Dysregulation of GSK3β-Target Proteins in Skin Fibroblasts of Myotonic Dystrophy Type 1 (DM1) Patients

Valentina Grande, Denisa Hathazi, Emily O'connor, Theo Marteau, Ulrike Schara-Schmidt, Andreas Hentschel, Genevieve Gourdon, Nikoletta Nikolenko, Hanns Lochmüller, Andreas Roos

Journal of Neuromuscular Diseases xx (2021) x–xx
DOI 10.3233/JND-200558
IOS Press

Dysregulation of GSK3β-Target Proteins in Skin Fibroblasts of Myotonic Dystrophy Type 1 (DM1) Patients

Valentina Grande[a], Denisa Hathazi[b,c], Emily O´Connor[d], Theo Marteau[a], Ulrike Schara-Schmidt[a], Andreas Hentschel[b], Genevieve Gourdon[e], Nikoletta Nikolenko[f], Hanns Lochmüller[d,g,h,i] and Andreas Roos[a,d,*]

[a]*Department of Neuropediatrics, University Hospital Essen, Duisburg-Essen University, Germany*
[b]*Leibniz-Institut für Analytische Wissenschaften -ISAS- e.V., Dortmund, Germany*
[c]*Department of Clinical Neurosciences, University of Cambridge School of Clinical Medicine, Cambridge, UK*
[d]*Children´s Hospital of Eastern Ontario Research Institute, University of Ottawa, Ottawa, ON, Canada*
[e]*Inserm UMR 974, Sorbonne Université, Centre de Recherche en Myologie, Association Institut de Myologie, Paris, France*
[f]*National Hospital for Neurology and Neurosurgery, Queen Square, University College London Hospitals NHS Foundation Trust, London, UK*
[g]*Division of Neurology, Department of Medicine, The Ottawa Hospital, Ottawa, ON, Canada*
[f]*Department of Neuropediatrics and Muscle Disorders, Medical Center - University of Freiburg, Faculty of Medicine, Freiburg, Germany*
[i]*Centro Nacional de AnálisisGenómico, Center for Genomic Regulation (CNAG-CRG), Barcelona Institute of Science and Technology (BIST), Barcelona, Catalonia, Spain*

Pre-press 1 March 2021

Abstract. Myotonic dystrophy type 1 (DM1) is the most common monogenetic muscular disorder of adulthood. This multisystemic disease is caused by CTG repeat expansion in the 3′-untranslated region of the DM1 protein kinase gene called *DMPK*. *DMPK* encodes a myosin kinase expressed in skeletal muscle cells and other cellular populations such as smooth muscle cells, neurons and fibroblasts. The resultant expanded (CUG)n RNA transcripts sequester RNA binding factors leading to ubiquitous and persistent splicing deregulation. The accumulation of mutant CUG repeats is linked to increased activity of glycogen synthase kinase 3 beta (GSK3β), a highly conserved and ubiquitous serine/threonine kinase with functions in pathways regulating inflammation, metabolism, oncogenesis, neurogenesis and myogenesis. As GSK3β-inhibition ameliorates defects in myogenesis, muscle strength and myotonia in a DM1 mouse model, this kinase represents a key player of DM1 pathobiochemistry and constitutes a promising therapeutic target. To better characterise DM1 patients, and monitor treatment responses, we aimed to define a set of robust disease and severity markers linked to GSK3βby unbiased proteomic profiling utilizing fibroblasts derived from DM1 patients with low (80–150) and high (2600–3600) CTG-repeats. Apart from GSK3β increase, we identified dysregulation of nine proteins (CAPN1, CTNNB1, CTPS1, DNMT1, HDAC2, HNRNPH3, MAP2K2, NR3C1, VDAC2) modulated by GSK3β. *In silico*-based expression studies confirmed expression in neuronal and skeletal muscle cells and revealed a relatively elevated abundance in fibroblasts. The potential impact of each marker in the myopathology of DM1 is discussed based on respective function to inform potential uses as severity markers or for monitoring GSK3β inhibitor treatment responses.

Keywords: GSK3β, fibroblast proteomics, CTPS1, CAPN1, HDAC2, CTNNB1

*Correspondence to: Andreas Roos, Tel.: +49 201 723 6570;
E-mail: andreas-roos@uk-essen.de.

INTRODUCTION

Myotonic dystrophy type 1 (DM1; MIM: 160900) is the most common monogenetic muscular disorder of adulthood with an estimated worldwide prevalence of 5/100,000, ranging from 0.5 in Taiwan up to 18.1/100,000 in Croatia [1]. In addition, DM1is highly prevalent in Canada (Saguenay-Lac-Saint-Jean) where its carrier rate reaches 1/550 [2, 3]. The disease is characterised by progressive muscle atrophy and weakness, often combined with myotonia, fatigue, respiratory insufficiency, and speech and swallowing difficulties [4, 5]. DM1 represents a multisystemic disorder and affects multiple organs including the central nervous system, heart, eye, skin, gastrointestinal and reproductive tracts, as well as the endocrine and immune systems [5]. Traditionally, DM1 is categorized according to phenotype and age of onset. However, this classification into congenital, juvenile, childhood, adult and late onset is complicated by significant intrafamilial and interfamilial variability [5, 6].

DM1 is an autosomal dominant disorder, caused by a CTG repeat expansion [(CTG)n] in the 3′-untranslated region of the DM1 protein kinase gene (*DMPK*). *DMPK* is located at position 19q13.32 and encodes a myosin kinase expressed in skeletal muscle [7]. Expanded alleles display microsatellite instability towards expansion in germline cells (causing genetic anticipation) and somatic cells (contributing to disease progression and phenotype variability [8–11]). The resultant expanded (CUG)n RNA transcripts cause the DM1 phenotype by sequestering RNA binding factors and leading to ubiquitous and persistent splicing deregulation [5, 12–17]. This toxic (CUG)n RNA effect is mediated by gain of function of combined CUGBPElav-like family member 1 (CELF1) and loss of function of muscleblind like splicing regulator (MBNL) due to clustering of both proteins [15–17]. Several aberrant splicing events have been linked to muscular [15, 16, 18–21], cardiac [15, 20, 22, 23], neurocognitive [24, 25] and endocrine [15, 16, 26–28] features of DM1.

Glycogen synthase kinase 3 beta (GSK3β) activity is significantly increased in the skeletal muscles of DM1 patients [29]. This increase in GSK3β activity is linked to the accumulation of mutant CUG repeats and, by promoting the phosphorylation of cyclin D3 at T283, leading to degradation and resultant in muscle weakness. GSK3β is a highly conserved and ubiquitous serine/threonine kinase with an important pleiotropic role in pathways regulating inflammation, metabolism, oncogenesis, neurogenesis and myogenesis [29–34]. Pharmacological inhibition of GSK3β ameliorates defects in myogenesis, muscle strength and myotonia in DM1 mice (HSA^{LR}) [29] further supporting the emergence of GSK3β as a novel and promising therapeutic target for DM1 [35]. To better characterise DM1 patients, and monitor treatment responses, it will be necessary to define a set of robust disease and severity markers.

PATIENTS, MATERIAL AND METHODS

In this study, we aimed to identify DM1 protein markers reflecting activation of pathways, such as GSK3β overactivation, in patient-derived fibroblasts, a suitable cell model to study the molecular etiology of neuromuscular diseases [36]. Proteomic profiling was performed on fibroblasts derived from DM1-patients with a mild/late onset [(CTG) 80–150] and a severe/congenital [(CTG) 2600–3600] DM1 phenotype. Obtained data were filtered for dysregulated proteins that are known to be modulated by GSK3β or involved in GSK3β-dependent processes.

Ethical considerations

This study has been ethically approved as a substudy as part of OPTIMISTIC (reference: NRES Committee North East - Sunderland 13.NE.0342) and PHENODM1 (reference: NRES Committee North East – Tyne & Wear South 15.NE.0178). Patients were consented and recruited at Newcastle upon Tyne NHS Foundation Trust and all procedures leading to these results complied with the Good Clinical Practices and the Declaration of Helsinki.For skin biopsies studies, informed consent was obtained from all patients.

Fibroblasts and cell culture

Fibroblasts were isolated from fresh donor skin biopsies following standardised EuroBioBank protocols (www.eurobiobank.org/biobanking-sops): biopsies were washed with sterile phosphate-buffered saline (PBS) and digested at 37°C for 15 minutes with 2.5% trypsin (Thermo Fisher Scientific) and a further 90 minutes with 0.5% collagenase (Type IV, Sigma-Aldrich). Fibroblasts were proliferated in Ham's F-10-Complete Medium (Thermo Fisher Scientific) supplemented with 20% foetal bovine serum (FBS, SeraLab – Bioreclamation IVT), 2% penicillin-streptomycin (Thermo Fisher Scientific), 1%

Table 1
Clinical findings for the six DM1 patients who provided fibroblasts for the proteomic profiling study. MIRS: Muscular impairment rating scale. Repeat expansions refer to DNA extracted from fibroblasts

Patient/ sample	CTG-repeats	Age at disease onset [years]	Age at time of study (skin biopsy)	Main DM symptoms (in addition to muscle weakness)	MIRS
1	84	34	50	Conduction defect, excessive daytime sleepiness	III
2	94	45	55	Excessive day-time sleepiness	II
3	140	49	58	Conduction defect, cataracts	IV
4	2431	9	43	Conduction defect, cataracts, breathing difficulties, excessive day-time sleepiness	IV
5	2683	16	35	Excessive day-time sleepiness, breathing difficulties	III
6	3187	25	60	Excessive day-time sleepiness, conduction defect, breathing difficulties, cataracts	V

GlutaMAX™ (Thermo Fisher Scientific) and 1% fungizone (Thermo Fisher Scientific). Once fibroblast cells attained sufficient confluency, they were frozen and stored long-term in liquid nitrogen. For further experiments including proteomic profiling, cells were cultured as described above until a confluence of 70%, harvested by scraping from culture flasks, washed twice with ice-cold PBS and cell pellets were snap-frozen in liquid nitrogen and stored at –80°C until further processing.

