Involvement of muscle satellite cell dysfunction in neuromuscular disorders: Expanding the portfolio of satellite cell-opathies

Massimo Ganassi, Peter S. Zammit

King’s College London, Randall Centre for Cell and Molecular Biophysics, Guy’s Campus, London, UK.

Abstract

Neuromuscular disorders are a heterogeneous group of acquired or hereditary conditions that affect striated muscle function. The resulting decrease in muscle strength and motility irreversibly impacts quality of life. In addition to directly affecting skeletal muscle, pathogenesis can also arise from dysfunctional crosstalk between nerves and muscles, and may include cardiac impairment. Muscular weakness is often progressive and paralleled by continuous decline in the ability of skeletal muscle to functionally adapt and regenerate. Normally, the skeletal muscle resident stem cells, named satellite cells, ensure tissue homeostasis by providing myoblasts for growth, maintenance, repair and regeneration. We recently defined 'Satellite Cell-opathies' as those inherited neuromuscular conditions presenting satellite cell dysfunction in muscular dystrophies and myopathies (doi:10.1016/j.yexcr.2021.112906). Here, we expand the portfolio of Satellite Cell-opathies by evaluating the potential impairment of satellite cell function across all 16 categories of neuromuscular disorders, including those with mainly neurogenic and cardiac involvement. We explore the expression dynamics of myopathogenes, genes whose mutation leads to skeletal muscle pathogenesis, using transcriptomic analysis. This revealed that 45% of myopathogenes are differentially expressed during early satellite cell activation (0 - 5 hours). Of these 271 myopathogenes, 83 respond to Pax7, a master regulator of satellite cells. Our analysis suggests possible perturbation of satellite cell function in many neuromuscular disorders across all categories, including those where skeletal muscle pathology is not predominant. This characterisation further aids understanding of pathomechanisms and informs on development of prognostic and diagnostic tools, and ultimately, new therapeutics.

Key Words: Muscle stem cell; satellite cell; neuromuscular disorder; primary; secondary; neuropathy; cardiomyopathy; satellite cell-opathy; myopathogene.
Satellite cell-opathies within neuromuscular disorders

Eur J Transl Myol 32 (1): 10064, 2022 doi: 10.4081/ejtm.2022.10064

Satellite cell dynamics during muscle growth and regeneration.

Satellite cells (SC) normally lay mitotically quiescent (green) between the basal lamina and plasmalemma of adult myofibres. In response to growth cues, trauma or injury (stimulus; blue arrowhead), quiescent satellite cells (green) activate (yellow) and proliferate to generate a population of muscle progenitor cells (MPCs) called myoblasts (orange). The majority of myoblasts enter the differentiation program to become myocytes (red) that will fuse either to pre-existing multinucleated muscle fibres, or together to form new myofibres. A fraction of satellite cell progeny withdraws from cell cycle and re-enters quiescence, to self-renew and reconstitute the stem cell pool, ensuring regenerative potential through life.

Differentiate and fuse either to pre-existing multinucleated myofibres, or to one another to form new myofibres (Figure 1). Simultaneously, a proportion of myoblasts instead commit to self-renewal to replenish the stem cell pool, providing life-long potential for muscle homeostasis, adaptation, repair and regeneration (Figure 1). Hence, loss-of-function in genes/proteins involved in satellite cell function can also lead to neuromuscular conditions.

Satellite Cell-opathies

We recently defined those muscle disorders where mutation in a myopathogene causes direct satellite cell dysfunction, thereby contributing to pathology, as Satellite Cell-opathies. Neur muscular conditions in which the mutation does not directly affect satellite cells are considered as non-satellite cell-opathy neuromuscular disorders. Diagnostic genome/exome sequencing broadened the classification of muscle conditions, often providing a precise genotype-to-phenotype correlation and expanding the range of molecular dysfunctions beyond the notional loss of myofibre integrity. To date, the pathogenesis of several muscle diseases has been experimentally linked to defects in satellite cells, due to the pathogenic mutation(s) affecting genes/proteins regulating satellite cell function.

Primary Satellite Cell-opathies

We defined Primary Satellite Cell-opathies as neuromuscular conditions in which the pathogenic mutation predominately/exclusively affects satellite cell function, with little/no direct effect on muscle fibres.
Mutations in myopathogenes that affect satellite cell quiescence, activation, proliferation or self-renewal can have a dramatic impact on skeletal muscle health and function. Prototypical is mutations in the transcription factor PAX7, a master regulator of satellite cell function, associated with Progressive congenital myopathy with scoliosis (MYOSCO, OMIM: 618578). Likewise, myopathogene mutations altering the ability of satellite cell progeny MPC/myoblasts to differentiate and/or fuse are likely to impede satellite cell-driven myofibre repair/replacement, alter growth and impair muscle adaptation. Mutations in genes controlling myoblast fusion such as MYOMAKER (MYMK), causing Carey-Fineman-Ziter syndrome (CFZS, OMIM: 254940) is archetypical.

Based on our analysis and/or the literature, we suggested that mutations in Pax7, SeleNon (formerly Sepn1), MEGF10, MYOD, MYMK, MYMX and probably Jag2 could cause disorders classified as Primary Satellite Cell-opathies.3 The associated neuromuscular diseases: MYOSCO (PAX7), Rigid Spine Muscular Dystrophy 1 (RSMD1; OMIM: 602771), Myopathy, Areflexia, Respiratory Distress, And Dysphagia, Early-Onset (EMARDD; OMIM: 614399) (MEGF10), Myopathy, Congenital, With Diaphragmatic Defects, Respiratory Insufficiency, And Dysmorphic Facies (MYODRIF; OMIM: 618975)14-10 (MYOD) and CFZS (MYMK) have clinical features in common. Primary Satellite Cell-opathies have congenital onset, and are characterised by general hypotonia, with facial, respiratory and trunk muscles particularly affected, but normal/mildly elevated serum levels of Creatine Kinase (CK). Muscle biopsies often show limited dystrophic features but altered satellite cell numbers.3

Secondary Satellite Cell-opathies

We classified Secondary Satellite Cell-opathies as those neuromuscular disorders where both satellite cells and muscle fibres are affected by the mutated myopathogene. Our analysis and/or the literature identified Secondary Satellite Cell-opathies caused by mutations in myopathogenes CAPN3 causing Muscular dystrophy, limb-girdle, autosomal dominant 4 (LGMD4D, OMIM: 618129) and LGMD Recessive 1 (LGMDR1, OMIM: 253600), LAMA2 associated with Muscular Dystrophy, Congenital Merosin-Deficient, 1a (MDC1A; OMIM: 607855) and LGMDR23 (OMIM: 618138), TTN in Myopathy, Myofibrillar, 9, with Early Respiratory Failure (MFM9; OMIM: 603689), COL6A1 and COL6A2 underlying Bethlem myopathy 1 (BTHLM1;OMIM: 158810) and Ulrich congenital muscular dystrophy 1 (UCMD1; OMIM: 254090) and MTMI in Myopathy, Centronuclear, X-Linked (CNMX or XLMTM/MTM1; OMIM: 310400). Secondary Satellite Cell-opathies display common clinical features, with alterations to myofibres/muscle such as size variation, necrosis and/or fat/immune infiltrates, together with altered satellite cell activity and/or number, and mild to elevated serum CK levels.3 Facioscapulohumeral muscular dystrophy 1 (FSHD1, OMIM: 158900) and FSHD2 (OMIM: 158901) can also be classified as Secondary Satellite Cell-opathies since genomic changes cause aberrant expression of the transcription factor DUX4 that affects both satellite cells and myofibres through gain-of-function mechanism. FSHD is characterised by progressive descending muscle weakness and wasting, concomitant with low levels of myofibre regeneration,17 indicating poor satellite cell function. Genetically, FSHD1 associates with substantial deletion of 3.3 kb D4Z4 units in the sub-telomere of chromosome 4q35,18,19 causing DUX4 upregulation which affects cell viability.20-22 However, transcriptomic analysis also reveals repression of PAX7-target genes that better correlates with disease progression than expression of DUX4-target genes.23,24 This implies that FSHD pathogenesis may have a component arising from FSHD/PAX7 mutual inhibition in satellite cells, likely facilitated by their highly similar homeodomain sequences.25

Further Candidate Satellite cell-opathies within the Full Range of Neuromuscular Disorders

Neuromuscular disorders encompass a wide range of conditions with the common feature of impairing skeletal muscle function: either directly affecting the muscle itself, or indirectly via affecting the peripheral nervous system and/or neuromuscular junctions. Muscle function depends on motor innervation at the neuromuscular junction (NMJ), the synapse that connects the motor neuron to the myofibre allowing conversion of electrical impulses generated by the nervous system, ultimately into force output.26,27 In turn, NMJ function is determined by formation and maintenance of its structure at the motor endplate through signals between nerve and muscle cell. Notably, NMJ and satellite cells are mutually dependent. Early studies demonstrate that satellite cells are enriched at the postsynaptic motor endplate in some muscles, suggesting an active role in NMJ homeostasis/repair.28 Indeed, depletion of satellite cells not only impairs myofibre repair, but also severely alters NMJ maintenance and its ability to regenerate properly after injury.29,30 Precocious activation of satellite cells severely delays reconstitution of NMJs, whereas chronic denervation blunts satellite cell-driven myonuclear addition to myofibres, with activated satellite cells undergoing defective regeneration or apoptosis.31. Hence, as satellite cells and NMJ mutually influence each other, it is likely that pathogenic mutations impairing the function of a gene/protein involved in satellite cell biology may also affect motor neurones and NMJ activity, and vice versa.

An example is Familial Amyotrophic lateral sclerosis 1 (ALS1; OMIM: 105400), where mutations in many genes, including SOD1, cause progressive motor neuron degeneration, leading to muscle weakness and wasting.
However, ALS1 patient-derived satellite cells also fail to undergo efficient myogenesis, suggesting that stem cell dysfunction may contribute to pathology, perhaps exacerbating NMJ dysfunction.\textsuperscript{32} Similarly, mutations in the Survival Motor Neuron protein (SMN1) associate with Spinal muscular atrophies (SMAs1-4; OMIMs: 253300, 253550, 253400, 271150) where loss of motor neurons causes muscle atrophy and weakness.\textsuperscript{33} Recent findings highlight that loss of SMN1 expression/function alters the fusion ability of myoblasts and expression of the fusogenic proteins MYOMAKER and MYOMIXER,\textsuperscript{34,35} likely impacting myofibre size and maintenance.

Myocardial involvement is frequent in patients affected by neuromuscular disorders and is the main cause of death in some conditions.\textsuperscript{36-38} Cardiomyopathies presenting with skeletal muscle involvement and associated neuromuscular symptoms are common, and in

---

[Image: Diagram showing distribution amongst neuromuscular disease classes of the 271 myopathogens differentially expressed during early satellite cell activation and their response to Pax7.]

