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

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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.
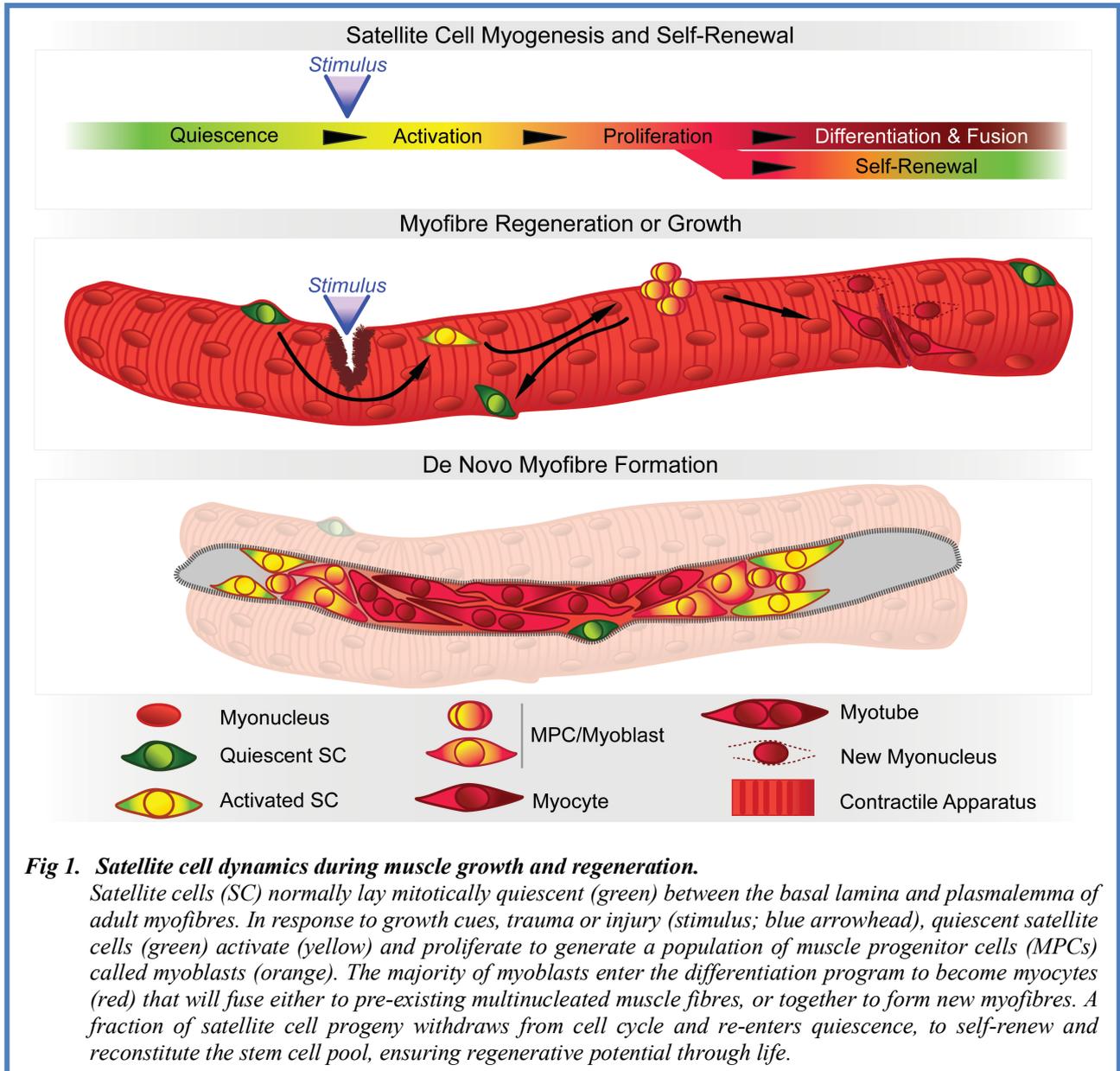
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Cellular and molecular interplay between the nervous system and cardiac/skeletal musculature dictates efficient striated muscle function.[1] Neuromuscular disorders are acquired or hereditary diseases that lead to poor muscle function, either by directly affecting the muscle or indirectly via pathology of the peripheral nervous system and/or neuromuscular junction, leading to impaired crosstalk between nerve and muscle.[2] Currently, mutations in over 600 genes, referred to by us as myopathogenes,[3] are linked to pathogenesis in specific neuromuscular disorders.[4] Several conditions present a more complex clinical pattern, involving one or more components of the neuromuscular system, indicating that genetic alterations impact directly or indirectly on several cell types/tissues.