In total 12 fibroblast samples were included in our proteomic study: 3 DM1 fibroblast lines from patients with early onset, severe disease and CTG-repeat expansions of more than 2000 (patients 4–6:2431, 2683 and 3187 CTG-repeats, respectively), 3 DM1 fibroblast lines from patients with late onset, mild disease and CTG-repeat expansions between 80 and 150 CTG-repeats (patients 1–3:84, 94 and 140 CTG-repeats, respectively), and a total of 6 sex- and age-matched controls (three controls per patient-group). DM1-patient derived cell lines were obtained from the "MRC Centre Neuromuscular Biobank" (Newcastle upon Tyne, UK) [37]. All patients were adults when the skin biopsies were taken. Clinical data for the individual patients are listed in Table 1.

Unbiased label-free LC-MS/MS and data analysis

The following reagents were used for LC-MS/MS: Ammonium hydrogen carbonate (NH_4HCO_3), anhydrous magnesium chloride ($MgCl_2$), guanidine hydrochloride (GuHCl), iodoacetamide (IAA), and urea (Sigma-Aldrich, Steinheim, Germany), tris base (AplichemBiochemica, Darmstadt, Germany), sodium dodecyl sulfate (SDS) (Carl Roth, Karlsruhe, Germany), dithiothreitol (DTT), EDTA-free protease inhibitor (Complete Mini) tablets (Roche Diagnostics, Mannheim, Germany), NaCl, $CaCl_2$ (Merck, Darmstadt, Germany), sequencing grade modified trypsin (Promega, Madison, WI USA), Benzonase® Nuclease (Novagen), a bicinchoninic acid assay (BCA) kit (Thermo Fisher Scientific, Dreieich, Germany). All chemicals for ultra-pure HPLC solvents such as formic acid (FA), trifluoroacetic acid (TFA) and acetonitrile (ACN) were obtained from Biosolve, Valkenswaard, The Netherlands.

Cell lysis, sample clean-up and proteolysis

Cell pellets were lysed in 100 µL of lysis buffer (50 mM Tris-HCl (pH 7.8), 150 mM NaCl, 1 % SDS, and Complete Mini) using a manual glass grinder. Extracts were centrifuged at 6000 g for 5 min at 4°C to separate cell debris from the protein lysate and protein concentration was determined by BCA assay (according to the manufacturer's protocol). Cysteines were reduced via the addition of 10 mM DTT followed by an immediate incubation at 56°C for 30 min. Alkylation of free thiol groups with 30 mM IAA was performed at room temperature (RT) in the dark for 30 min.

Sample preparation was performed using filter-aided sample preparation (FASP) [38, 39] with some minor changes: 100 µg of protein lysate was diluted 10-fold with freshly prepared 8 M urea/100 mM Tris-HCl (pH 8.5) buffer and placed on a PALL microsep centrifugal device (30 KDa cut off) and centrifuged at 13,500 g at RT for 20 min (all the following centrifugation steps were performed under the same conditions). Three washing steps were carried out with 100 µL of 8 M urea/100 mM Tris-HCl (pH 8.5). For buffer exchange, the device was washed three times with 100 µL of 50 mM NH_4HCO_3 (pH 7.8). The digestion buffer (final volume of 100 µL) composed of trypsin (1:25 w/w, protease to substrate), 0.2 M GuHCl and 2 mM $CaCl_2$ in 50 mM NH_4HCO_3

(pH 7.8), was added to the concentrated proteins and samples incubated at 37°C for 14 h. The resulting tryptic peptides were recovered by centrifugation with 50 µL of 50 mM NH_4HCO_3 followed by 50 µL of ultra-pure water. Peptides were acidified (pH < 3 by addition of 10 % TFA (v/v)). All digests were quality controlled as described previously [40].

LC-MS/MS analysis

1 µg of each sample was analyzed using an Ultimate 3000 nano RSLC system coupled to an Orbitrap Fusion Lumos mass spectrometer (both Thermo Scientific) in a randomized order to minimize systematic errors. Peptides were preconcentrated on a 100 µm × 2 cm C18 trapping column for 10 min using 0.1 % TFA (v/v) at a flow rate of 20 µL/min followed by separation on a 75 µm × 50 cm C18 main column (both Pepmap, Thermo Scientific) with a 95 min LC gradient ranging from 3–35 % of 84 % ACN, 0.1 % FA (v/v) at a flow rate of 250 nL/min. MS survey scans were acquired in the Orbitrap from 300 to 1500 *m/z* at a resolution of 120,000 using the polysiloxane ion at *m/z* 445.12002 as lock mass [41], an automatic gain control target value of 2.0×10^5 and maximum injection times of 50 ms. TopSmost intense signals were selected for fragmentation by higher-energy collisional dissociation with an energy of 30 % and MS/MS spectra were acquired in the Iontrap using an automatic gain control target value of 2.0×10^3 ions, a maximum injection time of 300 ms, a dynamic exclusion of 15 s and an isolation window of 1.2 (*m/z*).

Label free data analysis

Data analysis of the acquired label free MS data was performed using the Progenesis LC-MS software from Nonlinear Dynamics (Newcastle upon Tyne, U.K.). Raw MS data was aligned by Progenesis, which automatically selected one of the LC-MS files as reference. After automatic peak picking, only features within retention time and *m/z* windows from 0–95 min and 300–1500 *m/z*, with charge states + 2, + 3, and + 4 were considered for peptide statistics and analysis of variance (ANOVA) and MS/MS spectra were exported as peak lists. Peak lists were searched against a concatenated target/decoy version of the human Uniprot database (downloaded on 22.07.2015 containing 20273 target sequences) using Mascot 2.4 (Matrix Science, Boston, MA, USA), MS-GF + (beta,v10282), X!Tandem (X!Tandem Vengeance, 2015.12.15.2) and MyriMatch (2.2.140) with the help of searchGUI 3.2.5 [42]. Trypsin was selected as the enzyme, with a maximum of two missed cleavages, carbamidomethylation of cysteine was set as fixed and oxidation of methionine was selected as variable modification. MS and MS/MS tolerances were set to 10 p.p.m and 0.5 Da, respectively.

To obtain a peptide-spectrum match and to maximize the number of identified peptides and proteins at a given quality, we used PeptideShaker software 1.4.0 (http://code.google.com/p/peptide-shaker/) [43]. Combined search results were filtered at a false discovery rate of 1% on the peptide and protein level and exported using the PeptideShaker features that allow direct re-import of the quality-controlled data into Progenesis. Peptide sequences containing oxidized methionine were excluded from further analysis. Only proteins quantified with unique peptides were exported. For each protein, average of the normalized abundances obtained from Progenesis was calculated to determine the ratios between the patient and control cells. Only proteins which were (*i*) commonly quantified in all the replicates with (*ii*) a minimum of one unique peptide, (*iii*) an ANOVA *p*-value of ≤ 0.05 (Progenesis) and (*iv*) an average ratio $\log_2 \leq 5.8$ or ≥ -0.73 for the low repeats (comparison 1) and an average ratio $\log_2 \leq 5.6$ or ≥ -2.3 for the high repeats (comparison 2) were considered as significantly regulated.

"STRING" (Search Tool for the Retrieval of Interacting Genes/Proteins; www.string-db.org) enables the delineation of direct and functionally related protein-protein interactions. Thus, to identify functional interdependences of proteins with altered abundances in diseased cells and tissues, we applied this *in silico* tool to decipher proteins interacting with the ten marker proteins identified by our proteomic profiling of DM1-patient derived skin fibroblasts.

The Genotype-Tissue Expression (GTEx; www.gtexportal.org) project, representing a comprehensive public resource to study tissue-specific gene expression, was used to address expression of GSK3β and identified markers in DM1 vulnerable tissues including different brain areas, peripheral nervous system, skeletal and cardiac muscle and skin/cultured fibroblasts. The data used for the analyses described in this manuscript were obtained from the GTEx Portal on 20/05.2020 dbGaP accession number phs000424.vN.pN on 12/10/2012.

Immunohistochemistry on murine muscles

Immunological staining of CAPN1 was performed utilizing cardiac and tibialis anterior muscle derived

from DMSXL mice (a suitable mouse model of DM1) [44] and wildtype littermates aged two and four months (two animals per group) as described previously [45]. For that purpose, a primary anti-Calpain 1 polyclonal antibody (Invitrogen: #PA5-86056) was used.

RESULTS

Expression of GSK3β in fibroblasts derived from patients with DM1

Proteomic profiles of fibroblasts derived from DM1 patients (early and adult disease onset; Table 1) and respective controls were generated. First, we analysed the proteomic signature for GSK3β within the whole protein extracts obtained from DM1-patient derived fibroblasts. This analysis revealed an increase in GSK3β expression in fibroblasts derived from DM1-patients of both groups; and this effect was more pronounced in cells derived from patients mildly affected by DM1 showing a 2.80-fold increase compared to a 1.62-fold increase in fibroblasts derived from severely affected DM1-patients (severe vs mild = 0.58, p-ANOVA = 0.110, statistically not significant; Table 2 &3; Fig. 1A).