Fig 2. Distribution amongst neuromuscular disease classes of the 271 myopathogens differentially expressed during early satellite cell activation and their response to Pax7.
we interrogate a wider selection of neuromuscular diseases, including those with neural and cardiac impairment, to define further disorders that fit within our new categorisation of Satellite Cell-opathies. We first retrieved all myopathogenes whose pathogenic variants are associated with hereditary neuromuscular conditions from the 2021 Gene Table of Neuromuscular Disorders (www.musclegenetable.fr). The list comprises 608 genes divided into 16 categories: muscular dystrophies, congenital muscular dystrophies, congenital myopathies, distal myopathies, other myopathies, myotonic syndromes, ion channel muscle diseases, malignant hyperthermia, metabolic myopathies, hereditary cardiomyopathies, congenital myasthenic syndromes, motor-neuron diseases, hereditary ataxias, hereditary motor and sensory neuropathies, hereditary paraplegias and other neuromuscular disorders (Figures 2 and 3). Some myopathogenes are associated with more than one disease (Figure 2).

Next, we evaluated the expression dynamics of these 608 myopathogenes in early satellite cell function exploiting transcriptomic analysis and comparison of publicly available datasets. Our analysis revealed that 45% (271/608) myopathogenes have differential expression during the first 5 hours of murine satellite cell activation, suggesting that these mutations may directly influence satellite cell activity, and thus their function in muscle homeostasis (Figure 2A-B). Strikingly, 32% (191/608) of myopathogenes are downregulated within 5 hours from activating stimulus, whereas 13% (80/608) are upregulated (Figure 2B). The remaining 55% (337/608) of myopathogenes do not show differential expression during the analysed time frame (Figure 2B), although we cannot rule out that those may oscillate during the analysed time frame and/or be required in later phases of satellite cell myogenesis.

Since the transcription factor PAX7 is recognised as a master regulator of satellite cells, we evaluated whether the myopathogenes showing differential expression in satellite cells could also be putative PAX7

Fig 2. Distribution amongst neuromuscular disease classes of the 271 myopathogenes differentially expressed during early satellite cell activation and their response to Pax7.
A. Quiescent/activated murine satellite cell gene-sets retrieved from (42) used to analyse involvement of the 608 myopathogenes described in (4) in satellite cell (SC) biology. Transcriptomic changes during early murine satellite cell activation highlights myopathogenes involved in satellite cell quiescence (downregulated between 0 - 5 hours (h); blue) and satellite cell activation (upregulated between 0 - 5 hours (h); orange) from activating stimulus (blue arrowhead). Venn diagram depicts overlap between significant differentially expressed (DE) genes (downregulated in blue circle and upregulated in orange circle) during the first 5 hours (T 0 h vs T 5 h) of satellite cell activation, and the 608 myopathogenes (pink circle). In this 5-hour period, 191 myopathogenes are downregulated and 80 are upregulated in satellite cells, while 337 are not differentially expressed. B. Pie chart showing the percentage of differentially expressed myopathogenes in the 5-hour period (left). Distribution of differentially expressed myopathogenes in satellite cells within each neuromuscular disease category as detailed above the graph, with the number of myopathogenes in each disease category reported (right panel). C. Pie chart (left) and graph (right) report the percentages of satellite cell downregulated differentially expressed myopathogenes responding to induction of Pax7 in murine embryonic stem cells (46) in each disease category. Number of myopathogenes included in each category is shown. D. As per C but reporting satellite cell-upregulated differentially expressed myopathogenes responding to Pax7 induction.

some cases cardiac dysfunction precedes muscular impairment. Mono or binucleate cardiomyocytes are linked together via the intercalated disc to form a functional syncytium. Contraction is initiated by pacemaker cells in the atroventricular node, with heart rate regulated by hormones and parasympathetic/sympathetic innervation. However, despite differing cellular architecture and regenerative capacity, heart and skeletal muscles share nearly identical molecular composition of the contraction machinery, with both equipped with sarcomeres and the Dystrophin-Associated Protein Complex (DAPC). Thus, pathogenic mutations affecting sarcomeric components would impinge on both cardiomyocytes and skeletal myofibres. Indeed, myopathogenes expressed in cardiomyocytes and whose mutation causes a cardiomyopathy, may also be expressed in satellite cells and concomitantly affect their function. This is the case with DAG1 encoding α-DYSTROGLYCAN and β-DYSTROGLYCAN, genes encoding proteins involved in post-translational modification of DYSTROGLYCAN, and DMD (encoding for DYSTROPHIN) all originally thought not to affect satellite cell function but now implicated. Thus, a broader analysis of potential Satellite Cell-opathies across all neuromuscular diseases is warranted.

Expanding the Portfolio of Satellite Cell-opathies
We previously developed a discovery tool to identify potential myopathogenes that we used to define Satellite Cell-opathies. Our multi-modal approach integrates:

1. differential myopathogene expression during satellite cell activation;
2. myopathogene regulation by the satellite cell-specific transcription factor PAX7;
3. determination of whether satellite cells are affected in the associated human disease and animal models.

Here, to expand the portfolio of Satellite Cell-opathies, we interrogate a wider selection of neuromuscular diseases, including those with neural and cardiac impairment, to define further disorders that fit within our new categorisation of Satellite Cell-opathies. We first retrieved all myopathogenes whose pathogenic variants are associated with hereditary neuromuscular conditions from the 2021 Gene Table of Neuromuscular Disorders (www.musclegenetable.fr). The list comprises 608 genes divided into 16 categories: muscular dystrophies, congenital muscular dystrophies, congenital myopathies, distal myopathies, other myopathies, myotonic syndromes, ion channel muscle diseases, malignant hyperthermia, metabolic myopathies, hereditary cardiomyopathies, congenital myasthenic syndromes, motor-neuron diseases, hereditary ataxias, hereditary motor and sensory neuropathies, hereditary paraplegias and other neuromuscular disorders (Figures 2 and 3). Some myopathogenes are associated with more than one disease (Figure 2).

Next, we evaluated the expression dynamics of these 608 myopathogenes in early satellite cell function exploiting transcriptomic analysis and comparison of publicly available datasets. Our analysis revealed that 45% (271/608) myopathogenes have differential expression during the first 5 hours of murine satellite cell activation, suggesting that these mutations may directly influence satellite cell activity, and thus their function in muscle homeostasis (Figure 2A-B). Strikingly, 32% (191/608) of myopathogenes are downregulated within 5 hours from activating stimulus, whereas 13% (80/608) are upregulated (Figure 2B). The remaining 55% (337/608) of myopathogenes do not show differential expression during the analysed time frame (Figure 2B), although we cannot rule out that those may oscillate during the analysed time frame and/or be required in later phases of satellite cell myogenesis.

Since the transcription factor PAX7 is recognised as a master regulator of satellite cells, we evaluated whether the myopathogenes showing differential expression in satellite cells could also be putative PAX7
target genes. We interrogated a publicly available dataset reporting transcriptomic changes in murine embryonic stem cells (ESCs) engineered to express Pax7 upon Doxycycline treatment. Expression of 41% (33/80) of satellite cell-upregulated myopathogenes and 26% (50/191) of satellite cell-downregulated myopathogenes changed in response to Pax7 accumulation, suggesting that they may have a direct role in satellite cell activity (Figure 2 C-D).

Finally, for our selected myopathogenes, we examined satellite cell numbers/function in the associated neuromuscular disease and/or animal models, as published data permitted. Below, we describe examples of satellite cell dysfunction in several conditions, and provide evidence of intimate connections among neuronal, cardiac and skeletal muscle tissues in the view of specific gene expression. Thus, satellite cell dysfunction may contribute to pathogenesis in many neuromuscular disorders, even in diseases where skeletal muscle impairment is not the predominant symptom. For example, the mutation may unbalance the homeostatic connection between satellite cells and neuromuscular components, further expanding the classification of Satellite cell-opathies.

---

**Fig 3. Myopathogenes differentially expressed during early satellite cell activation and modulated by Pax7.**

Diagram schematises the transcriptomic dataset from 0 - 5 hours of murine satellite cell activation (42, 46) used for the expression analysis of myopathogenes (top). Table reports all differentially expressed myopathogenes showing either downregulation (blue column) or upregulation (orange column) during the 5-hour period from satellite cell activating stimulus, divided into the 16 neuromuscular disease categories (4). Some myopathogenes appear in more than one disease category as they are associated with multiple neuromuscular disorders. For each category, myopathogenes showing expression changes upon Pax7 induction in murine embryonic stem cells are indicated in red text. Myopathogenes that are underlined indicate the 46 myopathogenes in muscular dystrophies, congenital muscular dystrophies, congenital myopathies and distal myopathies whose expression was found differentially regulated during 0 - 5 hours of satellite cell activation in our previous study.
Identifying New Myopathogenes Associated with Muscular Dystrophies and Myopathies and Regulated by PAX7

We previously identified 63 myopathogenes potentially associated with Satellite Cell-opathies within the neuromuscular disease categories of muscular dystrophies, congenital muscular dystrophies, congenital myopathies and distal myopathies by assessing dynamic expression within 0 - 3 hours of murine satellite cell activation and response to PAX7. As expected, this new analysis again highlighted myopathogenes (46/63) that we previously found contributing to Satellite Cell-opathies in these 4 categories. Included were MEGF10 and PAX7 causing Primary Satellite Cell-opathies and DAG1, POMT1, FKTN, POGLUT1, POMK, POMGNT2, ISPD and B4GAT1 (dystroglycanopathies), as well as MTM1 and TTN, all associated with likely Secondary Satellite Cell-opathies (Figure 3). Our expanded analysis encompassing differential regulation during 0-5 hours of satellite cell activation also identifies 8 new myopathogenes in these 4 categories that could affect satellite cell function, namely FH1L1, MPDU1, RXYLT1, COL12A1, LARGE1, RYR3, ACTN2 and MYPN (Figure 3). Importantly, most of these myopathogenes (except RXYLT1 and LARGE1) are regulated by PAX7, which given the role of PAX7 in satellite cell biology, suggests that many could fit the Satellite Cell-opathy category (Figure 2C-D and Figure 3). Intriguingly, 13/17 myopathogenes differentially regulated during satellite cell activation in the disease category ‘Congenital Myopathy’ are regulated by PAX7 (Figure 3).

Myopathogenes Associated with all 16 Neuromuscular Disease Categories are Expressed in Satellite Cells

Overall our new analysis reveals a further 225 (225/271) myopathogenes that may contribute to satellite cell dysfunction in many neuromuscular diseases (Figure 3). For example, in the Myotonic Syndrome class is DMPK, and its pathogenic variants associate with Myotonic Dystrophy 1 (DM1; OMIM: 160900), which presents with myotonia, weakness at distal muscles that later progresses proximally and normal to mildly elevated serum CK. DM1 resembles features of MYOSCO and CFZS such as involvement of facial muscles and respiratory distress, especially in the congenital form. Muscle biopsies display limited myofibre damage/alterations, but increased number of satellite cells due to reduced proliferative capacity and poor myogenic potential, suggesting a dysfunction that would classify DM1 as Satellite Cell-opathy, most likely Secondary as DMPK is also expressed in myofibres. DM1 patients may present concomitant cardiac dysfunction and neuropathy. Indeed, even diseases with prevalent neurogenic dysfunction, such as motor neuron diseases, ataxias and neuropathies, are associated with genes showing differential expression in satellite cells that respond to PAX7 upregulation (Figure 2C - D).