Among the many cell types comprising the neuromuscular system, the resident population of muscle stem cells, named satellite cells, are key players in the growth and homeostasis of skeletal muscle, contributing to functional adaptation, repair and regeneration (Figure 1).[5-7] Given this key role of satellite cells, it is crucial that this stem cell pool is maintained throughout life.

The functional unit of skeletal muscle is the muscle fibre (myofibre), a syncytial cell containing myofibrils composed of sarcomeres that generate force via interaction of actin and myosin (Figure 1). Historically, the pathogenesis of most muscular dystrophies and myopathies has been predominantly associated with genetic defects in either the contractile apparatus or membrane integrity, causing impaired myofibre structure, function or maintenance.[8,9] Such damage to myofibres, as well as unusual muscle use or hypertrophic stimuli, activates the mitotically quiescent satellite cells to withdraw from quiescence and proliferate, generating a population of muscle progenitor cells (MPCs) called myoblasts. Following rapid proliferation, myoblasts

Fig 1. 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).[5] Simultaneously, a proportion of myoblasts instead commit to self-renewal to replenish the stem cell pool,[10-12] 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.[3] Neuromuscular 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.[13]

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.[3]

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) (*SELENON*), 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)[14-16] (*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 (LGMDD4, 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 *MTM1* 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 DUX4/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.

Fig 2. *Distribution amongst neuromuscular disease classes of the 271 myopathogenes differentially expressed during early satellite cell activation and their response to Pax7.*