Identification of dysregulated proteins related to GSK3β

Next, we screened these profiles for detection of one of the ∼100 proteins known to be modulated by GSK3β or involved in GSK3β -dependent processes, allowing the identification of 25dysregulated proteins [31, 30]: 9 of these dysregulated proteins were quantified with a statistically significant p-ANOVA (≤ 0.05) in both patient groups (Table 2 & Fig. 1A) and 16 with a statistically significant p-ANOVA (≤ 0.05) in only one of the defined groups (Supplementary Table 1). Out of the nine proteins, four are decreased (DNMT1, VDAC2, HNRNPH3 & CTNNB1) and five (MAP2K2, HDAC2, NR3C1, CTPS1 & CAPN1) are increased in both patient groups as compared to controls (Table 2 & Fig. 1A). To further define a disease marker as a marker of severity, the statistical significance of the change in fold-regulation for each protein between mild and severe patient groups was taken into account. This resulted in the definition of three severity markers (Table 2): HDAC2 is 2.42-fold upregulated in cells derived from mild patients, and 4.86-fold increased in severe patients (p-ANOVA 0.005). Similarly, CTPS1 is 1.99-fold

Table 2
Dysregulation of GSK3β and associated proteins in DM1-patient derived fibroblasts

Accession number	Protein name	Gene symbol	*p*-ANOVA mild group	*p*-ANOVA severe group	Fold of regul. mild group	Fold of regul. severe group	Disease marker	Severity marker
P49841	Glycogensynthase kinase-3 beta	*GSK3B*	0.044	0.030	2.80	1.62	x	
P26358	DNA (cytosine-5)-methyltransferase 1	*DNMT1*	0.036	0.001	0.18	0.27	x	
P35222	Catenin beta-1	*CTNNB1*	0.012	0.010	0.35	0.77	x	
P31942	Heterogeneous nuclear ribonucleoprotein H3	*HNRNPH3*	0.013	0.004	0.37	0.48	x	
P45880	Voltage-dependent anion-selective channel protein 2	*VDAC2*	0.042	0.001	0.55	0.59	x	
P07384	Calpain-1 catalytic subunit	*CAPN1*	0.007	0.000	1.39	1.71		x
P17812	CTP synthase 1	*CTPS1*	0.033	0.005	1.99	2.55		x
P04150	Glucocorticoid receptor	*NR3C1*	0.037	0.001	2.02	2.01	x	
Q92769	Histone deacetylase 2	*HDAC2*	0.042	0.008	2.42	4.86		x
P36507	Dual specificity mitogen-activated protein kinase kinase 2	*MAP2K2*	0.034	0.002	4.77	4.10	x	

Table 3
Abundance of each GSK3β-related protein identified in the proteomics of DM1 patient-derived fibroblasts, as compared to expression levels of GS3Kβ

	Average intensities			Ratio of each protein compared to GS3Kβ		
Gene symbol	Severegroup	Mild group	Controls	Severegroup	Mild group	Controls
GSK3B	1.14E+06	2.55E+06	9.10E+05	1.00	1.00	1.00
DNMT1	4.40E+05	3.38E+05	1.74E+06	0.39	0.13	1,92
CTNNB1	4.79E+06	5.53E+06	1.09E+07	4.19	2.17	12.00
HNRNPH3	7.71E+06	6.27E+06	1.65E+07	6.75	2.46	18.15
VDAC2	2.15E+07	3.02E+07	4.53E+07	18.84	11.87	49.81
CAPN1	5.82E+06	7.99E+06	4.57E+06	5.10	3.14	5.03
CTPS1	2.11E+06	2.58E+06	1.06E+06	1.85	1.01	1.16
NR3C1	1.27E+06	1.49E+06	6.73E+05	1.11	0.58	0.74
HDAC2	1.59E+06	3.70E+06	4.03E+06	1.39	1.45	4.43
MAP2K2	7.08E+05	5.61E+05	1.45E+05	0.62	0.22	0.16

increased in mild patient fibroblasts and 2.55-fold in cells of the severe patient group (p-ANOVA 0.04). Moreover, CAPN1 is 1.39-fold upregulated in cells derived from mild patients, and 1.71-fold increased in severe patients (p-ANOVA 0.002).

Change in expression of dysregulated proteins in relation to GSK3β

To investigate potential stoichiometric changes between GSK3β and the nine substrates dysregulated in DM1-patient derived fibroblasts, we compared the abundance of each protein with GSK3β expression. This comparison revealed decreased ratios for DNMT1, MAP2K2, VDAC2, HDAC2, HNRNPH3 and CTNNB1 in both patient groups compared to controls. For NR3C1 and CTPS1 decreased ratios were observed in the mild group and increased ratios in the severe DM1-patient group. For CAPN1, decreased ratios were identified by comparing control and mild patient group, but ratios remained almost unchanged between the controls and the group of severely affected DM1 patients. All protein intensities and calculated ratios to GSK3β are shown in Fig. 1B and listed in Table 3.

Protein network analysis

"STRING" protein-network analyses revealed a predicted functional interaction of GSK3β, MAP 2K2, CTNNB1, HDAC2, NR3C1, DNMT1& VDAC2 (Fig. 1C). Expanding the interaction network to include first level interactors of our dysregulated proteins revealed a further predicted interplay of 13 proteins, with the addition of CDH1, FYN, CTNNA1, APC and BCL9 as well as connecting CAPN1 to the functional network (Fig. 1C). Considering second level binding partners, a functional interplay of 18 proteins was delineated, with the addition of AXIN1, FER, AKT1, AKT3 and CTNNBIP1 (Fig. 1C).

In silico tissue expression analysis of dysregulated proteins

To address tissue expression of the identified dysregulated proteins, the GTEx database was used, with a focus on DM1-associated tissues, such as different areas of the central nervous system (amygdala, anterior cingulate cortex, frontal cortex, basal ganglia, cerebellum and cerebellar hemisphere, hippocampus, hypothalamus, substantia nigra and spinal cord), tibial nerve, skeletal and cardiac muscle as well as skin and cultured fibroblasts. Of note, among the different brain areas, all proteins show highest abundance in the cerebellum, including the cerebellar hemisphere. For CAPN1 and MAP2K2 increased abundance was also observed in cortex (Fig. 2). In addition, the nine markers and GSK3β are highly expressed in the tibial nerve, skin and cultured fibroblasts. Compared to the other tissues addressed, moderate abundance in skeletal muscle was observed for GSK3β, VDAC2, CAPN1, CTPS1, NR3C1 and MAP2K2 (Fig. 2).

Calpain-1 expression analysis in murine muscle

To verify our proteomic findings, expression CAPN1 as a paradigmatic protein was studied in cardiac and tibialis anterior muscle derived from DMSXL mice and wildtype littermates (two and four months of age). For both tissues elevated sarcoplasmic CA PN1 level were observed at two and four months of age, respectively (Fig. 3).

