&""  
 (""&R[32]C[23]&"" index) in heterozygous state  
 ""&IF(SUM(R[28]C[23]:R[30]C[24])>0, ""(associated with pathogenic or likely pathogenic  
 variant""&IF(SUM(R[28]C[23]:R[30]C[24])>0, ""s"", """"&""  
 ""&R[28]C[27]&""""&"" from the Timone Dysferlinopathy  
 cohort.""&IF(SUM(R[20]C[23]:R[25]C[23])>0, R[-3]C[14], """"&IF(SUM(R[20]C[24]:R[25]C[24])>0, R[-2]C[14], """"& \_  
 ""  
 ""  
 Range("AM6").Select  
 ActiveCell.FormulaR1C1 = "=R[2]C[33]"  
 Range("AQ6").Select  
 ActiveCell.FormulaR1C1 = "=R[2]C[30]"  
 Range("AP1").Select  
 ActiveCell.FormulaR1C1 = \_  
 ""="", associated in ""&R[7]C[2]&"" case""&IF(R[7]C[2]>1, ""s"", """"&"" (PMID: ""&R[7]C[24]&"" with  
 an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. """"  
 Range("AP2").Select  
 ActiveCell.FormulaR1C1 = \_

"=", associated in ""&R[6]C[3]&"" case""&IF(R[6]C[3]>1,""s"", """"")&"" (PMID: ""&R[6]C[25]&"" ) with a decrease of Dysferlin on Western Blot / ImmunoHistoChemistry. ""

Range("AP3").Select

ActiveCell.FormulaR1C1 = \_

"=", associated in ""&R[5]C[-1]&"" case""&IF(R[5]C[-1]>1,""s"", """"")&"" (PMID: ""&R[5]C[22]&"" ) with an absence of Dysferlin on Western Blot / ImmunoHistoChemistry. ""

Range("AP4").Select

ActiveCell.FormulaR1C1 = \_

"=", associated in ""&R[4]C&"" case""&IF(R[4]C>1,""s"", """"")&"" (PMID: ""&R[4]C[23]&"" ) with a decrease of Dysferlin on Western Blot / ImmunoHistoChemistry. ""

Range("AP5").Select

Range("S4").Select

ActiveCell.FormulaR1C1 = \_

"=""This variant has previously been reported in heterozygous state in ""&R[4]C[16]&"" case""&IF(R[4]C[16]>1,""s"", """"")&"" in the literature (associated with ""&R[4]C[50]&"" PMID: ""&R[2]C[24]&"").""&IF(R[4]C[22]>0,R[-1]C[23], """"")&IF(R[4]C[23]>0,RC[23], """"")

Range("B2").Select

ActiveWorkbook.Save

Workbooks(fs).Close False

fs = Dir

Loop

End Sub

## ABBREVIATION

ACMG-AMP : American College of Medical Genetics & the Association for Molecular Pathology

ADN : Acide DésoxyriboNucléique

AON : Antisense OligoNucleotide (oligonucléotides antisens)

ARN : Acide RiboNucléique

BP : Benign Poor

BS : Benign Strong

BA : Benign stand Alone

DHPLC : Denaturing High Performance Liquid Chromatography (chromatographie liquide à haute performance sur gel dénaturant)

ESHG : European Society of Human Genetics

FILNEMUS : Filière Nationale des Maladies Rares Neuromusculaires

IHC : ImmunoHistoChimie

LGMD2B : Limb-Girdle Muscular Dystrophy 2B (dystrophie musculaire des ceintures de type 2B)

MLPA : Multiplex Ligation-dependent probe amplification (amplification multiplex de sondes dépendant d'une ligation)

MM : Myopathie de Miyoshi

NGS : Next Generation Sequencing

NMD : Nonsense-Mediated Decay

PCR : Polymerase Chain Reaction (amplification en chaine par polymérase)

PM : Pathogenic Moderate

PP : Pathogenic Poor

PS : Pathogenic Strong

PVS : Pathogenic Very Strong

SSCP : Single Strand Conformation Polymorphism (polymorphisme de conformation des acides nucléiques simple brin)

WB : Western Blot

W.E.S. : Whole-Exome Sequencing (séquençage d'exome entier)

W.G.S. : Whole-Genome Sequencing (séquençage de génome entier)

# Au moment d'être admis à exercer la médecine, je promets et je jure d'être fidèle aux lois de l'honneur et de la probité.

Mon premier souci sera de rétablir, de préserver ou de promouvoir la santé dans tous ses éléments, physiques et mentaux, individuels et sociaux.