Myopathogenes Encoding Proteins of the DAPC may also Affect Satellite Cells

Primary and Secondary Satellite cell-opathies are diseases where muscle is predominantly affected, but other neuromuscular disorders display concomitant dysfunction of neuromuscular components. Disruption of the NMJ leads to defective neurotransmission from the motor neurons and consequent decline in muscle force production. In parallel, myofibres exhibit alterations such as muscle fibre type transition observed in myopathies, or atrophy, more characteristic of dystrophic muscles; hence it is not surprising that NMJ dysfunction is common in neuromuscular disorders, correlating with decreased muscle function and integrity. DAG1 is a myopathogene identified as being regulated during satellite cell activation and responding to PAX7 (Figure 3). DAG1 encodes a precursor polypeptide that by post-translational cleavage generates α-DYSTROGLYCAN and β-DYSTROGLYCAN, central components of the DAPC. α-DYSTROGLYCAN activity is modulated by glycosylation, which regulates its interaction with several ligands. Notably, α-DYSTROGLYCAN hypo-glycosylation causes dystroglycanopathies, a class of congenital muscular dystrophies that are classified depending on whether the causative mutation is in DAG1 itself (primary) or in genes encoding for α-DYSTROGLYCAN-glycosylating enzymes (secondary or tertiary). Patients affected by dystroglycanopathy often present dysfunctional NMJ, demonstrating the importance of α-DYSTROGLYCAN and the DAPC. α-DYSTROGLYCAN is expressed in both myofibres and satellite cells, suggesting that its loss-of-function could affect either cell type, and impact directly or indirectly on the NMJ, especially given the function of α-DYSTROGLYCAN in the nervous system. Satellite cells and NMJ activity could also be compromised in secondary and tertiary dystroglycanopathies, since our analysis confirmed dynamic expression of α-DYSTROGLYCAN-glycosylating enzymes in early satellite cell myogenesis (Figures 2 and 3). For example, satellite cell-specific deletion of Fukutin (Fktn) in mouse leads to a more severe muscle wasting than when Fktn is deleted in myofibres, resembling the phenotype of Muscular Dystrophy-Dystroglycanopathy (Congenital With Brain And Eye Anomalies), Type A, 4 (MDDGA4) (OMIM: 253800). However, Fukutin also functions in synapse formation and Fktn-deficient mice have impaired NMJs. FKTN mutation in human can cause dilated cardiomyopathy (Figure 3), implying a role for FUKUTIN in the cardiac/neuromuscular apparatus. Loss of POGLUT1 (protein O-glucosyltransferase 1) causes LGMDR21 (OMIM: 617232), with a severe reduction in

Identifying New Myopathogenes Associated with Muscular Dystrophies and Myopathies and Regulated by PAX7

We previously identified 63 myopathogenes potentially associated with Satellite Cell-opathies within the neuromuscular disease categories of muscular dystrophies, congenital muscular dystrophies, congenital myopathies and distal myopathies by assessing dynamic expression within 0 - 3 hours of murine satellite cell activation and response to PAX7. As expected, this new analysis again highlighted myopathogenes (46/63) that we previously found contributing to Satellite Cell-opathies in these 4 categories. Included were MEGF10 and PAX7 causing Primary Satellite Cell-opathies and DAG1, POMT1, FKTN, POGLUT1, POMK, POMGNT2, ISPD and B4GAT1 (dystroglycanopathies), as well as MTM1 and TTN, all associated with likely Secondary Satellite Cell-opathies (Figure 3). Our expanded analysis encompassing differential regulation during 0-5 hours of satellite cell activation also identifies 8 new myopathogenes in these 4 categories that could affect satellite cell function, namely FH1L1, MPDU1, RXYLT1, COL12A1, LARGE1, RYR3, ACTN2 and MYPN (Figure 3). Importantly, most of these myopathogenes (except RXYLT1 and LARGE1) are regulated by PAX7, which given the role of PAX7 in satellite cell biology, suggests that many could fit the Satellite Cell-opathy category (Figure 2C-D and Figure 3). Intriguingly, 13/17 myopathogenes differentially regulated during satellite cell activation in the disease category ‘Congenital Myopathy’ are regulated by PAX7 (Figure 3).

Myopathogenes Associated with all 16 Neuromuscular Disease Categories are Expressed in Satellite Cells

Overall our new analysis reveals a further 225 (225/271) myopathogenes that may contribute to satellite cell dysfunction in many neuromuscular diseases (Figure 3). For example, in the Myotonic Syndrome class is DMPK, and its pathogenic variants associate with Myotonic Dystrophy 1 (DM1; OMIM: 160900), which presents with myotonia, weakness at distal muscles that later progresses proximally and normal to mildly elevated serum CK. DM1 resembles features of MYOSCO and CFZS such as involvement of facial muscles and respiratory distress, especially in the congenital form. Muscle biopsies display limited myofibre damage/alterations, but increased number of satellite cells due to reduced proliferative capacity and poor myogenic potential, suggesting a dysfunction that would classify DM1 as Satellite Cell-opathy, most likely Secondary as DMPK is also expressed in myofibres. DM1 patients may present concomitant cardiac dysfunction and neuropathy. Indeed, even diseases with prevalent neurogenic dysfunction, such as motor neuron diseases, ataxias and neuropathies, are associated with genes showing differential expression in satellite cells that respond to PAX7 upregulation (Figure 2C - D).

Myopathogenes Encoding Proteins of the DAPC may also Affect Satellite Cells

Primary and Secondary Satellite cell-opathies are diseases where muscle is predominantly affected, but other neuromuscular disorders display concomitant dysfunction of neuromuscular components. Disruption of the NMJ leads to defective neurotransmission from the motor neurons and consequent decline in muscle force production. In parallel, myofibres exhibit alterations such as muscle fibre type transition observed in myopathies, or atrophy, more characteristic of dystrophic muscles; hence it is not surprising that NMJ dysfunction is common in neuromuscular disorders, correlating with decreased muscle function and integrity. DAG1 is a myopathogene identified as being regulated during satellite cell activation and responding to PAX7 (Figure 3). DAG1 encodes a precursor polypeptide that by post-translational cleavage generates α-DYSTROGLYCAN and β-DYSTROGLYCAN, central components of the DAPC. α-DYSTROGLYCAN activity is modulated by glycosylation, which regulates its interaction with several ligands. Notably, α-DYSTROGLYCAN hypo-glycosylation causes dystroglycanopathies, a class of congenital muscular dystrophies that are classified depending on whether the causative mutation is in DAG1 itself (primary) or in genes encoding for α-DYSTROGLYCAN-glycosylating enzymes (secondary or tertiary). Patients affected by dystroglycanopathy often present dysfunctional NMJ, demonstrating the importance of α-DYSTROGLYCAN and the DAPC. α-DYSTROGLYCAN is expressed in both myofibres and satellite cells, suggesting that its loss-of-function could affect either cell type, and impact directly or indirectly on the NMJ, especially given the function of α-DYSTROGLYCAN in the nervous system. Satellite cells and NMJ activity could also be compromised in secondary and tertiary dystroglycanopathies, since our analysis confirmed dynamic expression of α-DYSTROGLYCAN-glycosylating enzymes in early satellite cell myogenesis (Figures 2 and 3). For example, satellite cell-specific deletion of Fukutin (Fktn) in mouse leads to a more severe muscle wasting than when Fktn is deleted in myofibres, resembling the phenotype of Muscular Dystrophy-Dystroglycanopathy (Congenital With Brain And Eye Anomalies), Type A, 4 (MDDGA4) (OMIM: 253800). However, Fukutin also functions in synapse formation and Fktn-deficient mice have impaired NMJs. FKTN mutation in human can cause dilated cardiomyopathy (Figure 3), implying a role for FUKUTIN in the cardiac/neuromuscular apparatus. Loss of POGLUT1 (protein O-glucosyltransferase 1) causes LGMDR21 (OMIM: 617232), with a severe reduction in
satellite cell numbers and impaired muscle regeneration, suggesting its classification as a Secondary Satellite Cell-opathy. POGlut1 also contributes to glycosylation of NOTCH1, an important regulator of both satellite cell and NMJ function, strengthening the hypothesis that neuromuscular disorders presenting NMJ impairment may have concurrent satellite cell dysfunction and vice versa.

Our new analysis reveals that the glycosylating enzyme LARGE1 is upregulated during satellite cell activation, suggesting that the congenital MDDGA6 (OMIM: 613154) caused by LARGE1 mutations, could be classified as a Satellite Cell-opathy. In line with this, a murine model of MDDGA6 presents increased satellite cell activation with reduced proliferation capacity compared to wild-type control. Thus, dystroglycanopathies can be considered Satellite cell-opathies.

Although not found in our analysis, the myopathogene DYSTROPHIN is implicated in satellite cell function, and so Duchenne Muscular dystrophy (DMD; OMIM: 310200) is a potential Satellite Cell-opathy. DYSTROPHIN is an essential component of the DAPC that connects the contractile apparatus of the myofibres to the extra-cellular matrix, ensuring myofibre integrity during contraction. DYSTROPHIN is also expressed in satellite cells and is reported to actively regulate their asymmetric division, maintaining the stem cell niche. DMD is characterised by muscle atrophy with fibrotic/fat infiltrations resulting from chronic muscle regeneration. However, muscle weakness is also attributed to severe deficits at the NMJ, observed both in patients and animal models. DYSTOPHIN accumulates at the NMJ, where the complex assists with maintenance of the motor endplate ensuring muscle excitability and optimal neurotransmission. Notably, muscle wasting in DMD biopsies is accompanied by increase in the number of PAX7-positive cells which could affect proper NMJ function/maintenance. Pathogenic mutations in DYSTROPHIN are also associated with Becker muscular dystrophy (BMD; OMIM: 300376), where NMJ dysfunction is also common. Thus, pathogenic DYSTROPHIN mutations alter the homeostasis of myofibres, satellite cells and neuromuscular junctions.

Candidate Satellite Cell-opathies with Neurogenic Features

Neuromuscular disorders presenting mainly with neurogenic impairment may also have satellite cell dysfunction, so could be candidate Satellite Cell-opathies. In congenital myasthenic syndromes, NMJ dysfunction is caused by pathogenic mutations in genes directly involved in NMJ development and function, leading to early onset progressive muscle weakness (50). We report here that 26 myopathogenes associated with congenital myasthenic syndromes show differential regulation during early satellite cell activation, with several including CHRND, MUSK, DOK7, SCN4A, SYNE1, TGFβ3 and JUP also responding to Pax7 induction (Figures 2 and 3), further suggesting interplay between satellite cells and the NMJ. Thus, compromised satellite cell function may also be directly involved in the pathogenesis of congenital myasthenic syndromes. Although myasthenia gravis is an autoimmune disease, there is an increased number of PAX7-positive cells in muscle biopsies from patients, indicating satellite cell dysfunction (71) and muscle fibres may be directly affected (72), suggesting that perturbed NMJ function may also indirectly affect satellite cells.