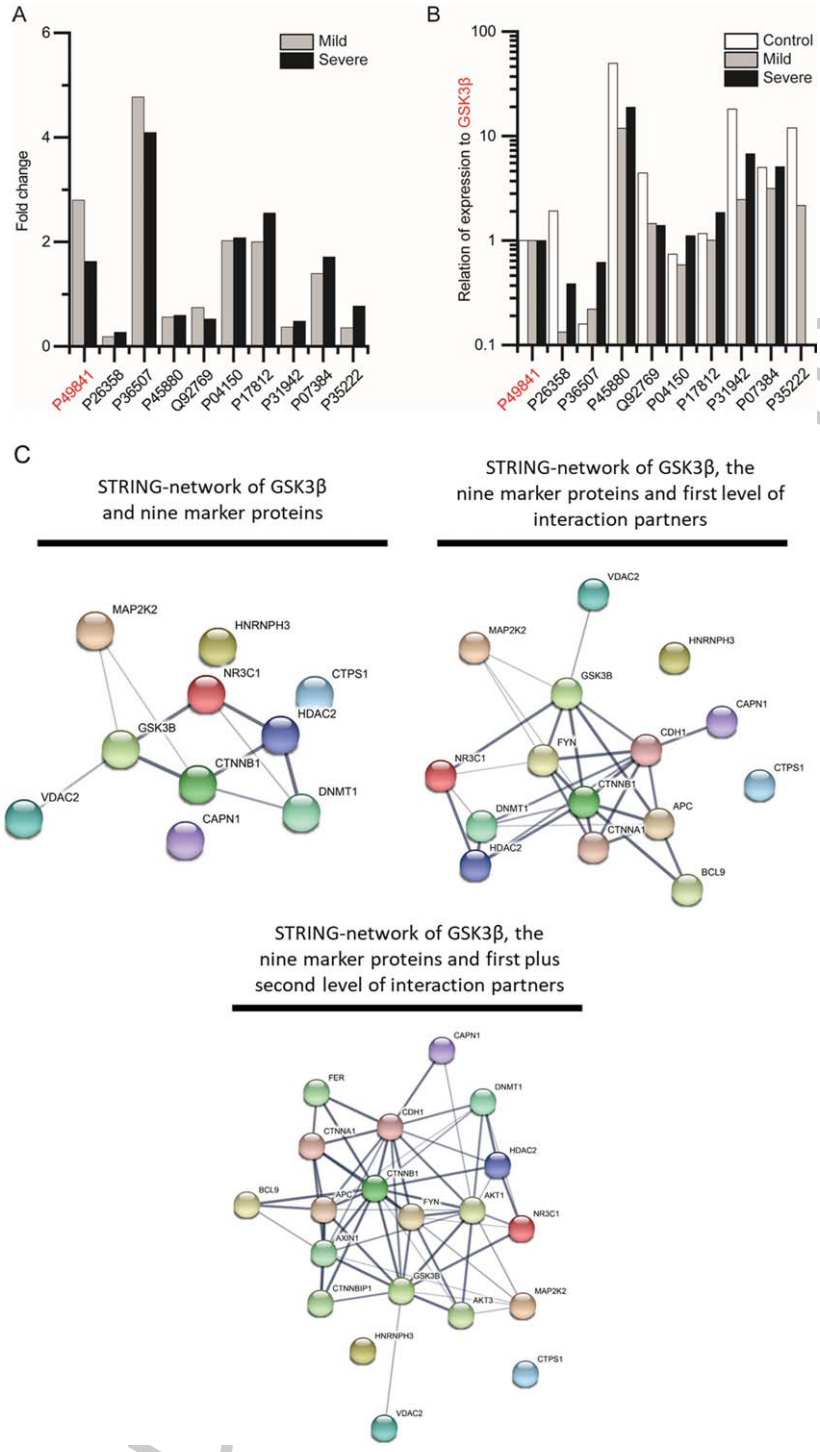

Fig. 1. Dysregulation of GSK3β and related proteins identified by unbiased proteomic profiling of DM1-patient derived skin fibroblasts (mildly and severely affected cases). (A) Altered relative expression of GSK3β (P49841; highlighted in red) and nine proteins known to be modulated by its function, thus serving as GSK3β-dependent cellular markers of DM1. (B) Changes in abundance of proteins expressed as a ratio with GSK3β expression in fibroblasts derived from mild and severely affected DM1 patients compared to controls. (C) STRING-based protein interaction networks reflecting interaction network of the nine markers identified, including first and second level binding partners. Thickness of the lines between the nodes refers to the confidence of interaction.

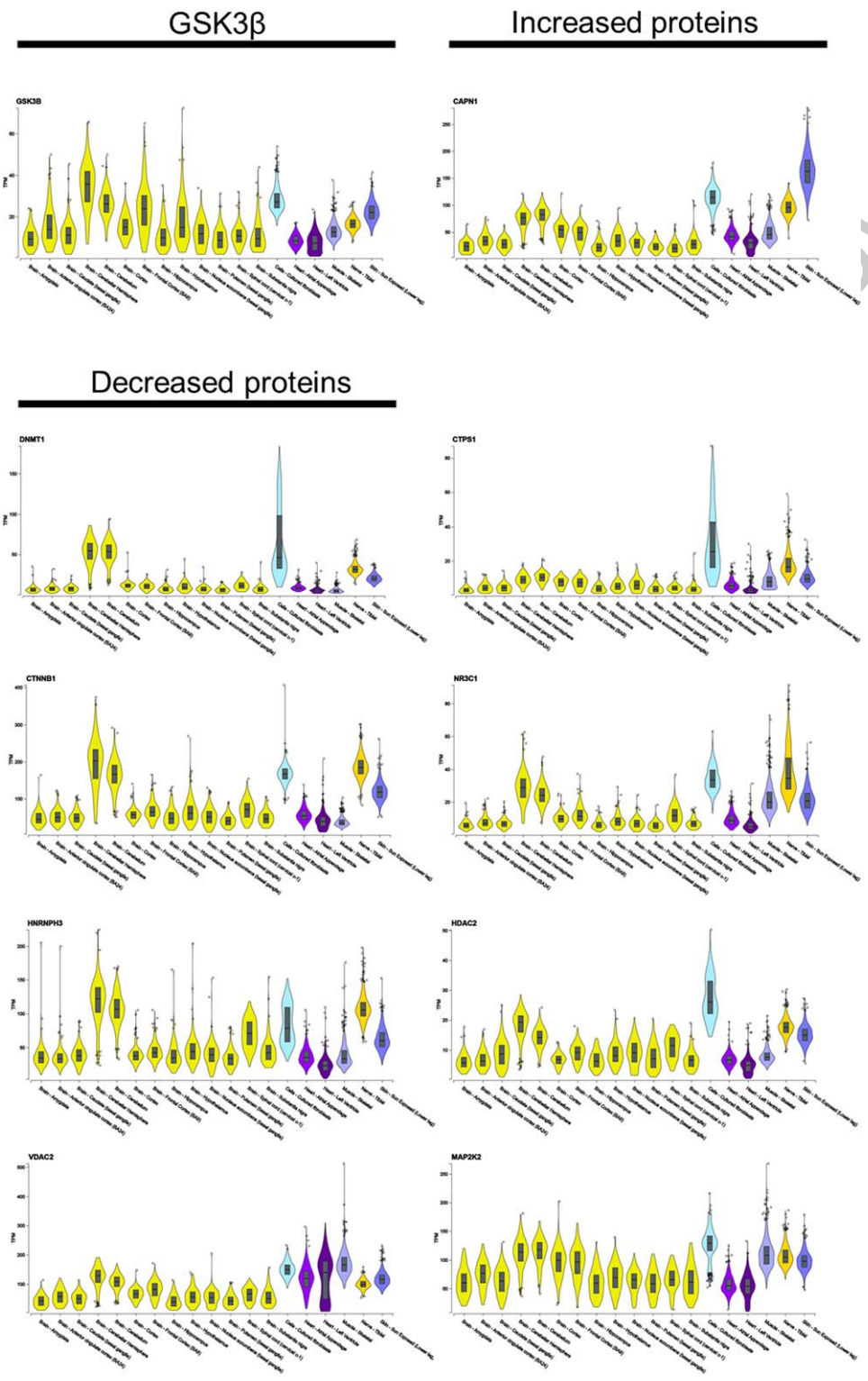

Fig. 2. GTEx-based *in silico* analysis of tissue expression of GSK3β and the nine associated proteins dysregulated in DM1-patient derived skin fibroblasts.

DISCUSSION

Proteomic profiling is a powerful tool for the unbiased identification of proteins contributing to neuromuscular disease pathology in cells and tissues derived from patients [46, 47]. Fibroblasts are known to be affected in DM1 [48], therefore, we performed LC-MS/MS-based analysis of the protein signature of DM1 patient-derived fibroblasts to identify proteins that may be markers of DM1 presence or severity.

Nearly 100 proteins are proposed to be substrates for GSK3β, highlighting the role of this kinase as a fundamental regulator of many cellular processes [31–49]. Given that GSK3β-overactivation is found in DM1-patient cells and tissues [29] and that inhibition of this overactivation represents a promising treatment strategy in DM1, we analysed GSK3β abundances in DM1-patient derived cells. This revealed an increase in GSK3β in both mild and severely affected patients. This effect was more pronounced in cells derived from mildly affected DM1-patients, thus in fibroblasts GSK3β protein level does not correlate with CTG-repeat expansion (and the resultant disease-severity classification). However, given that GSK3β-overactivation is modulated by post-translational modification, studies of GS3Kβ-phosphorylation are needed to draw a conclusion regarding a correlation of GSK3β-activation and CTG-repeat expansion/disease severity in DM1-fibroblasts.

After filtering out proteomic data for proteins modulated by GSK3β, we were left with nine proteins that may be potential markers of DM1 disease or severity. To the best of our knowledge, none of the nine proteins has been described as a DM1 blood biomarker thus far. *In silico*-based interaction studies suggested a down-stream impact on pathways perturbed in DM1, such as AKT-signalling (AKT1 & AKT3) [50]. The known ubiquitous expression of the nine cellular markers hints toward a potential overall involvement in DM1-pathophysiology and moreover suggests that further functional studies or pre-clinical intervention concepts can be performed in DM1-patient derived skin fibroblasts. This postulate is further supported by the proven high expression in human cultured skin fibroblasts as exemplified by or *in silico*-based expression studies. A comparison of their relative abundance with GSK3β levels showed many proteins had expression that varied with severity. This finding suggests a pathophysiological shift of GSK3β, and some of its substrates, that may allow their use for tracking progression of disease or response to GSK3β inhibitor therapy. The relevance of each GSK3β-related dysregulated protein to DM1 pathophysiology is discussed in subsequent sections:

DNA (cytosine-5)-methyltransferase 1 (DNMT1; decreased in DM1 fibroblasts): DNA (cytosine-5)-methyltransferase 1, encoded by the *DNMT1* gene, maintains DNA methylation profiles after DNA replication and cell division [51]. Koh and colleagues linked DNMT1 to GSK3β in fibroblasts: they found that when GSK3β is inactivated by phosphorylation, DNMT1 levels are elevated by TGF-beta1 [52]. Recent evidence shows that DNMT11 is required for correct myogenesis, a process impaired in DM1, leading to defective muscle regeneration and to muscle weakness and wasting [53, 54]. Studies performed in mice with muscle-specific deletion of *Dnmt1* revealed a reduced ability of myoblasts to differentiate [55]. Dnmt1-knock down in HT1080 cells, a human fibrosarcoma cell line carrying a copy of a human HPRT minigene inactivated by a $(CAG)_{95}$ repeat tract, destabilizes CAG-repeats, suggesting a fundamental role of Dnmt1 in CAG-repeat instability [56].

DNMT1 expression is more pronounced in "young" human fibroblasts than in passage-aged, and DNMT11-knockdown induces a senescence phenotype in "young" fibroblasts [57].The premature aging in DM1 patients [58] could link to the decreased DN MT1 protein level in DM1 patient derived fibroblasts (Table 1), presumably caused by GSK3β-overactivation.