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.[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.[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,[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.[36-38] Cardiomyopathies presenting with skeletal muscle involvement and associated neuromuscular symptoms are common, and in
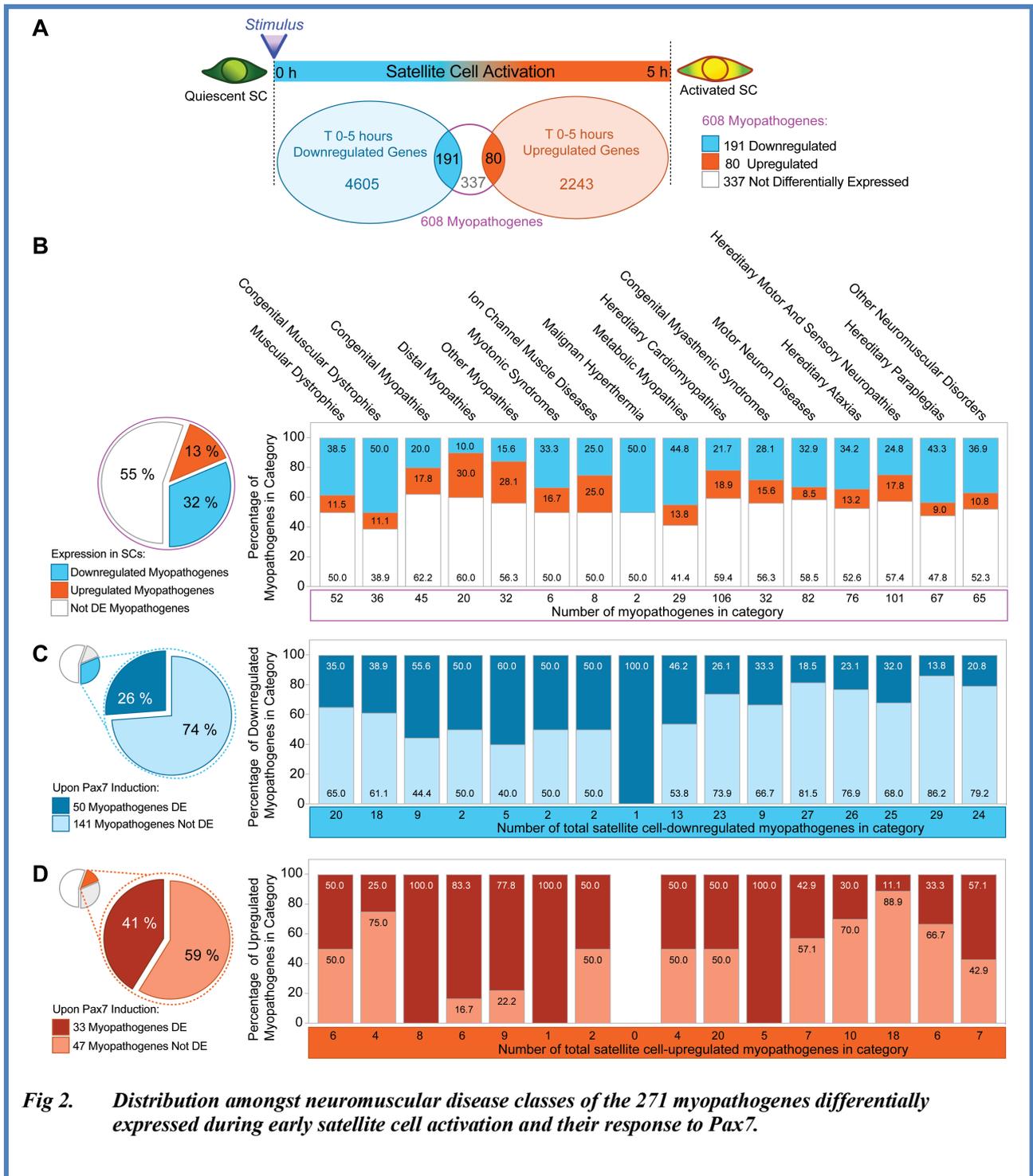
> **Fig 2.** **Distribution amongst neuromuscular disease classes of the 271 myopathogenes differentially expressed during early satellite cell activation and their response to Pax7.**
> *A. Quiescent/activated murine satellite cell gene-sets retrieved from (42) used to analyse involvement of the 608 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.[39] Mono or binucleate cardiomyocytes are linked together via the intercalated disc to form a functional syncytium. Contraction is initiated by pacemaker cells in the atrioventricular node, with heart rate regulated by hormones and parasympathetic/sympathetic innervation.[40] 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).[41] 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.[3]

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.[3] Our multi-modal approach integrates:
i) differential myopathogene expression during satellite cell activation;
ii) myopathogene regulation by the satellite cell-specific transcription factor PAX7;
iii) 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).[4] 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).[4] 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.[42] 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,[43-45] 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.[46] 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.

Satellite Cell Myogenesis — 0 h Downregulated → Upregulated 5 h — Myopathogenes regulated by Pax7

Muscular Dystrophies

Downregulated		Upregulated
FHL1	TRAPPC11	LMNA
SYNE1	ISPD	COL6A3
COL6A1	POGLUT1	CAV3
COL6A2	LAMA2	TTN
SGCA	POMGNT2	BVES
SGCB	GAA	POPDC3
TRIM32	LIMS2	
POMT1	DPM3	
FKTN	PYROXD1	
DAG1	POMK	

Congenital Muscular Dystrophies

Downregulated		Upregulated
B4GAT1	MSTO1	COL12A1
COL6A1	POMGNT2	COL6A3
COL6A2	POMK	LARGE1
DAG1	POMT1	LMNA
FKTN	RXYLT1	
GOLGA2	RYR1	
INPP5K	TRAPPC11	
ISPD	TRIP4	
LAMA2		
MPDU1		