We also found 34 myopathogenes differentially regulated during satellite cell activation in the Motor Neurone Disease category of neuromuscular disorders, with some being controlled by Pax7 (Figure 3). For example, ALS is characterised by motor neural death and compromised NMJs resulting from proteostatic imbalance and impaired unfolded protein response (UPR) involving several genes/proteins. Strikingly, our new analysis shows that ALS myopathogenes Neki, Tub4a4, Sqstm1, Tia1, Hspb1, Cryab and Sigmal are differentially regulated in satellite cells (Figure 2 and Figure 3). This indicates that ALS may have underlying satellite cell dysfunction, as suggested by recent studies. Our analysis also shows that other Motor Neurone Diseases may exhibit concomitant motor neuron, NMJ and satellite cell dysfunction due to Hspb1 mutations in Neuronopathy, distal hereditary motor, type IIB (Hmn2b; OMIM: 608634), Hspb8 mutations in Charcot-Marie-Tooth disease (Cmt2l; OMIM: 608673), Bag3 mutations in myofibrillar myopathy 6 (Mfm6; OMIM: 612954), Sqstm1 mutations in Myopathy, distal, with rimmed vacuoles (Dmrv; Omim 617158) and Tia1 mutations in Wandel distal myopathy (Wdm; Omim: 604454) (Figures 2 and 3), so all may also have satellite cell dysfunction and be Satellite Cell-opathies.
Satellite cell-opathies within neuromuscular disorders
Eur J Transl Myol 32 (1): 10064, 2022 doi: 10.4081/ejtm.2022.10064

inefficient muscle regeneration, likely due to poor satellite cell activation, consistent with mutant GAA expression in satellite cells. GSD2 also display NMJ deterioration. Given the interplay across NMJ and satellite cells, improving satellite cell function may not only ameliorate GSD2 pathogenesis in muscle, but also enhance NMJ function. Notably, cardiomyopathy is observed in GSD2, suggesting a common metabolic/molecular network connecting heart, skeletal muscle and nerves.

Our new analysis reveals that other myopathogenes associated with metabolic myopathies are differentially expressed during satellite cell activation and some response to Pax7 including ENO3, FLAD1, PGMI and PRKAG2 (Figure 3) arguing that the related diseases could also present features of Satellite Cell-opathies.

**Satellite Cell-opathies with Cardiac Impairment**
We discovered that circa 40% (43/106) of myopathogenes associated with hereditary cardiomyopathies display dynamic expression during early satellite cell myogenesis (Figure 2). Furthermore, over half of these satellite cell-expressed myopathogenes respond to Pax7 induction, suggesting that pathogenic mutations in genes associated with cardiomyopathies may also impinge on satellite cell function (Figure 3). Conversely, if a mutated myopathogene is found to affect satellite cell function, and it is also expressed in heart, it may also adversely affect cardiomyocyte function. Prototypes are myopathogenes such as LMNA and TTN causing muscular dystrophies or myopathies that we classed as Satellite Cell-opathies, which also associate with neuromuscular disorders presenting mainly cardiomyopathic phenotypes.

Contraction of cardiac and skeletal muscle elicits changes in gene expression through mechanical stimuli. The nuclear envelope is a pivotal player in mechanotransduction, nuclear stability and chromatin organisation, so pathogenic mutations altering the nuclear envelope may have profound effects on overall muscle health. Lamin A and C, encoded by the LMNA gene, are nuclear intermediate filament proteins that contribute to nuclear architectural integrity and function as part of the nuclear lamina, which contributes to orchestrating gene expression. Mutations in LMNA are associated with a variety of neuromuscular conditions including cardiomyopathies, presenting cardiac conduction deficiency and hypertrophy in Cardiomyopathy, Dilated, 1A (CMD1A; OMIM: 115200), motor-neuropathy in CMT2B1 (OMIM: 605588) and three muscular dystrophies with both skeletal and cardiac phenotypes in Emery-Dreifuss Muscular Dystrophy 2, Autosomal Dominant (EDMD2, OMIM: 181350), Emery-Dreifuss Muscular Dystrophy 3, Autosomal Recessive (EDMD3, OMIM: 616516) and Muscular Dystrophy, Congenital, Lmna-Related (MDCL, OMIM: 613205). Hence, mutant LaminA/C alters performance of both striated muscle and nerve, with cardiac dysfunction and muscle weakness often present together. We have previously defined MDCL as a bone-tide Secondaty Satellite Cell-opathies, given the role/expression of LMNA in satellite cells and myofibres. Expression analysis is consistent, revealing that LMNA expression increases as satellite cells exit from quiescence, in line with nuclear remodelling and augmented transcriptional activity (Figure 2). Furthermore, LMNA expression dynamically responds to Pax7 modulation (Figure 3). Of note, EDMD2 patients display increased numbers of PAX7-positive cells. Despite the molecular/cellular mechanism for such an increase remaining unclear, Lmna-null murine satellite cells activate poorly, have reduced proliferation and inefficient differentiation. Similarly, human MDCL satellite cells fail to fuse in vitro, whereas overexpression of pathogenic missense Lamin A/C variants in healthy myoblasts alters fusion and blunts expression of fusogens MyoD and MyoG. Lmna-null murine myofibres also have less myonuclei suggesting reduced fusion of satellite cell-derived myoblasts. Together, these observations may explain why dysfunctional satellite cells accumulate in EDMD2 muscles. Notably, Lmna-null mouse models have alterations in NMJ structure, resembling the EDMD2 muscle phenotype. Finally, given that NMJ and satellite cells show mutual and synergic influence and that loss of LMNA can functionally impair both, it is likely that even CMT2B1 neuropathy, where motor neuron conductivity is altered, may also have satellite cell dysfunction. As skeletal and cardiac muscles share nearly identical contractile apparatus, pathogenic variants of a gene involved in sarcomere function/maintenance could impinge broadly on the neuromuscular system. Somewhat surprisingly, we find several genes involved in sarcomeres are also differentially expressed during satellite cell activation and react to Pax7 induction, including TTN, MYPN, ACTN2, FLNC and FLNA (Figure 3) suggesting an early role in satellite cell function.

Mutations in TTN encoding TITIN are associated with a wide range of neuromuscular disorders: muscular dystrophy in LGMDR10 (OMIM: 608807), congenital and distal myopathies Myopathy, Myofibrillar, 9, With Early Respiratory Failure (MFM9; OMIM: 603689) and Tibial Muscular Dystrophy, Tardive (TMD, OMIM: 600334), motor neuron disease in Lethal Congenital Contracture Syndrome, and cardiomyopathies in Cardiomyopathy, Familial Hypertrophic, 9 (CMH9; OMIM: 613765) and CMD1G (OMIM: 604145) demonstrating the importance of TITIN function in homeostasis of the neuromuscular apparatus. Consistent with this hypothesis, mice bearing mutation in Ttn have increased satellite cell numbers, and although similar evaluation has not been performed for human, we suggested Titinopathies could also include perturbed satellite cell function. MYPN, encoding for the sarcomeric component MYOPALLADIN, is upregulated in the 0 - 5 hour time-
frame, and also regulated by Pax7 (Figure 3), suggesting that diseases associated with MYPN mutations (several cardiomyopathies including CMD1KK (OMIM: 615248) and a congenital form of slowly progressing nemaline myopathy with myofibre size variation and evident atrophy (NEM1; OMIM: 617336) may present satellite cells dysfunction. CAV3 is also notable as a myopathogene associated with hereditary cardiomyopathies that is differentially expressed during satellite cell activation and regulated by Pax7 (Figure 3). Mutations in CAV3 associate with CMH1 (OMIM: 192600). However, CAV3 mutations also lead to skeletal muscle disorders that can present with cardiomyopathy such as muscular dystrophy Rippling Muscle Disease 2 (RMD2; OMIM: 606072), Myopathy, Distal, Tateyama Type (MPDT; OMIM: 614321) and Creatine Phosphokinase, Elevated Serum (OMIM: 123320) - a myotonic syndrome characterised by persistently elevated serum levels of CK. CAV3 localises persistently to the sarcoplemma along with the DAPC, and contributes to cytoskeletal remodelling during differentiation and later, to myocyte fusion. As CAV3 is upregulated during satellite cell activation (Figure 3), its pathogenic mutation may impinge directly on the ability of satellite cells to regenerate myofibres, although satellite cell status/activity in patients is yet to be reported. Remarkably, given that binucleation in cardiomyocytes occurs by cell fusion in vertebrates, it is plausible that CAV3 dysfunction may also blunt formation of binucleated cardiomyocytes in human. Not only does CAV3 colocalise with the DAPC, but it also contributes to its integrity, as altered CAV3 expression disrupts the complex and result in decreased Dystrophin accumulation. CAV3 is also crucial for NMJ formation and function. Thus, CAV3 dysfunction potentially impacts several cellular structures/populations of the neuromuscular system, and given CAV3 upregulation during satellite cell activation, CAV3-associated conditions are likely to also display features of Satellite Cellopathies. HCN4, KCND3, KCNE1, KCNQ1 and CACNB2 that encode for ion-activated channels involved in skeletal/cardiac muscle contraction are notable among myopathogenes associated with hereditary cardiomyopathies that display differential regulation during satellite cell activation (Figure 3). The contribution of ion channels in satellite cells function, myocyte fusion and myofibre formation further prompts analysis of satellite cell status/function in this category of neuromuscular disorders.

More Myopathogenes Potentially Affecting Satellite Cell Function

The final categories of the 16 neuromuscular disorders are hereditary ataxias, hereditary motor and sensory neuropathies, hereditary paraplegias and other neuromuscular disorders. Our analysis reveals that many genes in these 4 categories are regulated during satellite cell activation, with some also being regulated by Pax7 (Figure 2 and 3), highlighting the important point that many neuromuscular disorders could have satellite cells dysfunction contributing to their pathogenesis. ATXN2 (hereditary ataxias), REEP1 (hereditary motor and sensory neuropathies) and IGHMP2 (hereditary paraplegias) in particular stand out as interesting candidates for affecting satellite cells function. ATXN2 regulates mTOR signaling, crucial for satellite cell activation, whereas mutations in REEP1 and IGHMP2 associate with MEGF10-like muscular phenotypes, so warranting further investigation.

Summary and Remarks

Satellite cells are essential for muscle homeostasis, mediating postnatal growth, turn-over/adaptation and myofibre repair and regeneration in adulthood (Figure 1). Hence, pathogenic mutations altering the activity of satellite cells can have dramatic effects on muscle health. As poor muscle homeostasis is a shared feature across many neuromuscular disorders, better characterisation of satellite cells status and activity, both at cellular and molecular level, is needed.

Several muscle conditions originate from mutations in genes that directly blunt satellite cells function such as in MYOSCO, EMARDD and CFZS, whereas other myopathogenes alter the function of both satellite cells, myofibres and NMJ such as in EDM2, DMD and dystroglycanopathies. Muscle impairment may accompany cardiac and/or neurogenic involvement, demonstrating the cellular/molecular overlap among satellite cells and other cellular populations in the neuromuscular system. Interestingly, some disorders mainly characterised by cardiac or neurogenic impairment also present declining satellite cell number or activity as observed in ALS, myasthenic syndromes and GSD2. Such satellite cell dysfunction may be a direct consequence of the mutation in the associated myopathogene, in addition to being an indirect consequence of perturbed skeletal muscle, cardiac muscle and/or the neurogenic system.