Catenin beta-1 (CTNNB1; decreased in DM1 fibroblasts): is a key component of the canonical Wnt signaling pathway controlling physiological processes like embryonic development, cellular proliferation and differentiation [59]. In the absence of Wnt ligands, β-catenin is part of a cytoplasmic complex also containing GSK3β. This complex facilitates the phosphorylation and ubiquitination of CTN NB1, thus controlling its activation and proteasome-mediated degradation. In skeletal muscle, Wnt/β-catenin signalling plays a central role in myogenesis, differentiation and myotube hypertrophy [60–62]. Consequently, CTNNB1 is considered a promising target in diseases such as cachexia or muscular dystrophies, characterized by muscle wasting. A chronic overactivation of Wnt pathways was found in skeletal muscles of DMD-patients, driving the activation of TGFβ2 and thus promoting fibrosis [63]. Of note, exogenous inhibition of the Wnt pathway was shown to reduce fibrosis in dystrophic muscles of the *mdx* mouse model [64]. In the disease etiology of DM1, CTNNB1-upregulation (via the Wnt signaling

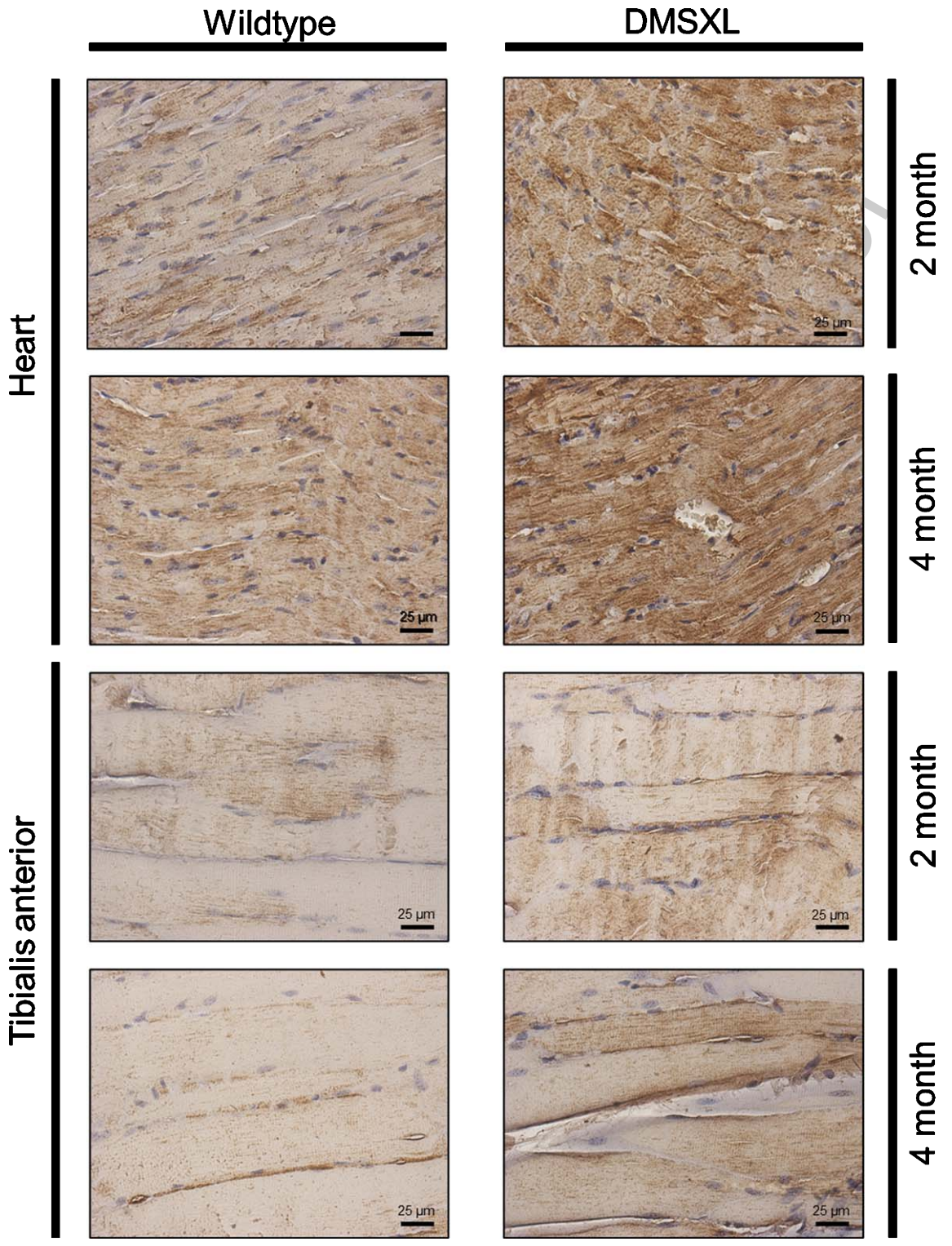

Fig. 3. Immunohistochemistry-based analysis of CAPN1 expression in cardiac and tibialis anterior muscle derived from DMSXL mice. Increased immunoreactivity of CAPN1 is detected in cardiac and tibialis anterior muscle (longitudinal sections) of diseased animals compared to controls at the age of two and four months.

pathway) was linked to increased cancer risk [65]. We identified decreased CTNNB1-levels in fibroblasts of both mild and severely affected patients, and in accordance with the expression level of GSK3β, this effect is more pronounced in mild patients rather than severe.

Heterogeneous nuclear ribonucleoprotein H3(hn RNPH3; decreased in DM1 fibroblasts): is an RNA binding protein involved in splicing, in particular by participating in early heat shock-induced splicing arrest [66]. Inhibition of GSK3β controls cellular levels of hnRNPH3, thus showing a functional interdependence of both proteins [67]. hnRNPH3 binds to splice regulator muscleblind 1 variants (MBNL1 CUG) that co-localize with CUG foci in DM1-patient-derived muscle cells [68]. Furthermore, hnRN PH3 levels are increased on average 3-fold in DM1 myoblasts [68], however, this is in contradiction with the decreased level observed in our study. This might be related to cell-type specific protein-composition of these foci, perhaps in turn influenced by cell type specific expression patterns of the related proteins.

Voltage-dependent anion-selective channel protein 2 (VDAC2; decreased in DM1 fibroblasts): localizes to the mitochondrial outer membrane where it constitutes a channel for the membrane transport of various substrates [69]. GSK3β is a major positive regulator of mitochondrial permeability during oxidative stress, a trigger of apoptosis, and interacts with VDAC2 [70]. Apart from its role in regulating the flux across the mitochondrial outer membrane, VDAC2 has a fundamental function in sequestering the proapoptotic protein BAK in the mitochondrial outer membrane and maintaining it in the inactive state [71].

Mitochondrial dysfunction and impaired oxidative metabolism are known pathophysiological mechanisms in DM1 [72, 73]. Impaired mitochondrial bioenergetics, accompanied by complex 1-dysfunction and decreased VDAC2 expression, were described in quadriceps and gastrocnemius muscle of *mdx* mice, an animal model for Duchenne muscular dystrophy [74]. Hence, one might assume that the VDAC2-decrease in DM1 contributes to mitochondrial dysfunction and the initiation of apoptosis.

Calpain-1 catalytic subunit (CAPN1; increased in DM1 fibroblasts): also called μ-calpain, belongs to a family of Ca^{2+}-dependent cysteine proteases [75]. It is expressed ubiquitously as a heterodimer [76]. Calpains are involved in several physiological functions such as cytoskeletal remodeling [77], signal transduction [78], apoptosis [79], cell cycle [80], regulation of gene expression [81] and long-term potentiation [82]. Truncation and activation of GS K3β is modulated by CAPN1 [83] and recent evidence showed that GSK3β phosphorylates desmin filaments (an essential event for myofibril destruction) in a CAPN1 dependent manner [84]. In skeletal muscle, increasedcalpain activation was described in atrophic conditions following disuse, denervation, glucocorticoid treatment and sepsis [85–87] as well as in necrotic fibers from *mdx* mice [88]. Moreover, elevated CAPN1 levels were found in sera samples of young DMD patients not yet treated with glucocorticoids, who also present with high levels of GSK3β that decline with aging [89]. Further evidence for a significant role of CAPN1 in muscle fibre integrity is given by *in vivo* studies: reduced muscle necrosis and, consequently, a significantly decreased regeneration was observed in *mdx* mice crossed with transgenic animals overexpressing calpastatin, a CAPN1-inhibitor [90]. Based on these findings, one might suppose that increased CAPN1 levels may be linked to the dystrophic conditions characterizing DM1. Indeed, immunohistochemical studies of CAPN1 on cardiac and tibialis anterior muscles derived from DMSXL mice, a suitable DM1 mouse model, revealed an increase compared to wildtype littermates in both tissues. This molecular finding suggests an applicability of our proteomic findings on tissues of major vulnerability in DM1 such as cardiac and skeletal muscle.

CTP synthase 1 (CTPS1; increased in DM1 fibroblasts): is responsible for *de novo* synthesis of CTP (cytidine triphosphate) and biosynthesis of phospholipids, nucleic acids and in sustaining the proliferation of activated lymphocytes [91–93]. GSK3β is a known regulator of CTPS1 activity [94]. We hypothesize that the increased level of CTPS1 is linked to the alterations in lipid composition or metabolism that can occur during skeletal and cardiac myopathies [95]. With regards to the insulin resistance in the cardiac and skeletal muscles of DM1 patients and animal models (see below), it is worth noting that this not only results in increased circulating glucose but also lipid levels [96]. Thus, an interdependent coregulation of both CTPS1 and NR3C1 in a GSK3β-dependent manner might be plausible.