Congenital Myopathies

Downregulated	Upregulated
CCDC78	ACTN2
MEGF10	HACD1
MTM1	KLHL40
PAX7	LMOD3
PYROXD1	MYPN
RYR1	SCN4A
RYR3	TPM3
STAC3	TTN
TRIM32	

Distal Myopathies

Downregulated	Upregulated
GNE	ACTN2
TIA1	CAV3
	FLNC
	HSPB8
	SQSTM1
	TTN

Other Myopathies

Downregulated	Upregulated	
FHL1	BAG3	MB
GIPC1	CAV3	MSTN
PYROXD1	CLN3	SVIL
RYR1	CRYAB	TTN
STIM1	FLNC	

Myotonic Syndromes

Downregulated	Upregulated
ATP2A1	CAV3
DMPK	

Ion Channel Muscle Disease

Downregulated	Upregulated
ATP1A2	KCNJ18
CACNA1A	SCN4A

Malignant Hyperthermia

Downregulated	Upregulated
RYR1	

Metabolic Myopathies

Downregulated		Upregulated
ACAD9	PHKA1	PGK1
AGL	PRKAG2	PGM1
CPT2	RBCK1	PNPLA2
ENO3		SLC16A1
ETFB		
FLAD1		
GAA		
GYG1		
LPIN1		
PFKM		

Hereditary Cardiomyopathies

Downregulated		Upregulated	
AARS2	MRPL44	ACTN2	KCND3
ABCC9	NDUFAF1	ANKRD1	KCNE1
CACNB2	NEXN	BAG3	KCNQ1
CALR3	PRKAG2	CAV3	LMNA
COX15	PSEN2	CRYAB	MYL4
EYA4	SCN1B	CTNNA3	MYPN
FKTN	SDHA	DTNA	PKP2
FLNA	SYNE1	FLNC	PRDM16
GAA	TAZ	HACD1	TTN
GATAD1	TGFB3	HCN4	VCL
GPD1L	TMPO		
JUP			

Congenital Myasthenic Syndromes

Downregulated		Upregulated
CHRNB1	NEXN	CHRND
AGRN	PRKAG2	MUSK
RAPSN	PSEN2	DOK7
ALG2	SCN1B	SCN4A
ALG14	SDHA	CHRNG
PREPL	SYNE1	
SLC25A1	TAZ	
VAMP1	TGFB3	
LAMB2	TMPO	
JUP		
MRPL44		
NDUFAF1		

Motor Neuron Diseases

Downregulated		Upregulated
ANG	NEK1	ATXN2
AR	NEK9	HSPB1
ASAH1	PIP5K1C	HSPB8
ASCC1	PLEKHG5	MUSK
CNTNAP1	PRUNE1	OPTN
DCTN1	REEP1	SQSTM1
FIG4	SIGMAR1	TTN
GLE1	SLC25A46	TUBA4A
HINT1	SLC52A2	
IGHMBP2	TRIP4	
MAPT	UBQLN2	
MCM3AP	VAPB	
MYBPC1	VRK1	
JMYH14		

Hereditary Ataxias

Downregulated		Upregulated
ANO10	STUB1	ATXN1
APTX	SYNE1	ATXN2
ATG5	TDP1	CACNA1G
ATM	THG1L	CACNB4
ATXN10	TK2	IFRD1
CACNA1A	TPP1	KCND3
COA7	TTPA	PCNA
ELOVL5	VWA3B	PUM1
FXN	WDR73	TGM6
MSTO1	WWOX	UBA5
PEX7	XRCC1	
PHYH		
PLD3		
SIL1		
SLC1A3		