Here we analysed literature and used publicly available transcriptomic datasets to examine myopathogene expression during early satellite cell dynamics to infer satellite cell dysfunction across neuromuscular disorders. Our study reveal that nearly half (45%; 271/608) of known myopathogenes from the 2021 gene table of neuromuscular disorders, display differential expression in the initial phases of satellite cell myogenesis (Figure 2). Moreover, 30% of satellite cell-expressed myopathogenes are regulated by Pax7, directly and/or indirectly (Figure 3). Such analysis could be refined by assessing regulation of selected myopathogenes by human PAX7 exploiting a publicly available RNA-seq dataset of wild-type PAX7-positive and PAX7-negative satellite cells isolated from healthy human biopsies and PAX7-null satellite cells from a MYOSCO patient.
Satellite cell-opathies within neuromuscular disorders
Eur J Transl Myol 32 (1): 10064, 2022 doi: 10.4081/ejtm.2022.10064

described previously. The 271 satellite cell-expressed myopathogenes, of which 225 are newly described here, are distributed across all 16 neuromuscular disease categories (Figures 2 and 3). This supports the hypothesis that many neuromuscular disorders may have some degree of underlying satellite cells dysfunction irrespective of whether or not they have overt muscle pathology. Indeed, those cardiomyopathies and neuropathies such as GSD2, Titinopathies and CAV3-associated disorders that are caused by myopathogenes also expressed by satellite cells are conditions with a more complex clinical presentation, where the associated myopathy only not affects both satellite cells and myofibres, but also compromises other neuromuscular components, such as motor neurons or cardiac cells. Our analysis indicates that evaluation of the number of satellite cells in patient biopsies and their functional status is desirable to determine the degree of satellite cell dysfunction in conditions caused by myopathogenes identified here and previously, even where the pathology is centered around neuronal or cardiac cells. Availability of antibodies against satellite cell markers such as PAX7, NCAM, M-Cadherin and CD56 facilitates ready assessment of satellite cells in human biopsies. Functional assessment of satellite cells is also eased by increasing availability of suitable tools for disease modelling including patient-derived primary cells, induced pluripotent stem cells (iPSCs) and immortalised myoblasts, together with tissue organoids and animal models.

It is important to note that the number of myopathogenes directly involved in satellite cell regulation may be higher than suggested by our analysis. To evaluate expression of myopathogenes during satellite cell activation we previously focussed on a 3-hour time-frame from satellite cell quiescence to activation to define Primary and Secondary Satellite Cell-opathies, whereas in this study we used a longer time frame of 5 hours from quiescence. However, other points during satellite cell myogenic progression can be investigated and may reveal further myopathogenes expressed at later phases of satellite cell myogenesis. For example, SELENON, MYMK or MYMX are not differentially regulated during either the first 3 or 5 hours of murine satellite cell activation, despite the well-described effects of their pathogenic mutation on satellite cell function. Similarly, we cannot rule out that expression of some myopathogenes may dynamically oscillate within the analysed time frame, yet not be identified as differentially expressed. In murine satellite cells, expression of the transcription factor MYOG encoding MYOGENIN, is significantly downregulated after 3 hours from activating stimulus, but is then rapidly upregulated 2 hours later, so that overall, expression of MYOG is not changed within the 5 hour time frame. This excluded MYOG from further analysis, but loss of MYOGENIN severely alters satellite cell number. Obviously, expression of genes crucial to satellite cell function may not oscillate, and so other criteria are required to filter them for examination. Interestingly, a quarter (17/63) of the myopathogenes previously identified as differentially expressed in the 3 hours from satellite cell activation, are not retrieved by our analysis over the first 5 hours here, indicating that their expression fluctuates between 0, 3 and 5 hours from quiescence withdraw (Figure 3).

“-Omics” technologies indicate molecular heterogeneity across satellite cell populations, and that individual stem cells may transition across behavioural stages to maintain a homeostatic equilibrium. It is also conceivable that some myopathogenes could be expressed temporarily and/or function in specific satellite cell subpopulations, both within, and between different, skeletal muscles. Analysis of myopathogenes could be also be refined further by exploiting recent datasets on human satellite cells.

As ever-growing diagnostic usage of DNA/RNA sequencing fosters discovery of new myopathogenes, examining their expression and function in satellite cells advances assessment of genotype-phenotype correlations to fully characterise neuromuscular disorders. Such analysis may also serve as a prognostic tool to improve diagnosis and management of certain neuromuscular conditions, and accelerate development of tailored treatments for neuromuscular disorders.

List of acronyms
ALS - Amyotrophic lateral sclerosis
CFZS - Carey-Fineman-Ziter Syndrome
CK - Creatine Kinase
DAPC - Dystrophin-Associated Protein Complex
DMD - Duchenne muscular dystrophy
EMARDD - Myopathy, Areflexia, Respiratory Distress, And Dysphagia, Early-Onset
FSHD - Facioscapulohumeral muscular dystrophy
GSD2 - Glycogen Storage Disease 2
LGMDR1 - Limb-Girdle Muscular Dystrophy
Recessive 1
MDC1A - Merosin-deficient Congenital Muscular Dystrophy
MPCs - Muscle Progenitors Cells
MYODRIF - Myopathy, Congenital, With Diaphragmatic Defects, Respiratory Insufficiency, And Dystrophic Facies
MYOSCO - Progressive Congenital Myopathy with Scoliosis
NMJ - Neuromuscular Junction

Contributions of Author
Acquisition of main funding: PZ. Conceptualisation: MG and PSZ. Data Curation and Analysis: MG. Writing Original Draft, Review and Editing: MG and PSZ.

Acknowledgments
We apologize to colleagues whose research papers have not been cited due to space constraints.
Funding

This work is supported by grants from the Medical Research Council to PSZ (MR/P023215/1 and MR/S002472/1), from Muscular Dystrophy UK (RA3/3052) to P.S.Z. and AMIS FSH (20210627-1) to MG.

Conflict of Interest

The authors confirm that I have read the Journal’s position on ethical publication issues and affirms that this report is consistent with those guidelines.

Corresponding Author

Massimo Ganassi, Randall Centre for Cell and Molecular Biophysics, King’s College London, London, SE1 1UL, UK. Phone/Fax: + 44-20-78486444 / + 44-20-78486435. ORCID ID: 0000-0003-3163-9707
Email: massimo.ganassi@kcl.ac.uk
Email and ORCID ID of Coauthor

Peter S. Zammit: peter.zammit@kcl.ac.uk
ORCID id: 0000-0001-9562-3072

References

1. Mukund K, Subramaniam S. Skeletal muscle: A review of molecular structure and function, in health and disease. Wiley Interdiscip Rev Syst Biol Med. 2020;12(1):e1462. Epub 2019/08/14. doi: 10.1002/wsbm.1462.
2. Dowling JJ, Weihl CC, Spencer MJ. Molecular and cellular basis of genetically inherited skeletal muscle disorders. Nat Rev Mol Cell Biol. 2021. Epub 2021/07/15. doi: 10.1038/s41580-021-00389-z.
3. Ganassi M, Muntoni F, Zammit PS. Defining and identifying satellite cell-opathies within muscular dystrophies and myopathies. Exp Cell Res. 2022;411(1):112906. Epub 2021/11/07. doi: 10.1016/j.yexcr.2021.112906.
4. Benarroch L, Bonne G, Rivier F, Hamroun D. The 2021 version of the gene table of neuromuscular disorders (nuclear genome). Neuromuscul Disord. 2020;30(12):1008-48. Epub 2020/12/02. doi: 10.1016/j.nmd.2020.11.009.
5. Fukada SI, Akimoto T; Sotiropoulos A. Role of damage and management in muscle hypertrophy: Different behaviors of muscle stem cells in regeneration and hypertrophy. Biochim Biophys Acta Mol Cell Res. 2020;1867(9):118742. Epub 2020/05/18. doi: 10.1016/j.bbamcr.2020.118742.
6. Mauro A. Satellite cell of skeletal muscle fibers. J Biophys Biochem Cytol. 1961;9:493-5. Epub 1961/02/01. doi: 10.1083/jcb.9.2.493.
7. Relaix F, Zammit PS. Satellite cells are essential for skeletal muscle regeneration: the cell on the edge returns centre stage. Development. 2012;139(16):2845-56. Epub 2012/07/27. doi: 10.1242/dev.069088.
8. Cardamone M, Darras BT, Ryan MM. Inherited myopathies and muscular dystrophies. Semin Neurol. 2008;28(2):250-9. Epub 2008/03/21. doi: 10.1055/s-2008-1062269.
9. Sewry CA, Wallgren-Pettersson C. Myopathy in congenital myopathies. Neuropathol Appl Neurobiol. 2017;43(1):5-23. Epub 2016/12/16. doi: 10.1111/nan.12369.
10. Collins CA, Olsen I, Zammit PS, Heslop L, Petrie A, Partridge TA, et al. Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. Cell. 2005;122(2):289-301. Epub 2005/07/30. doi: 10.1016/j.cell.2005.05.010.
11. Collins CA, Partridge TA. Self-renewal of the adult skeletal muscle satellite cell. Cell Cycle. 2005;4(10):1338-41. Epub 2005/09/24. doi: 10.4161/cc.4.10.2114.
12. Moss FP, Leblond CP. Satellite cells as the source of nuclei in muscles of growing rats. Anat Rec. 1971;170(4):421-35. Epub 1971/08/01. doi: 10.1002/ar.1091700405.
13. Morgan J, Butler-Browne G, Muntoni F, Patel K, skeletal muscle stem cells involvement in pathology study g. 240th ENMC workshop: The involvement of skeletal muscle stem cells in the pathology of muscular dystrophies 25-27 January 2019, Hoofddorp, The Netherlands. Neuromuscul Disord. 2019;29(9):704-15. Epub 2019/08/27. doi: 10.1016/j.nmd.2019.07.003.
14. Lopes F, Miguet M, Mucha BE, Gauthier J, Saillour V, Nguyen CE, et al. MYOD1 involvement in myopathy. Eur J Neurol. 2018;25(12):e12347. Epub 2018/11/08. doi: 10.1111/ejnp.13782.
15. Shukla A, Narayanan DL, Asher U, Girisha KM. A novel bi-allelic loss-of-function variant in MYOD1: Further evidence for gene-disease association and phenotypic variability in MYOD1-related myopathy. Clin Genet. 2019;96(3):276-7. Epub 2019/07/02. doi: 10.1111/cge.13596.
16. Watson CM, Crinnion LA, Murphy H, Newbould M, Harrison SM, Lascelles C, et al. Deficiency of the myogenic factor MyoD causes a perinatally lethal fetal akinesia. J Med Genet. 2016;53(4):264-9. Epub 2016/01/07. doi: 10.1136/jmedgenet-2015-103620.
17. Banerji CRS, Henderson D, Tawil RN, Zammit PS. Skeletal muscle regeneration in facioscapulohumeral muscular dystrophy is correlated with pathological severity. Hum Mol Genet. 2020;29(16):2746-60. Epub 2020/08/04. doi: 10.1093/hmg/ddaa164.
18. Hewitt JE, Lyle R, Clark LN, Valleley EM, Wright TJ, Wijmenga C, et al. Analysis of the tandem repeat locus D4Z4 associated with facioscapulohumeral muscular dystrophy. Hum
Satellite cell-opathies within neuromuscular disorders

Eur J Transl Myol 32 (1): 10064, 2022 doi: 10.4081/ejtm.2022.10064

Mol Genet. 1994;3(8):1287-95. Epub 1994/08/01. doi: 10.1093/hmg/3.8.1287.

19. van Deutkom JC, Wijmenga C, van Tienhoven EA, Gruter AM, Hewitt JE, Padberg GW, et al. FSHD associated DNA rearrangements are due to deletions of integral copies of a 3.2 kb tandemly repeated unit. Hum Mol Genet. 1993;2(12):2037-42. Epub 1993/12/01. doi: 10.1093/hmg/2.12.2037.