Glucocorticoid receptor (NR3C1; increased in DM1 fibroblasts): is a member of the superfamily of nuclear receptors (acting as modulators of gene transcription) that, in the absence of glucocorticoid/cortisol, localizes to the cytosol in a chaperone-containing multiprotein complex [97]. GSK3β is

involved in ligand-dependent activation of transcription and cellular localization of this receptor [98]. A study performed in a large cohort of DM1 patients revealed alterations in glucocorticoid metabolism with an increased reactivation of cortisone to cortisol, mainly in male patients [99]. An excess of glucocorticoids leads to insulin resistance, which is also present in 5–17% of DM1 cases [100, 101]. In skeletal muscle, insulin resistance leads to impaired glucose uptake into the muscle fibers where it is required as an energy source. Due to insulin resistance and subsequent lipid metabolism alterations, DM1 patients can present with cardiac and vascular defects [101]. Therefore, increased NR3C1 might serve as a marker for the presence of DM1.

Histone deacetylase 2 (HDAC2; increased in DM1 fibroblasts): is a chromatin modifying enzyme that prevents gene-transcription by removal of acetyl groups from histones and deacetylates proteins such as transcription factors and those involved in the control of cell growth, differentiation and apoptosis [102]. HDAC2 expression and function is known to be controlled by GSK3β [103, 104]. In the context of DM1 pathophysiology it is important to note that two small molecule HDAC inhibitors, ISOX and vorinostat, increase MBNL1 expression, partially rescuing aberrant splicing in DM1 patient-derived fibroblasts [105]. HDAC2 might represent a marker protein in DM1-patient derived fibroblasts that plays a direct role in DM1 pathophysiology.

Dual specificity mitogen-activated protein kinase kinase 2 (MAP2K2; increased in DM1 fibroblasts): also known as MEK2, is a ubiquitously expressed protein that catalyses the phosphorylation of tyrosine and threonine in target proteins participating in the RAS–RAF–MEK–ERK signal transduction cascade. Consequently, via this cascade, MEK2 is involved in the regulation of a variety of processes including apoptosis, cell cycle progression, cell migration, differentiation, metabolism, and proliferation [106]. It also modulates phosphorylation and thus function of GSK3β via ERK [34]. Of particular interest for the muscle vulnerability of DM1, the MEK–ERK pathway is important in muscle cell differentiation [107]. Sustained activation of this pathway presumably also includes MEK2, as found in DM1 myoblasts, and can be considered as a contributor to the abnormal myoblast differentiation observed [108]. Moreover, RAS-ERK pathway activation is known to regulate alternative splicing [109], the main pathophysiological process in DM1. One of the most common symptoms in DM1, myotonia, is based on aberrant splicing of the skeletal muscle chloride channel (*CLCN1*) transcript [110, 111] and RAS pathway inhibitors correct this alternative splicing, alleviating some DM1 features in mice [112].

Although a transfer of the relevance of the proteomic data acquired from human skin fibroblasts could be demonstrated for CAPN1 in skeletal muscle utilizing a DM1 mouse model, further biochemical investigations would be needed to draw the same conclusion for the other marker proteins highlighted in this study. Along this line, confirmational studies on fibroblasts derived from further DM1 patients would be needed to substantiate the robustness of the proposed cellular marker proteins.

CONCLUSIONS

Overall, in this study we have identified nine proteins associated with GSK3β that may provide much-needed biomarkers for DM1 disease identification using easily obtained patient fibroblasts. Furthermore, we have also identified three proteins (CTPS1, CAPN1and HDAC2) that increase with severity of the disease, providing potential biomarkers that can be used in categorizing patients, monitoring disease progression and response to treatment. Validation of such biomarkers and their hypothesized roles in DM1 and GSK3β-associated pathophysiology will be required in future studies. Given that recent clinical trials in DM1 are based on GSK3β-inhibition [113], as the proteins defined in our study, due to their interaction with GSK3β,may serve as suitable markers to monitor success of the tested treatment concepts.

DECLARATIONS

Funding

Financial support by the Ministerium für Innovation, Wissenschaft und Forschung des Landes Nordrhein-Westfalen, the Senatsverwaltung für Wirtschaft, Technologie und Forschung des Landes Berlin and the Bundesministerium für Bildung und Forschung is gratefully acknowledged. This work was also supported by an unconditional grant from AMO pharma as well as a grant from the French Muscular Dystrophy Association (AFM-Téléthon; #21466) to AR. HL receives support from the Canadian Institutes of Health Research (Foundation Grant FDN-167281), the Canadian Institutes of Health Research and Muscular Dystrophy Canada (Network

Catalyst Grant for NMD4C), the Canada Foundation for Innovation (CFI-JELF 38412), and the Canada Research Chairs program (Canada Research Chair in Neuromuscular Genomics and Health, 950-232279).

ACKNOWLEDGMENTS

We thank Mr. Daniel Cox (MRC biobank Newcastle) for expert technical assistance.

CONFLICTS OF INTEREST/ COMPETING INTERESTS

All authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

AR, DH and HL designed the study. NN collected skin biopsies and provided clinical information. DH performed proteomic studies. AH performed data analysis and *in silico* studies. VG, EOC, USS, HL & AR drafted the manuscript. GG provided the tissue of the DM1 mouse model. All authors declare that the work described has not been published before and is also not under consideration for publication anywhere else. The final manuscript-draft has been approved by all co-authors.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/JND-200558.

REFERENCES

[1] Theadom A, Rodrigues M, Roxburgh R, Balalla S, Higgins C, Bhattacharjee R, et al. Prevalence of muscular dystrophies: A systematic literature review. Neuroepidemiology. 2014;43(3-4):259-68. https://doi.org/10.1159/000369343.

[2] Mathieu J, De Braekeleer M, Prévost C.Genealogical reconstruction of myotonic dystrophy in the Saguenay-Lac-Saint- Jean area (Quebec, Canada). Neurology. 1990;40(5):839. https://doi.org/10.1212/WNL.40.5.839.

[3] Yotova V, Labuda D, Zietkiewicz E, Gehl D, Lovell A, Lefebvre JF et al. Anatomy of a founder effect: Myotonic dystrophy in Northeastern Quebec. HUM GENET. 2005;117(2-3):177-87. https://doi.org/10.1007/s00439-005-1298-8.

[4] Pinessi L, Bergamini L, Cantello R, Di Tizio C. Myotonia congenita and myotonic dystrophy: Descriptive epidemiological investigation in Turin, Italy (1955-1979). Ital J Neurol Sci. 1982;3(3):207-10. https://doi.org/10.1007/BF02043311.

[5] Meola G and Cardani R. Myotonic dystrophies: An update on clinical aspects, genetic, pathology, and molecular pathomechanisms. Biochim Biophys Acta. 2015;1852(4):594-606. https://doi.org/10.1016/j.bbadis.2014.05.019.

[6] De Antonio M, Dogan C, Hamroun D, Mati M, Zerrouki S, Eymard B, et al. French Myotonic Dystrophy Clinical Network. Unravelling the myotonic dystrophy type 1 clinical spectrum: A systematic registry-based study with implications for disease classification. Rev Neurol (Paris). 2016;172(10):572-80. https://doi.org/10.1016/j.neurol.2016.08.003.

[7] Brook JD, McCurrach ME, Harley HG, Buckler AJ, Church D, Aburatani H, et al. Molecular basis of myotonic dystrophy: Expansion of a trinucleotide (CTG) repeat at the 3' end of a transcript encoding a protein kinase family member. Cell. 1992;69(2):385. https://doi.org/10.1016/0092-8674(92)90154-5.

[8] Harley HG, Rundle SA, MacMillan JC, Myring J, Brook JD, Crow S, et al. Size of the unstable CTG repeat sequence in relation to phenotype and parental transmission in myotonic dystrophy. Am J Hum Genet. 1993;52(6):1164-74.

[9] De Temmerman N, Sermon K, Seneca S, De Rycke M, Hilven P, Lissens W, et al.Intergenerational instability of the expanded CTG repeat in the DMPK gene: Studies in human gametes and preimplantation embryos. Am J Hum Genet. 2004;75(2):325-9. http://dx.doi.org/10.1086/422762.

[10] Yum K, Wang ET, Kalsotra A. Myotonic dystrophy: Disease repeat range, penetrance, age of onset, and relationship between repeat size and phenotypes. Curr Opin Genet Dev. 2017;44:30-7. http://dx.doi.org/10.1016/j.gde.2017.01.007.

[11] Harley HG, Rundle SA, Reardon W, Myring J, Crow S, Brook JD, et al. Unstable DNA sequence in myotonic dystrophy. Lancet. 1992;339(8802):1125-8. http://dx.doi.org/10.1016/0140-6736(92)90729-m.

[12] Klein AF, Gasnier E, Furling D. Gain of RNA function in pathological cases: Focus on myotonic dystrophy. Biochimie. 2011;93(11):2006-12. http://dx.doi.org/10.1016/j.biochi.2011.06.028.

[13] Thomas JD, Sznajder LJ, Bardhi O, Aslam FN, Anastasiadis ZP, Scotti MM, et al.Disrupted prenatal RNA processing and myogenesis in congenital myotonic dystrophy. Genes Dev. 2017;31(11):1122-33. http://dx.doi.org/10.1101/gad.300590.117.

[14] Holt I, Jacquemin V, Fardaei M, Sewry CA, Butler-Browne GS, Furling D, et al. Muscleblind-like proteins: Similarities and differences in normal and myotonic dystrophy muscle. Am J Pathol. 2009;174(1):216-27. http://dx.doi.org/10.2353/ajpath.2009.080520.

[15] Lin X, Miller JW, Mankodi A, Kanadia RN, Yuan Y, Moxley RT, et al. Failure of MBNL1-dependent post-natal splicing transitions in myotonic dystrophy. Hum Mol Genet. 2006;15(13):2087-97. http://dx.doi.org/10.1093/hmg/ddl132.

[16] Cardani R, Bugiardini E, Renna LV, Rossi G, Colombo G, Valaperta R, et al.Overexpression of CUGBP1 in skeletal muscle from adult classic myotonic dystrophy type 1 but not from myotonic dystrophy type 2. PLoS One. 2013;8(12):e83777. https://doi.org/10.1371/journal.pone.0083777.