Je respecterai toutes les personnes, leur autonomie et leur volonté, sans aucune discrimination selon leur état ou leurs convictions. J'interviendrai pour les protéger si elles sont affaiblies, vulnérables ou menacées dans leur intégrité ou leur dignité. Même sous la contrainte, je ne ferai pas usage de mes connaissances contre les lois de l'humanité.

J'informerai les patients des décisions envisagées, de leurs raisons et de leurs conséquences.

Je ne tromperai jamais leur confiance et n'exploiterai pas le pouvoir hérité des circonstances pour forcer les consciences.

Je donnerai mes soins à l'indigent et à quiconque me les demandera. Je ne me laisserai pas influencer par la soif du gain ou la recherche de la gloire.

Admis dans l'intimité des personnes, je tairai les secrets qui me seront confiés. Reçu à l'intérieur des maisons, je respecterai les secrets des foyers et ma conduite ne servira pas à corrompre les mœurs.

Je ferai tout pour soulager les souffrances. Je ne prolongerai pas abusivement les agonies. Je ne provoquerai jamais la mort délibérément.

Je préserverai l'indépendance nécessaire à l'accomplissement de ma mission. Je n'entreprendrai rien qui dépasse mes compétences. Je les entretiendrai et les perfectionnerai pour assurer au mieux les services qui me seront demandés.

J'apporterai mon aide à mes confrères ainsi qu'à leurs familles dans l'adversité.

Que les hommes et mes confrères m'accordent leur estime si je suis fidèle à mes promesses ; que je sois déshonoré et méprisé si j'y manque.





# Résumé

## Anglais

Recent evolution of sequencing technologies and the development of international standards in variant interpretation have profoundly changed the diagnostic approaches in clinical genetics. As a consequence, many variants that were initially claimed to be disease-causing have been reclassified in light of the new data available. Unfortunately, the misclassified variants are still present in the scientific literature and variant databases, greatly interfering with interpretation of diagnostic sequencing results. Despite the urgent need, large-scale efforts to update the classifications of these variants are still not sufficient. We have retrospectively analyzed 176 *DYSF* gene variants that were identified in dysferlinopathy patients referred to the Marseille Medical Genetics Department for diagnostic sequencing since 2001. In parallel, we tested the diagnostic capabilities of the new classification tools. Since the majority of these variants were returned before implementation of ACMG classifications (American College of Medical Genetics and Genomics, 2015), we re-classified them into five-tier pathogenicity classes, leading to downgraded pathogenicity for 18 variants. We then updated the information for the variants that have been previously published and submitted to variant databases. We also submitted 35 novel *DYSF* variants to a variant database (LOVD, Leiden Online Variation Database). The new classification tools should remain only a diagnostic aid because their margin of error is too large. Besides direct benefit for dysferlinopathy diagnostics, our study contributes to the much needed effort to re-analyze variants from previously published cohorts and to work with curators of variant databases to update the entries for erroneously classified variants.

## Français

L'arrivée récente du NGS et le développement de recommandations internationales pour l'interprétation des variants ont profondément changé l'approche diagnostique en génétique clinique. Par conséquent beaucoup de variants initialement considérés comme pathogènes ont été déclassés à la lumière de ces nouvelles données. Malheureusement des variants mal classés sont toujours présents dans la littérature et les bases de données, ce qui interfère grandement avec l'interprétation des résultats de séquençage. Malgré l'urgence, les efforts à grande échelle pour mettre à jour les classifications de ces variants ne sont toujours pas suffisants. Nous avons reclassé rétrospectivement 176 variants du gène *DYSF* identifiés depuis 2001 chez les patients français atteints de dyferlinopathies dans la cohorte du Département de Génétique Médicale de Marseille. En parallèle, nous avons testé les capacités d'aide au diagnostic des nouveaux outils de classification. Comme la majorité des variants a été initialement classée avant l'implémentation de la classification ACMG (American College of Medical Genetics and Genomics, 2015), ce sont 18 de ces variants qui ont été reclassés en variants de signification inconnue et (probablement) bénins. Nous avons alors mis à jour les informations de ces variants dans les bases de données, et soumis les 35 nouveaux variants dans la base de données LOVD (Leiden Online Variation Database). Les nouveaux outils de classification ne doivent rester qu'une aide au diagnostic car leur marge d'erreur est trop importante. Outre le bénéfice direct pour le diagnostic des dysferlinopathies, notre étude contribue à l'effort nécessaire pour réanalyser les variants des cohortes publiées précédemment et pour travailler avec les curateurs des bases de données de variants afin de mettre à jour les entrées des variants classés de manière erronée.

Mots clés : reclassification, variant faux-sens, hotspot mutationnel, exonique, épissage, DYSFERLINE, *DYSF*, LGMD2B, MIYOSHI MYOPATHY, maladies neuromusculaires