20. Greco A, Goossens R, van Engelen B, van der Maarel SM. Consequences of epigenetic derepression in facioscapulohumeral muscular dystrophy. Clin Genet. 2020;97(6):799-814. Epub 2020/02/23. doi: 10.1111/cge.13726.

21. Lim KRQ, Nguyen Q, Yokota T. DUX4 Signalling in the Pathogenesis of Facioscapulohumeral Muscular Dystrophy. Int J Mol Sci. 2020;21(3). Epub 2020/01/26. doi: 10.3390/ijms21030729.

22. Heher P, Ganassi M, Weidinger A, Engquist EN, Pruller J, Nguyen TH, et al. Interplay between mitochondrial reactive oxygen species, oxidative stress and hypoxic adaptation in facioscapulohumeral muscular dystrophy: Metabolic stress as potential therapeutic target. Redox Biology. 2022;102251. doi: https://doi.org/10.1016/j.redox.2022.102251.

23. Banerji CRS, Panamorova M, Hebaishi H, White RB, Relax F, Severini S, et al. PAX7 target genes are globally repressed in facioscapulohumeral muscular dystrophy skeletal muscle. Nat Commun. 2017;8(1):2152. Epub 2017/12/20. doi: 10.1038/s41467-017-01200-4.

24. Banerji CRS, Zammit PS. PAX7 target gene repression is a superior FSHD biomarker than DUX4 target gene activation, associating with pathological severity and identifying FSHD at the single-cell level. Hum Mol Genet. 2019;28(13):2224-36. Epub 2019/05/09. doi: 10.1093/hmg/ddz043.

25. Bosnakovski D, Toso EA, Hartweck LM, Magli A, Lee HA, Thompson ER, et al. The DUX4 homeodomains mediate inhibition of myogenesis and are functionally exchangeable with the Pax7 homeodomain. J Cell Sci. 2017;130(21):3685-97. Epub 2017/09/25. doi: 10.1242/jcs.205427.

26. Omar A, Marwaha K, Bollu PC. Physiology, Neuromuscular Junction. StatPearls. Treasure Island (FL)2021.

27. Jimsheleishvili S, Marwaha K, Sherman A. Physiology, Neuromuscular Transmission. StatPearls. Treasure Island (FL)2021.

28. Kelly AM. Persynaptic satellite cells in the developing and mature rat soleus muscle. Anat Rec. 1978;190(4):891-903. Epub 1978/04/01. doi: 10.1002/ar.1091900409.

29. Liu W, Wei-Lapierre L, Klose A, Dirksen RT, Chakkalakal JV. Inducible depletion of adult skeletal muscle stem cells impairs the regeneration of neuromuscular junctions. Elife. 2015;4. Epub 2015/08/28. doi: 10.7554/eLife.09221.

30. Sanes JR, Marshall LM, McMahen UJ. Reinnervation of muscle fiber basal lamina after removal of myofibers. Differentiation of regenerating axons at original synaptic sites. J Cell Biol. 1978;78(1):176-98. Epub 1978/07/01. doi: 10.1083/jcb.78.1.176.

31. Bruusgaard JC, Gundersen K. In vivo time-lapse microscopy reveals no loss of murine myonuclei during weeks of muscle atrophy. J Clin Invest. 2008;118(4):1450-7. Epub 2008/03/05. doi: 10.1172/JCI34022.

32. Scaramozza A, Marchese V, Papa V, Salaroli R, Soraru G, Angelini C, et al. Skeletal muscle satellite cells in myotrophic lateral sclerosis. Ultrastruct Pathol. 2014;38(5):295-302. Epub 2014/08/01. doi: 10.3109/01913123.2014.937842.

33. Burr P, Reddivari AKR. Spinal Muscle Atrophy. StatPearls. Treasure Island (FL)2021.

34. McCormack NM, Villanon E, Viollet C, Soltis AR, Dalgard CL, Lorson CL, et al. Survival motor neuron deficiency slows myoblast fusion through reduced myomaker and myomixer expression. J Cachexia Sarcopenia Muscle. 2021;12(4):1098-116. Epub 2021/06/12. doi: 10.1002/jcsm.12740.

35. Hellbach N, Peterson S, Haehnke D, Shankar A, LaBarge S, Pivaroff C, et al. Impaired myogenic development, differentiation and function in hESC-derived SMA myoblasts and myotubes. PLoS One. 2018;13(10):e0205589. Epub 2018/10/12. doi: 10.1371/journal.pone.0205589.

36. Bushby K, Muntoni F, Bourke JP. 107th ENMC international workshop: the management of cardiac involvement in muscular dystrophy and myotonic dystrophy. 7th-9th June 2002, Naarden, the Netherlands. Neuromuscul Disord. 2003;13(2):166-72. Epub 2003/02/05. doi: 10.1016/s0960-8966(02)00213-4.

37. Muntoni F. Cardiac complications of childhood myopathies. J Child Neurol. 2003;18(3):191-202. Epub 2003/05/07. doi: 10.1177/088303030180030301.

38. Verhaert D, Richards K, Rafael-SV. Cardiac involvement in patients with muscular dystrophies: magnetic resonance imaging phenotype and genotypic considerations. Circ Cardiovasc Imaging. 2011;4(1):67-76. Epub 2011/01/20. doi: 10.1161/CIRCIMAGING.110.960740.

39. Kostareva A, Sejersen T, Sjoberg G. Genetic spectrum of cardiomyopathies with neuromuscular phenotype. Front Biosci (Schol Ed). 2013;5:325-40. Epub 2013/01/02. doi: 10.2741/s375.

40. Ripa R, George T, Sattar Y. Physiology, Cardiac Muscle. StatPearls. Treasure Island (FL)2021.

41. Sweeney HL, Hammers DW. Muscle Contraction. Cold Spring Harb Perspect Biol. 2018;10(2). Epub 2018/02/09. doi: 10.1101/cshperspect.a023200.
42. Machado L, Estevés de Lima J, Fabre O, Proux C, Legendre R, Szegedi A, et al. In Situ Fixation Redefines Quiescence and Early Activation of Skeletal Muscle Stem Cells. Cell Rep. 2017;21(7):1982-93. Epub 2017/11/16. doi: 10.1016/j.celrep.2017.10.080.

43. Relaix F, Rocancourt D, Mansouri A, Buckingham M. Divergent functions of murine Pax3 and Pax7 in limb muscle development. Genes Dev. 2004;18(9):1088-105. Epub 2004/05/11. doi: 10.1101/gad.301004.

44. Seale P, Sabourin LA, Girgis-Gabardo A, Mansouri A, Gruss P, Rudnicki MA. Pax7 is required for the specification of myogenic satellite cells. Cell. 2000;102(6):777-86. Epub 2000/10/13. doi: 10.1016/s0092-8674(00)00066-0.

45. Zammit PS, Relaix F, Nagata Y, Ruiz AP, Collins CA, Partridge TA, et al. Pax7 and myogenic progression in skeletal muscle satellite cells. PLoS One. 2017;12(4):e0176190. Epub 2017/04/26. doi: 10.1371/journal.pone.0176190.

46. Jain A, Al Khalili Y. Congenital Myotonic Dystrophy. StatPearls. Treasure Island (FL)2022.

47. Thornell LE, Lindstrom M, Renault V, Klein A, Mouly V, Ansvård T, et al. Satellite cell dysfunction contributes to the progressive muscle atrophy in myotonic dystrophy type 1. Neuropathol Appl Neurobiol. 2009;35(6):603-13. Epub 2009/02/12. doi: 10.1111/j.1365-2990.2009.01014.x.

48. Furling D, Coiffier L, Mouly V, Barbet JP, St Guily JL, Taneja K, et al. Defective satellite cells in congenital myotonic dystrophy. Hum Mol Genet. 2001;10(10):2079-87. Epub 2001/10/09. doi: 10.1038/hmg.10.2079.

49. Iyer SR, Shah SB, Lovering RM. The Neuromuscular Junction: Roles in Aging and Neuromuscular Disease. Int J Mol Sci. 2021;22(15). Epub 2021/08/08. doi: 10.3390/ijms22158058.

50. Ng SY, Ljubicic V. Recent insights into neuromuscular junction biology in Duchenne muscular dystrophy: Impacts, challenges, and opportunities. EBioMedicine. 2020;61:103032. Epub 2020/10/12. doi: 10.1016/j.ebiom.2020.103032.

51. Michele DE, Barresi R, Kanagawa M, Saito F, Cohn RD, Satz JS, et al. Post-translational disruption of dystroglycan-ligand interactions in congenital muscular dystrophies. Nature. 2002;418(6896):417-22. Epub 2002/07/26. doi: 10.1038/nature00837.

52. Brancaccio A. A molecular overview of the primary dystroglycanopathies. J Cell Mol Med. 2019;23(5):3058-62. Epub 2019/03/07. doi: 10.1111/jcmm.14218.

53. Gonzalez-Perez P, Smith C, Sebetka WL, Gedlinske A, Perlman S, Mathews KD. Clinical and electrophysiological evaluation of myasthenic features in an alpha-dystroglycanopathy cohort (FKRP-predominant). Neuromuscul Disord. 2020;30(3):213-8. Epub 2020/03/03. doi: 10.1016/j.nmd.2020.01.002.

54. Cohn RD, Henry MD, Michele DE, Barresi R, Saito F, Moore SA, et al. Disruption of DAG1 in differentiated skeletal muscle reveals a role for dystroglycan in muscle regeneration. Cell. 2002;110(5):639-48. Epub 2002/09/17. doi: 10.1016/s0092-8674(02)00907-8.

55. Beedle AM, Turner AJ, Saito Y, Lueck JD, Foltz SJ, Fortunato MJ, et al. Mouse fukutin deletion impairs dystroglycan processing and recapitulates muscular dystrophy. J Clin Invest. 2012;122(9):3330-42. Epub 2012/08/28. doi: 10.1172/JCI63004.

56. Furling D, Coiffier L, Mouly V, Barbet JP, St Guily JL, Taneja K, et al. Defective satellite cells in congenital myotonic dystrophy. Hum Mol Genet. 2001;10(10):2079-87. Epub 2001/10/09. doi: 10.1038/hmg.10.2079.

57. Iyer SR, Shah SB, Lovering RM. The Neuromuscular Junction: Roles in Aging and Neuromuscular Disease. Int J Mol Sci. 2021;22(15). Epub 2021/08/08. doi: 10.3390/ijms22158058.

58. Ng SY, Ljubicic V. Recent insights into neuromuscular junction biology in Duchenne muscular dystrophy: Impacts, challenges, and opportunities. EBioMedicine. 2020;61:103032. Epub 2020/10/12. doi: 10.1016/j.ebiom.2020.103032.

59. Michele DE, Barresi R, Kanagawa M, Saito F, Cohn RD, Satz JS, et al. Post-translational disruption of dystroglycan-ligand interactions in congenital muscular dystrophies. Nature. 2002;418(6896):417-22. Epub 2002/07/26. doi: 10.1038/nature00837.

60. Arimura T, Hayashi YK, Murakami T, Oya Y, Funabe S, Arikawa-Hirasea E, et al. Mutational analysis of fukutin gene in dilated cardiomyopathy and hypertrophic cardiomyopathy. Circ J. 2009;73(1):158-61. Epub 2008/11/19. doi: 10.1253/circj-cj-08-0722.