[17] Gomes-Pereira M, Cooper TA, Gourdon G. Myotonic dystrophy mouse models: Towards rational therapy development. Trends Mol Med. 2011;17(9):506-17. http://dx.doi.org/10.1016/j.molmed.2011.05.004.

[18] Choi J, Personius KE, DiFranco M, Dansithong W, Yu C, Srivastava S, et al. Muscleblind-Like 1 and Muscleblind-Like 3 Depletion Synergistically Enhances Myotonia by Altering Clc-1 RNA Translation. EBio Medicine. 2015;2(9):1034-47. http://dx.doi.org/10.1016/j.ebiom.2015.07.028.

[19] Kino Y, Washizu C, Oma Y, Onishi H, Nezu Y, Sasagawa N, et al. MBNL and CELF proteins regulate alternative splicing of the skeletal muscle chloride channel CLCN1. Nucleic Acids Res. 2009;37(19):6477-90. http://dx.doi.org/10.1093/nar/gkp681.

[20] Bachinski LL, Baggerly KA, Neubauer VL, Nixon TJ, Raheem O, Sirito M, et al. Most expression and splicing changes in myotonic dystrophy type 1 and type 2 skeletal muscle are shared with other muscular dystrophies. Neuromuscul Disord. 2014;24(3):227-40. http://dx.doi.org/10.1016/j.nmd.2013.11.001.

[21] Santoro M, Piacentini R, Masciullo M, Bianchi ML, Modoni A, Podda MV, et al. Alternative splicing alterations of Ca2+handling genes are associated with Ca2+signal dysregulation in myotonic dystrophy type 1 (DM1) and type 2 (DM2) myotubes. Neuropathol Appl Neurobiol. 2014;40(4):464-76. http://dx.doi.org/10.1111/nan.12076.

[22] Vihola A, Bachinski LL, Sirito M, Olufemi SE, Hajibashi S, Baggerly KA, et al. Differences in aberrant expression and splicing of sarcomeric proteins in the myotonic dystrophies DM1 and DM2. Acta Neuropathol. 2010;119(4):465-79. http://dx.doi.org/10.1007/s00401-010-0637-6.

[23] Freyermuth F, Rau F, Kokunai Y, Linke T, Sellier C, Nakamori M, et al.Splicing misregulation of SCN5A contributes to cardiac-conduction delay and heart arrhythmia in myotonic dystrophy. Nat Commun. 2016;7:11067. http://dx.doi.org/10.1038/ncomms11067.

[24] Suenaga K, Lee KY, Nakamori M, Tatsumi Y, Takahashi MP, Fujimura H, et al. Muscleblind-like 1 knockout mice reveal novel splicing defects in the myotonic dystrophy brain. PLoS One. 2012;7(3):e33218. http://dx.doi.org/10.1371/journal.pone.0033218.

[25] Charizanis K, Lee KY, Batra R, Goodwin M, Zhang C, Yuan Y, et al. Muscleblind-like 2-mediated alternative splicing in the developing brain and dysregulation in myotonic dystrophy. Neuron. 2012;75(3):437-50. http://dx.doi.org/10.1016/j.neuron.2012.05.029.

[26] Paul S, Dansithong W, Kim D, Rossi J, Webster NJ, Comai L, et al. Interaction of muscleblind, CUG-BP1 and hnRNP H proteins in DM1-associated aberrant IR splicing. EMBO J. 2006;25(18):4271-83. http://dx.doi.org/10.1038/sj.emboj.7601296.

[27] Sen S, Talukdar I, Webster NJ. SRp20 and CUG-BP1 modulate insulin receptor exon 11 alternative splicing. Mol Cell Biol. 2009;29(3):871-80. http://dx.doi.org/10.1128/MCB.01709-08.

[28] Sen S, Talukdar I, Liu Y, Tam J, Reddy S, Webster NJ.Muscleblind-like 1 (Mbnl1) promotes insulin receptor exon 11 inclusion via binding to a downstream evolutionarily conserved intronic enhancer. J Biol Chem. 2010;285(33):25426-37. http://dx.doi.org/10.1074/jbc.M109.095224.

[29] Jones K, Wei C, Iakova P, Bugiardini E, Schneider-Gold C, Meola G, et al. GSK3beta mediates muscle pathology in myotonic dystrophy. J Clin Invest. 2012;122(12):4461-72. http://dx.doi.org/10.1172/JCI64081.

[30] Beurel E, Grieco SF, Jope RS.Glycogen synthase kinase-3 (GSK3): Regulation, actions, and diseases. Pharmacol Ther. 2015;148:114-31. http://dx.doi.org/10.1016/j.pharmthera.2014.11.016.

[31] Sutherland C. What Are the bona fide GSK3 Substrates? Int J Alzheimers Dis. 2011;2011:505607. http://dx.doi.org/10.4061/2011/505607.

[32] Verhees KJ, Pansters NA, Schols AM, Langen RC. Regulation of skeletal muscle plasticity by glycogen synthase kinase-3beta: A potential target for the treatment of muscle wasting. Curr Pharm Des. 2013;19(18):3276-98. http://dx.doi.org/10.2174/1381612811319180011.

[33] Pardo M, Abrial E, Jope RS, Beurel E. GSK3beta isoform-selective regulation of depression, memory and hippocampal cell proliferation. Genes Brain Behav. 2016;15(3):348-55. http://dx.doi.org/10.1111/gbb.12283.

[34] Bradley CA, Peineau S, Taghibiglou C, Nicolas CS, Whitcomb DJ, Bortolotto ZA, et al. A pivotal role of GSK-3 in synaptic plasticity. Front Mol Neurosci. 2012;5:13. http://dx.doi.org/10.3389/fnmol.2012.00013.

[35] Wei C, Jones K, Timchenko NA, Timchenko L. GSK3beta is a new therapeutic target for myotonic dystrophy type 1. Rare Dis. 2013;1:e26555. http://dx.doi.org/10.4161/rdis.26555.

[36] Hentschel A, Czech A, Münchberg U, Freier E, Schara-Schmidt U, Sickmann A, et al. Protein Signature of Human Skin Fibroblasts Allows the Study of the Molecular Etiology of Rare Neurological Diseases. Research Square; 2020. http://dx.doi.org/10.21203/rs.3.rs-48014/v1.

[37] Reza M, Cox D, Phillips L, Johnson D, Manoharan V, Grieves M, et al. MRC Centre Neuromuscular Biobank (Newcastle and London): Supporting and facilitating rare and neuromuscular disease research worldwide. Neuromuscular Disord. 2017;27(11):1054-64. http://dx.doi.org/10.1016/j.nmd.2017.07.001.

[38] Manza LL, Stamer SL, Ham AJ, Codreanu SG, Liebler DC. Sample preparation and digestion for proteomic analyses using spin filters. Proteomics. 2005;5(7):1742-5. http://dx.doi.org/10.1002/pmic.200401063.

[39] Wisniewski JR, Zougman A, Nagaraj N, Mann M. Universal sample preparation method for proteome analysis. Nat Methods. 2009;6:359-362. https://doi.org/10.1038/nmeth.1322.

[40] Burkhart JM, Schumbrutzki C, Wortelkamp S, Sickmann A, Zahedi RP. Systemic and quantitative comparison of digest efficiency and specificity reveals the impact of trypsin quality on MS-based proteomics. J Proteomics. 2012;75:1454-62. http://dx.doi.org/10.1016/j.jprot.2011.11.016.

[41] Phan V, Schmidt J, Matyash V, Malchow S, Thanisch M, Lorenz C, et al.Characterization of Naïve and Vitamin C-Treated Mouse Schwann Cell Line MSC80: Induction of the Antioxidative Thioredoxin Related Transmembrane Protein 1. J Proteome Res. 2018;17(9):2925-36. http://dx.doi.org/10.1021/acs.jproteome.8b00022.

[42] Vaudel M, Barsnes H, Berven FS, Sickmann A, Martens L. SearchGUI: An open-source graphical user interface for simultaneous OMSSA and X!Tandem searches. Proteomics. 2011;11:996-9. http://dx.doi.org/10.1002/pmic.201000595.

[43] Vaudel M, Burkhart JM, Zahedi RP, Oveland E, Berven FS, Sickmann A, et al. PeptideShaker enables reanalysis of MS-derived proteomics data sets. Nat Biotechnol. 2015;33(1):22-4. http://dx.doi.org/10.1038/nbt.3109.

[44] Huguet A, Medja F, Nicole A, Vignaud A, Guiraud-Dogan C, Ferry A, et al. Molecular, physiological, and motor performance defects in DMSXL mice carrying >1,000 CTG repeats from the human DM1 locus. PLoS Genet. 2012;8(11):e1003043. http://dx.doi.org/10.1371/journal.pgen.1003043.

[45] Roos A, Buchkremer S, Kollipara L, Labisch T, Gatz C, Zitzelsberger M, et al. Myopathy in Marinesco-Sjögren syndrome links endoplasmic reticulum chaperone dysfunction to nuclear envelope pathology. Acta Neuropathol. 2014;127(5):761-77. http://dx.doi.org/10.1007/s00401-013-1224-4.

[46] Roos A, Thompson R, Horvath R, Lochmüller H, Sickmann A. Intersection of Proteomics and Genomics to "Solve the Unsolved" in Rare Disorders such as Neurodegenerative and Neuromuscular Diseases. PROTEOM CLIN APPL. 2017;12(2):1700073. http://dx.doi.org/10.1002/prca.201700073.