Hereditary Motor and Sensory Neuropathies

Downregulated		Upregulated	
AIFM1	PLEKHG5	ATP1A1	RAB7A
ARHGEF10	PMP22	BAG3	SGPL1
ATL1	PNKP	C1orf194	SPTLC2
ATL3	SCN11A	CTDP1	
CNTNAP1	SLC12A6	DGAT2	
DCAF8	SORD	EGR2	
DHTKD1	SURF1	FLVCR1	
FGD4	TRIM2	HSPB1	
FIG4	VRK1	HSPB8	
HINT1	ZFHX2	INF2	
IGHMBP2		LITAF	
ITPR3		LMNA	
LRSAM1		NDRG1	
MPV17		NGF	
NAGLU		PRPS1	

Hereditary Paraplegias

Downregulated		Upregulated
ALDH3A2	HACE1	ALDH18A1
AP4B1	KIDINS220	CHP1
AP4M1	KIF1C	DDHD1
AP5Z1	NIPA1	HSPD1
ATL1	PLP1	UBAP1
ATP13A2	PNPLA6	UCHL1
C12orf65	REEP1	
C19orf12	RTN2	
CAPN1	SLC33A1	
CPT1C	SPG21	
CYP2U1	SPG7	
ERLIN2	TECPR2	
FARS2	VAMP1	
GBA2	ZFYVE26	
GJC2		

Other Neuromuscular Disorders

Downregulated		Upregulated
AGRN	RYR1	DOK7
AIFM1	SGCE	KIF21A
ASCC1	SLC25A4	MET
COQ2	SLC25A42	MUSK
COQ4	SUCLG1	SCN4A
COQ7	SYNE1	TMEM65
COQ9	TIMM22	TNNT3
DGUOK	TK2	
FBXL4	TOP3A	
MSTO1		
MYBPC1		
OPA1		
RAPSN		
RNASEH1		
RRM2B		

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 - 3 hours of satellite cell activation in our previous study.[3]*

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.[3] 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).[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 *FHL1*, *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.[47] Muscle biopsies display limited myofibre damage/alterations, but increased number of satellite cells due to reduced proliferative capacity and poor myogenic potential,[48,49] 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;[50] hence it is not surprising that NMJ dysfunction is common in neuromuscular disorders, correlating with decreased muscle function and integrity.[51]

DAG1 is a myopathogene identified as being regulated during satellite cell activation and responding to PAX7 (Figure 3).[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.[52] 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).[53] Patients affected by dystroglycanopathy often present dysfunctional NMJ, demonstrating the importance of α-DYSTROGLYCAN and the DAPC.[54] 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.[55]

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).[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).[56] However, Fukutin also functions in synapse formation and *Fktn*-deficient mice have impaired NMJs.[57-59] *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,[61,62] suggesting its classification as a Secondary Satellite Cell-opathy.[3] 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.[63] 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.[51,64] *DYSTROPHIN* is also expressed in satellite cells and is reported to actively regulate their asymmetric division, maintaining the stem cell niche.[65,66] 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.[51] DYSTROPHIN accumulates at the NMJ, where the complex assists with maintenance of the motor endplate ensuring muscle excitability and optimal neurotransmission.[67,68] Notably, muscle wasting in DMD biopsies is accompanied by increase in the number of PAX7-positive cells which could affect proper NMJ function/maintenance.[69] Pathogenic mutations in *DYSTROPHIN* are also associated with Becker muscular dystrophy (BMD; OMIM: 300376), where NMJ dysfunction is also common.[70] 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, TGFB3 and JUP also responding to Pax7 induction (Figures 2 and 3),[42,46] 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.[73,74] Strikingly, our new analysis shows that ALS myopathogenes *NEK1, TUBA4A, SQSTM1, TIA1, HSPB1, CRYAB* and *SIGMAR1* are differentially regulated in satellite cells (Figure 2 and Figure 3).[3,74,75] This indicates that ALS may have underlying satellite cell dysfunction, as suggested by recent studies.[32,76,77] 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 Welander 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 Metabolic Myopathies