61. Servian-Morilla E, Cabrera-Serrano M, Johnson K, Pandey A, Ito A, Rivas E, et al. POGZLUT1 biallelic mutations cause myopathy with reduced satellite cells, alpha-dystroglycan hypoglycosylation and a distinctive radiological pattern. Acta Neuropathol. 2020;139(3):565-82. Epub 2020/01/04. doi: 10.1007/s00401-019-02117-6.

62. Servian-Morilla E, Takeuchi H, Lee TV, Clarimon J, Mavillard F, Area-Gomez E, et al. A POGZLUT1 mutation causes a muscular dystrophy with reduced Notch signaling and satellite cell loss. EMBO Mol Med. 2016;8(11):1289-309. Epub 2016/11/04. doi: 10.1016/j.ebiom.2020.02.1032.
Lee HO, et al. Ganassi M, Mateju D, Bigi I, Mediani L, Galli V, Carra AD, Bigi I, Vinet J, Mediani M, et al. The chaperone HSPB8 reduces the accumulation of truncated TDP-43 species in cells and protects against TDP-43-mediated toxicity. Hum Mol Genet. 2016;25(18):3908-24. Epub 2016/07/29. doi: 10.1039/hmg/ddw232.

67. Mediani L, Galli V, Carra AD, Bigi I, Vinet J, Mediani M, et al. BAG3 and BAG6 differentially affect the dynamics of stress granules by targeting distinct subsets of defective polypeptides released from ribosomes. Cell Stress Chaperones. 2020;25(6):1045-58. Epub 2020/07/23. doi: 10.1007/s12192-020-01141-w.

68. Sancar F, Tourtoutine D, Gao S, Oh HJ, Gentrel M, Bessereau JL, et al. The dystrophin-dependent K(+) (BK) channel regulates their polarity and asymmetric division. Nat Med. 2019;25(11):1455-63. Epub 2019/11/17. doi: 10.1038/s41591-019-0455-6.

69. Kong J, Anderson JE. Dystrophin is required for organizing large acetylcholine receptor aggregates. Brain Res. 1999;839(2):298-304. Epub 1999/10/16. doi: 10.1016/S0006-8993(99)01737-0.

70. Fukuhara N, Suzuki M, Tsubaki T, Kushiro S, Takasawa N. Ultrastructural studies on the neuromuscular junctions of Becker's muscular dystrophy. Acta Neuropathol. 1985;66(4):283-91. Epub 1985/01/01. doi: 10.1007/BF00690960.

71. Attia M, Maurer M, Robinet M, Le Grand F, Fadel E, Le Panse R, et al. Muscle satellite cells are functionally impaired in myasthenia gravis: consequences on muscle regeneration. Acta Neuropathol. 2017;134(6):869-88. Epub 2017/08/02. doi: 10.1007/s00401-017-1754-2.

72. Vilquin JT, Bayer AC, Le Panse R, Berrih-Aknin S. The Muscle Is Not a Passive Target in Myasthenia Gravis. Front Neurol. 2019;10:1343. Epub 2020/01/11. doi: 10.3389/fneur.2019.01343.

73. Cicardi ME, Marrone L, Azzouz M, Troiti D. Proteostatic imbalance and protein spreading in amyotrophic lateral sclerosis. EMBO J. 2021;40(10):e106389. Epub 2021/04/02. doi: 10.15252/embj.2020106389.

74. Ganassi M, Mateju D, Bigi I, Mediani L, Poser I, Lee HO, et al. A Surveillance Function of the HSPB8-BAG3-HSP70 Chaperone Complex Ensures Stress Granule Integrity and Dynamism. Mol Cell. 2016;63(5):796-810. Epub 2016/08/30. doi: 10.1016/j.molcel.2016.07.021.

75. Crippa V, Cicardi ME, Ramesh N, Seguin SJ, Ganassi M, Bigi I, et al. The chaperone HSPB8 recapitulates hallmarks of disease progression. Proc Natl Acad Sci U S A. 2016;113(24):6642-7. doi: 10.1073/pnas.1524553113.

76. Badu-Mensah A, Guo X, McAleer CW, Rumsey JW, Hickman JJ. Functional skeletal muscle model derived from SOD1-mutant ALS patient iPSCs recapitulates hallmarks of disease progression. EMBO J. 2018;37(4):e1001141. doi: 10.15252/embj.2016106389.

77. Hoffman EP, Brown RH, Jr., Kunkel LM. The muscular dystrophy locus. Cell. 1987;51(6):919-35. Epub 1987/12/24. doi: 10.1016/0092-8674(87)90579-4.

78. Zhang M, McLennan IS. Use of antibodies to identify satellite cells with a light microscope. Muscle Nerve. 1994;17(9):987-94. Epub 1994/09/01. doi: 10.1002/mus.880170905.

79. Dumont NA, Wang YX, von Maltzahn J, Pasut A, Bentzinger CF, Brun CE, et al. Dystrophin expression in muscle stem cells regulates their polarity and asymmetric division. Nat Med. 2015;21(12):1455-63. Epub 2015/11/17. doi: 10.1038/nm.3990.

80. Coo IF, van Doorn PA, et al. Lack of robust satellite cell activation and muscle regeneration during the progression of Pompe disease. Acta Neuropathol Commun. 2015;3:65. Epub 2015/10/30. doi: 10.1038/s12243-015-00620-9.

81. Schaaf GJ, Canibano-Fraile R, van Gestel TJM, van der Ploeg AT, Pijnappel W. Restoring the regenerative balance in neuromuscular disorders: satellite cell activation as therapeutic target in Pompe disease. Ann Transl Med. 2019;7(13):280. Epub 2019/08/09. doi: 10.21037/atm.2019.04.48.

82. Schaaf GJ, van Gestel TJ, Brusse E, Verdijk RM, de Coo IF, van Doorn PA, et al. Lack of robust satellite cell activation and muscle regeneration during the progression of Pompe disease. Acta Neuropathol Commun. 2015;3:65. Epub 2015/10/30. doi: 10.1186/s40478-015-0243-x.

83. Schaaf GJ, van Gestel TJM, In 't Groen SLM, de Jong B, Boomaars B, Tarallo A, et al. Satellite cells maintain regenerative capacity but fail to repair disease-associated muscle damage in mice with Pompe disease. Acta Neuropathol Commun. 2018;6(1):119. Epub 2018/11/09. doi: 10.1186/s40478-018-0620-3.
Satellite cell-opathies within neuromuscular disorders

Eur J Transl Myol 32 (1): 10064, 2022 doi: 10.4081/ejtm.2022.10064

84. Soliman OI, van der Beek NA, van Doorn PA, Vletter WB, Nemes A, Van Dalen BM, et al. Cardiac involvement in adults with Pompe disease. J Intern Med. 2008;264(4):333-9. Epub 2008/04/10. doi: 10.1111/j.1365-2796.2008.01966.x.

85. de Leeuw R, Gruenbaum Y, Medalia O. Nuclear Lamins: Thin Filaments with Major Functions. Trends Cell Biol. 2018;28(1):34-45. Epub 2017/09/13. doi: 10.1016/j.tcb.2017.08.004.

86. Donnaloja F, Carnevali F, Jabchetti E, Raimondi MT. Lamin A/C Mechanotransduction in Laminopathies. Cells. 2020;9(5). Epub 2020/05/28. doi: 10.3390/cells9051306.

87. Scharner J, Brown CA, Bower M, Iannaccone ST, Khatri IA, Escobar D, et al. Novel LMNA mutations in patients with Emery-Dreifuss muscular dystrophy and functional characterization of four LMNA mutations. Hum Mutat. 2011;32(2):152-67. Epub 2010/09/18. doi: 10.1002/humu.21361.

88. Owens DJ, Messeant J, Moog S, Viggars M, Ferry A, Mamchaoui K, et al. Lamin-Related Congenital Muscular Dystrophy Alters Mechanical Signaling and Skeletal Muscle Growth. Int J Mol Sci. 2020;22(1). Epub 2021/01/06. doi: 10.3390/ijms22010306.

89. Favreau C, Higuet D, Courvalin JC, Buendia B. Expression of a mutant lamin A that causes Emery-Dreifuss muscular dystrophy inhibits in vitro differentiation of C2C12 myoblasts. Mol Cell Biol. 2004;24(4):1481-92. Epub 2004/01/30. doi: 10.1128/MCB.24.4.1481-1492.2004.

90. Gnocchi VF, Scharner J, Huang Z, Brady K, Lee JS, White RB, et al. Uncordinated transcription and compromised muscle function in the lmna-null mouse model of Emery-Emery-Dreifuss muscular dystrophy. PLoS One. 2011;6(2):e16651. Epub 2011/03/03. doi: 10.1371/journal.pone.0016651.

91. Ignatieva EV, Ivanova OA, Komarova MY, Khromova NV, Polev DE, Kostareva AA, et al. LMNA Mutations G232E and R482L Cause Dysregulation of Skeletal Muscle Differentiation, Bioenergetics, and Metabolic Gene Expression Profile. Genes (Basel). 2020;11(9). Epub 2020/09/11. doi: 10.3390/genes11091057.

92. Markiewicz E, Ledran M, Hutchison CJ. Remodelling of the nuclear lamina and nucleoskeleton is required for skeletal muscle differentiation in vitro. J Cell Sci. 2005;118(Pt 2):409-20. Epub 2005/01/18. doi: 10.1242/jcs.01630.

93. Pilat U, Dechat T, Bertrand AT, Woisetschlager N, Gotic I, Spilka R, et al. The muscle dystrophy-causing DeltaK32 lamin A/C mutant does not impair the functions of the nucleoplasmic lamin-A/C-LAP2alpha complex in mice. J Cell Sci. 2013;126(Pt 8):1753-62. Epub 2013/02/28. doi: 10.1242/jcs.115246.

94. Mejat A, Decostre V, Li J, Renou L, Kesari A, Hanttai D, et al. Lamin A/C-mediated neuromuscular junction defects in Emery-Dreifuss muscular dystrophy. J Cell Biol. 2009;184(1):31-44. Epub 2009/01/07. doi: 10.1083/jcb.200811035.

95. Oyston LJ, Lin YQ, Khuong TM, Wang QP, Lau MT, Clark T, et al. Neuronal Lamin regulates motor circuit integrity and controls motor function and lifespan. Cell Stress. 2018;2(9):225-32. Epub 2019/06/22. doi: 10.15698/cst2018.09.152.

96. De Sandre-Giovannoli A, Chaouch M, Kozlov S, Vallat JM, Tazir M, Kassouri N, et al. Homozygous defects in LMNA, encoding lamin A/C nuclear-envelope proteins, cause autosomal recessive axonal neuropathy in human (Charcot-Marie-Tooth disorder type 2) and mouse. Am J Hum Genet. 2002;70(3):726-36. Epub 2002/01/19. doi: 10.1086/339274.

97. Chervinsky E, Khayat M, Soltsman S, Habiballa H, Elpeleg O, Shalev S. A homozygous TTN gene variant associated with lethal congenital contracture syndrome. Am J Med Genet A. 2018;176(4):1001-5. Epub 2018/03/27. doi: 10.1002/ajmg.a.38639.

98. Heimann P, Menke A, Rothkegel B, Jockusch H. Overshooting production of satellite cells in murine skeletal muscle affected by the mutation "muscular dystrophy with myositis" (mdm, Chr 2). Cell Tissue Res. 1996;283(3):435-41. Epub 1996/03/01. doi: 10.1007/s004400500554.

99. Pradhan BS, Proszyński TJ. A Role for Caveolin-3 in the Pathogenesis of Muscular Dystrophies. Int J Mol Sci. 2020;21(22). Epub 2020/11/25. doi: 10.3390/ijms21228736.

100. Song KS, Scherer PE, Tang Z, Okamoto T, Li S, Chafl M, et al. Expression of caveolin-3 in skeletal, cardiac, and smooth muscle cells. Caveolin-3 is a component of the sarcosome and co-fractionates with dystrophin and dystrophin-associated glycoproteins. J Biol Chem. 1996;271(25):15160-5. Epub 1996/06/21. doi: 10.1074/jbc.271.25.15160.

101. Volonté D, Peoples AJ, Galbiati F. Modulation of myoblast fusion by caveolin-3 in dystrophic skeletal muscle cells: implications for Duchenne muscular dystrophy and limb-girdle muscular dystrophy-1C. Mol Biol Cell. 2003;14(10):4075-88. Epub 2003/10/01. doi: 10.1091/fnc.03-03-0161.

102. Ali SR, Menendez-Montes I, Warshaw J, Xiao F, Sadek HA. Homotypic Fusion Generates Multinucleated Cardiomyocytes in the Murine Heart. Circulation. 2020;141(23):1940-2. Epub 2020/06/09. doi: 10.1161/CIRCULATIONAHA.119.043530.

103. Sawamiphak S, Kontarakis Z, Filosa A, Reischauer S, Stainier DYR. Transient cardiomyocyte fusion regulates cardiac development in zebrafish. Nat Commun. 2017;8(1):1525. Epub 2017/11/17. doi:
Satellite cell-opathies within neuromuscular disorders

Eur J Transl Myol 32 (1): 10064, 2022 doi: 10.4081/etjm.2022.10064

10.1038/s41467-017-01555-8.

104. Soonpaa MH, Kim KK, Pajak L, Franklin M, Field LJ. Cardiomyocyte DNA synthesis and binucleation during murine development. Am J Physiol. 1996;271(5 Pt 2):H2183-9. Epub 1996/11/01. doi: 10.1152/ajpheart.1996.271.5.H2183.

105. Herrmann R, Straub V, Blank M, Kutzick C, Franke N, Jacob EN, et al. Dissociation of the dystroglycan complex in caveolin-3-deficient limb girdle muscular dystrophy. Hum Mol Genet. 2000;9(15):2335-40. Epub 2000/09/26. doi: 10.1093/oxfordjournals.hmg.a018926.

106. Hezel M, de Groot WC, Galbiati F. Caveolin-3 promotes nicotinic acetylcholine receptor clustering and regulates neuromuscular junction activity. Mol Biol Cell. 2010;21(2):302-10. Epub 2009/11/27. doi: 10.1091/mbc.E09-05-0381.

107. Chen L, Hassani Nia F, Stauber T. Ion Channels and Transporters in Muscle Cell Differentiation. Int J Mol Sci. 2021;22(24). Epub 2021/12/25. doi: 10.3390/ijms22243615.

108. Ortuste Quiroga HP, Ganassi M, Yokoyama S, Nakamura K, Yamashita T, Raimbach D, et al. Fine-Tuning of Piezo1 Expression and Activity Ensures Efficient Myoblast Fusion during Skeletal Myogenesis. Cells. 2022;11(3):393.

109. Tsuchiya M, Hara Y, Okuda M, Itoh K, Nishioka R, Shiomi A, et al. Cell surface flip-flop of phosphatidylserine is critical for PIEZO1-mediated myotube formation. Nat Commun. 2018;9(1):2049. Epub 2018/05/26. doi: 10.1038/s41467-018-04436-w.

110. Lastres-Becker I, Nonis D, Eich F, Klinkenberg M, Gorospe M, Kotter P, et al. Mammalian axin-2 modulates translation control at the pre-initiation complex via PI3K/mTOR and is induced by starvation. Biochim Biophys Acta. 2016;1862(9):1558-69. Epub 2016/06/01. doi: 10.1016/j.bbadis.2016.05.017.

111. Rodgers JT, King KY, Brett JO, Crombie MJ, Charville GW, Maguire KK, et al. mTORC1 controls the adaptive transition of quiescent stem cells from G0 to G(Alert). Nature. 2014;510(7505):393-6. Epub 2014/05/30. doi: 10.1038/nature13255.

112. Schottmann G, Seelov D, Seifert F, Morales-Gonzalez S, Gill E, von Au K, et al. Recessive REEP1 mutation is associated with congenital axonal neuropathy and diaphragmatic palsy. Neuror Genet. 2015;1(4):e32. Epub 2016/04/12. doi: 10.1212/NXG.0000000000000032.

113. Biressi S, Filareto A, Rando TA. Stem cell therapy for muscular dystrophies. J Clin Invest. 2020;130(11):5652-64. Epub 2020/09/19. doi: 10.1172/JCI142031.

114. Marg A, Escobar H, Kanaiskos N, Grunwald SA, Metzler E, Kieshauer J, et al. Human muscle-derived CLEC14A-positive cells regenerate muscle independent of PAX7. Nat Commun. 2019;10(1):5776. Epub 2019/12/20. doi: 10.1038/s41467-019-13650-z.

115. Jalal S, Dastidar S, Tedesco FS. Advanced models of human skeletal muscle differentiation, development and disease: Three-dimensional cultures, organoids and beyond. Curr Opin Cell Biol. 2021;73:92-104. Epub 2021/08/14. doi: 10.1016/j.celbi.2021.06.004.

116. Mamchaoui K, Trottel C, Bigot A, Negroni E, Chaouch S, Wolff A, et al. Immortalized pathological human myoblasts: towards a universal tool for the study of neuromuscular disorders. Skelet Muscle. 2011;1:34. Epub 2011/03/02. doi: 10.1186/2044-5040-1-34.

117. Monge C, DiStasio N, Rossi T, Sebastien M, Sakai H, Kalman B, et al. Quiescence of human muscle stem cells is favored by culture on natural biopolymeric films. Stem Cell Res Ther. 2017;8(1):104. Epub 2017/05/04. doi: 10.1186/s13287-017-0556-8.

118. Thorley M, Duguez S, Mazza EMC, Valsoni S, Bigot A, Mamchaoui K, et al. Skeletal muscle characteristics are preserved in hTERT/cdk4 human myogenic cell lines. Skelet Muscle. 2016;6(1):43. Epub 2016/12/10. doi: 10.1186/s13395-016-0115-5.

119. Ganassi M, Badodi S, Ortuste Quiroga HP, Zammit PS, Hints Y, Hughes SM. Myogenin promotes myocyte fusion to balance fibre number and size. Nat Commun. 2018;9(1):4232. Epub 2018/10/14. doi: 10.1038/s41467-018-06583-6.

120. Ganassi M, Badodi S, Wanders K, Zammit PS, Hughes SM. Myogenin is an essential regulator of adult myofibre growth and muscle stem cell homeostasis. Elife. 2020;9. Epub 2020/10/02. doi: 10.7554/eLife.60445.

121. Nabeshima Y, Hanaoka K, Hayasaka M, Esumi E, Li S, Nonaka I, et al. Myogenin gene disruption results in perinatal lethality because of severe muscle defect. Nature. 1993;364(6437):532-5. Epub 1993/03/08. doi: 10.1038/364532a0.

122. Zammit PS. Function of the myogenic regulatory factors Myf5, MyoD, Myogenin and MRF4 in skeletal muscle, satellite cells and regenerative myogenesis. Semin Cell Dev Biol. 2017;72:19-32. Epub 2017/11/12. doi: 10.1016/j.semcdb.2017.11.011.

123. Ganassi M, Zammit PS, Hughes SM. Isolation of Myofibres and Culture of Muscle Stem Cells from Adult Zebrafish. Bio Protoc. 2021;11(17):e4149. Epub 2021/10/05. doi: 10.21769/BioProtoc.4149.

124. Day K, Shefer G, Shearer A, Yablonka-Reuveni Z. The depletion of skeletal muscle satellite cells with age is concomitant with reduced capacity of single progenitors to produce reserve progeny. Dev Biol. 2010;340(2):330-43. Epub 2010/01/19. doi:
10.1016/j.ydbio.2010.01.006.

125. Feldman JL, Stockdale FE. Skeletal muscle satellite cell diversity: satellite cells form fibers of different types in cell culture. Dev Biol. 1991;143(2):320-34. Epub 1991/02/01. doi: 10.1016/0012-1606(91)90083-f.

126. Lagord C, Soulet L, Bonavaud S, Bassaglia Y, Rey C, Barlovatz-Meimon G, et al. Differential myogenicity of satellite cells isolated from extensor digitorum longus (EDL) and soleus rat muscles revealed in vitro. Cell Tissue Res. 1998;291(3):455-68. Epub 1998/04/18. doi: 10.1007/s004410051015.

127. Molnar G, Ho ML, Schroedl NA. Evidence for multiple satellite cell populations and a non-myogenic cell type that is regulated differently in regenerating and growing skeletal muscle. Tissue Cell. 1996;28(5):547-56. Epub 1996/10/01. doi: 10.1016/s0040-8166(96)80057-7.

128. Ono Y, Boldrin L, Knopp P, Morgan JE, Zammit PS. Muscle satellite cells are a functionally heterogeneous population in both somite-derived and branchiomieric muscles. Dev Biol. 2010;337(1):29-41. Epub 2009/10/20. doi: 10.1016/j.ydbio.2009.10.005.

129. Ono Y, Masuda S, Nam HS, Benezra R, Miyagoe-Suzuki Y, Takeda S. Slow-dividing satellite cells retain long-term self-renewal ability in adult muscle. J Cell Sci. 2012;125(Pt 5):1309-17. Epub 2012/02/22. doi: 10.1242/jcs.096198.

130. Beauchamp JR, Heslop L, Yu DS, Tajbakhsh S, Kelly RG, Wernig A, et al. Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells. J Cell Biol. 2000;151(6):1221-34. Epub 2000/12/21. doi: 10.1083/jcb.151.6.1221.

131. Porpiglia E, Samusik N, Ho ATV, Cosgrove BD, Mai T, Davis KL, et al. High-resolution myogenic lineage mapping by single-cell mass cytometry. Nat Cell Biol. 2017;19(5):558-67. Epub 2017/04/18. doi: 10.1038/nccellbio.3507.

132. Zammit PS, Golding JP, Nagata Y, Hudson V, Partridge TA, Beauchamp JR. Muscle satellite cells adopt divergent fates: a mechanism for self-renewal? J Cell Biol. 2004;166(3):347-57. Epub 2004/07/28. doi: 10.1083/jcb.200312007.

133. Chow LS, Bosnakovski D, Mashek DG, Kyba M, Perlingeiro RCR, Magli A. Chromatin accessibility profiling identifies evolutionary conserved loci in activated human satellite cells. Stem Cell Res. 2021;55:102496. Epub 2021/08/20. doi: 10.1016/j.scr.2021.102496.

134. Barruet E, Garcia SM, Striedinger K, Wu J, Lee S, Byrnes L, et al. Functionally heterogeneous human satellite cells identified by single cell RNA sequencing. Elife. 2020;9. Epub 2020/04/03. doi: 10.7554/eLife.51576.

Submitted: October 31, 2021
Revision submitted: February 11, 2022
Accepted for publication: February 11, 2022