Muscle conditions can originate from metabolic disturbances affecting the neuromuscular system that could also alter satellite cell status/number.[78] We found that 17 myopathogenes in the metabolic myopathy class were differentially regulated during activation in satellite cells (Figure 3). The *GAA* gene encodes for acid alpha Glucosidase and is mutated in Glycogen Storage Disease 2 (GSD2/Pompe disease, OMIM: 232300, but formerly LGMD2V), leading to accumulation of glycogen in both myofibres and motor neurons,[79] suggesting that mutant *GAA* has a wider impact on neuromuscular homeostasis. *GAA* is dynamically regulated during early satellite cell myogenesis (Figure 2)[42] although the number of PAX7-positive cells is unchanged in muscle biopsies.[80] Classification of GSD2 as either a LGMD or metabolic myopathy is still debated, as muscle biopsies show some myofibre damage, but limited fibrosis and inflammation. Both GSD2 patients and *Gaa*-null mice display

inefficient muscle regeneration, likely due to poor satellite cell activation,[81,82] consistent with mutant GAA expression in satellite cells. GSD2 also display NMJ deterioration.[83] 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,[84] 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 respond to Pax7 including *ENO3*, *FLAD1*, *PGM1* 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,[3] 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 mechano-transduction, 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.[85,86] 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).[87] Hence, mutant LaminA/C alters performance of both striated muscle and nerve,

with cardiac dysfunction and muscle weakness often present together.[38] We have previously defined MDCL as a bone-fide Secondary Satellite Cell-opathies, given the role/expression of *LMNA* in satellite cells and myofibres.[3] 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).[42] Furthermore, *LMNA* expression dynamically responds to Pax7 modulation (Figure 3).[46] 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,[88] whereas overexpression of pathogenic missense Lamin A/C variants in healthy myoblasts alters fusion and blunts expression of fusogens *Mymk* and *Mymx*. *Lmna*-null murine myofibres also have less myonuclei suggesting reduced fusion of satellite cell-derived myoblasts.[88-93] Together, these observations may explain why dysfunctional satellite cells accumulate in EDMD2 muscles. Notably, *Lmna*-null mouse models have alterations in NMJ structure,[94] 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.[95,96]

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,[97] 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.[4] Consistent with this hypothesis, mice bearing mutation in Ttn have increased satellite cell numbers,[98] and although similar evaluation has not been performed for human, we suggested Titinopathies could also include perturbed satellite cell function.[3]

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 (NEM11; 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.[99] CAVEOLIN 3 localises mainly at the sarcolemma along with the DAPC,[100] and contributes to cytoskeletal remodelling during differentiation and later, to myocyte fusion.[101] 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 CAVEOLIN 3 dysfunction may also blunt formation of binucleated cardiomyocytes in human.[102-104] Not only does CAVEOLIN 3 colocalise with the DAPC, but it also contributes to its integrity, as altered *CAV3* expression disrupts the complex and result in decreased Dystrophin accumulation.[105] CAVEOLIN 3 is also crucial for NMJ formation and function.[106] Thus, CAVEOLIN 3 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 Cell-opathies.

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 (107-109) 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,[110] crucial for satellite cell activation,[111] whereas mutations in *REEP1* and *IGHMP2* associate with MEGF10-like muscular phenotypes,[112] 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,[113] 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 EDMD2, 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,[4] 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,[114] as

described previously.[3] 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 myopathogene not only 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,[3] 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.[115-118]

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,[3] whereas in this study we used a longer time frame of 5 hours from quiescence.[42] 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,[119-122] is significantly downregulated after 3 hours from activating stimulus,[3,42] 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.[120,123] 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).[3]

"-Omics" technologies indicate molecular heterogeneity across satellite cell populations,[124-129] and that individual stem cells may transition across behavioural stages to maintain a homeostatic equilibrium.[10,130-132] 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.[133,134]

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 Dysmorphic Facies
MYOSCO - Progressive Congenital Myopathy with Scoliosis
NMJ - Neuromuscular Junction

Contributions of Author

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