[47] Thompson R, Spendiff S, Roos A, Bourque PR, Warman Chardon J, Kirschner J, et al. Advances in the diagnosis of inherited neuromuscular diseases and implications for therapy development. Lancet Neurol. 2020;19(6):522-32. http://dx.doi.org/10.1016/S1474-4422(20)30028-4.

[48] Rodríguez R, Hernández-Hernández O, Magaña JJ, González-Ramírez R, García-López ES, Cisneros B. Altered nuclear structure in myotonic dystrophy type 1-derived fibroblasts. Mol Biol Rep. 2014;42(2):479-88. http://dx.doi.org/10.1007/s11033-014-3791-4.

[49] Jope RS and Johnson GVW. The glamour and gloom of glycogen synthase kinase-3. Trends in Biochemical Sciences. 2004;29(2):95-102. https://doi.org/10.1016/j.tibs.2003.12.004.

[50] Morriss GR, Rajapakshe K, Huang S, Coarfa C and Cooper TA. Mechanisms of skeletal muscle wasting in a mouse model for myotonic dystrophy type 1.Hum Mol Genet. 2018;27(16):2789-804. https://doi.org/10.1093/hmg/ddy192.

[51] Gujar H, Weisenberger DJ, Liang G. The Roles of Human DNA Methyltransferases and Their Isoforms in Shaping the Epigenome. Genes. 2019;10(2):172. http://dx.doi.org/10.3390/genes10020172.

[52] Koh HB, Scruggs AM, Huang SK. Transforming Growth Factor-β1 Increases DNA Methyltransferase 1 and 3a Expression through Distinct Post-transcriptional Mechanisms in Lung Fibroblasts. J BIOL CHEM. 2016;291(37):19287-98. http://dx.doi.org/10.1074/jbc.m116.723080.

[53] Amack JD, Mahadevan MS. Myogenic defects in myotonic dystrophy. Dev Biol. 2004;265(2):294-301. http://dx.doi.org/10.1016/j.ydbio.2003.07.021.

[54] André LM, Ausems CRM, Wansink DG, Wieringa B. Abnormalities in Skeletal Muscle Myogenesis, Growth, and Regeneration in Myotonic Dystrophy. Front Neurol. 2018;9:368. http://dx.doi.org/10.3389/fneur.2018.00368.

[55] Liu R, Kim KY, Jung YW, Park IH. Dnmt1 regulates the myogenic lineage specification of muscle stem cells. Sci Rep. 2016;6:35355. http://dx.doi.org/10.1038/srep35355.

[56] Dion V, Lin Y, Hubert L, Waterland RA, Wilson JH. Dnmt1 deficiency promotes CAG repeat expansion in the mouse germline. Hum Mol Genet. 2008;17(9):1306-317. http://dx.doi.org/10.1093/hmg/ddn019.

[57] Xie H, Liu Y, Du R, Wang B, Chen M, Zhang Y, et al. miR-377 induces senescence in human skin fibroblasts by targeting DNA methyltransferase 1.CELL DEATH DIS. 2017;8(3):e2663-e2663. http://dx.doi.org/10.1038/cddis.2017.75.

[58] Campione E, Botta A, Di Prete M, Rastelli E, Gibellini M, Petrucci A, et al. Cutaneous features of myotonic dystrophy types 1 and 2: Implication of premature aging and vitamin D homeostasis. Neuromuscul Disord. 2017;27(2):163-9. http://dx.doi.org/10.1016/j.nmd.2016.11.004.

[59] Girardi F, Le Grand F. Wnt signaling in skeletal muscle development and regeneration. Progress in Molecular Biology and Translational Science. 2018;153:157-79. https://doi.org/10.1016/bs.pmbts.2017.11.026.

[60] van der Velden JLJ, Langen RCJ, Kelders MCJM, Wouters EFM, Janssen-Heininger YMW, Schols AMWJ Inhibition of glycogen synthase kinase-3β activity is sufficient to stimulate myogenic differentiation. AmJ Physiol Cell Physiol. 2006;290(2):C453-C462. https://doi.org/10.1152/ajpcell.00068.2005.

[61] Han XH, Jin Y-R, Seto M, Yoon JK. A WNT/β-catenin signaling activator, R-spondin, plays positive regulatory roles during skeletal myogenesis. J Biol Chem. 2011;286(12):10649-59. https://doi.org/10.1074/jbc.M110.169391.

[62] Rochat A, Fernandez A, Vandromme M, Molès JP, Bouschet T, Carnac G, Lamb NJC. Insulin and Wnt1 pathways cooperate to induce reserve cell activation in differentiation and myotube hypertrophy. MolBiolCell. 2004;15(10):4544-55. https://doi.org/10.1091/mbc.E03-11-0816.

[63] Biressi S, Miyabara EH, Gopinath SD, Carlig PMM, Rando TA. A Wnt-TGFβ2 axis induces a fibrogenic program in muscle stem cells from dystrophic mice. SciTransl Med. 2014;6(267):267ra176. https://doi.org/10.1126/scitranslmed.3008411.

[64] Trensz F, Haroun S, Cloutier A, Richter MV, Grenier G. A muscle resident cell population promotes fibrosis in hindlimb skeletal muscles of mdx mice through the Wnt canonical pathway. Am J Physiol Cell Physiol. 2010;299(5):C939-C947. https://doi.org/10.1152/ajpcell.00253.2010.

[65] Mueller CM, Hilbert JE, Martens W, Thornton CA, Moxley RT, Greene MH. Hypothesis: Neoplasms in myotonic dystrophy. Cancer Causes Control. 2009;20(10):2009-20. https://doi.org/10.1007/s10552-009-9395-y.

[66] Mahe´ D, Mähl P, Gattoni R, Fischer N, Mattei MG, Stevenin J, Fuchs JP. Cloning of Human 2H9 Heterogeneous Nuclear Ribonucleoproteins. Relation With Splicing and Early Heat Shock-Induced Splicing Arrest. J Biol Chem. 1997;272(3):1827-36. http://dx.doi.org/10.1074/jbc.272.3.1827.

[67] Wang Z, Pandey A, Hart GW. Dynamic Interplay betweenO-LinkedN-Acetylglucosaminylation and Glycogen Synthase Kinase-3-dependent Phosphorylation. MOL CELLPROTEOMICS. 2007;6(8):1365-79. http://dx.doi.org/10.1074/mcp.m600453-mcp200.

[68] Paul S, Dansithong W, Jog SP, Holt I, Mittal S, Brook JD, et al. Expanded CUG repeats Dysregulate RNA splicing by altering the stoichiometry of the muscleblind 1 complex. J Biol Chem. 2011;286:38427-38. http://dx.doi.org/10.1074/jbc.M111.255224.

[69] Naghdi S, Hajnóczky G. VDAC2-specific cellular functions and the underlying structure. Biochim Biophys Acta. 2016;1863(10):2503-514. http://dx.doi.org/10.1016/j.bbamcr.2016.04.020.

[70] Tanno M, Kuno A, Ishikawa S, Miki T, Kouzu H, Yano T, et al. Translocation of Glycogen Synthase Kinase-3β (GSK-3β), a Trigger of Permeability Transition, Is Kinase Activity-dependent and Mediated by Interaction with Voltage-dependent Anion Channel 2 (VDAC2). J BIOL CHEM. 2014;289(42):29285-96. http://dx.doi.org/10.1074/jbc.m114.563924.

[71] De Pinto V, Reina S, Gupta A, Messina A, Mahalakshmi R. Role of Cysteines in Mammalian VDAC Isoforms' Function. Biochim Biophys Acta. 2016;1857(8):1219-27. http://dx.doi.org/10.1016/j.bbabio.2016.02.020.

[72] Toscano A, Messina S, Campo GM, Di Leo R, Musumeci O, Rodolico C, et al. Oxidative stress in myotonic dystrophy type 1. Free Radic Res. 2005;39(7):771-76. http://dx.doi.org/10.1080/10715760500138932.

[73] Gramegna LL, Giannoccaro MP, Manners DN, Testa C, Zanigni S, Evangelisti S, et al. Mitochondrial dysfunction in myotonic dystrophy type 1. Neuromuscul Disord. 2018;28(2):144-9. http://dx.doi.org/10.1016/j.nmd.2017.10.007.

[74] Hughes MC, Ramos SV, Tumbull PC, Rebalka IA, Cao A, Monaco CMF, et al. Early Myopathy in Duchenne Muscular Dystrophy Is Associated With Elevated Mitochondrial H_2O_2 Emission During Impaired Oxidative Phosphorylation. J Cachexia Sarcopenia Muscle. 2019;10(3):643-61. http://dx.doi.org/10.1002/jcsm.12405.

[75] Goll DE, Thompson VF, Li H, Wei W, Cong J. The calpain system. Physiol Rev. 2003;83:731-801. https://doi.org/10.1152/physrev.00029.2002.

[76] Lin GD, Chattopadhyay D, Maki M, Wang KK, Carson M, Jin L, et al. Crystal structure of calcium bound domain VI of calpain at 1.9 A resolution and its role in enzyme assembly, regulation, and inhibitor binding. Nat Struct Biol. 1997;4:539-47. https://doi.org/10.1038/nsb0797-539.

[77] Mazères G, Leloup L, Daury L, Cottin P, Brustis JJ. Myoblast attachment and spreading are regulated by different patterns by ubiquitous calpains. Cell Motil Cytoskeleton. 2006;63:193-207. https://doi.org/10.1002/cm.20116.

[78] Jungwirth U, Gojo J, Tuder T, Walko G, Holcmann M, Schöfl T, et al. Calpain-mediated integrin deregulation as a novel mode of action for the anticancer gallium compound KP46. Mol Cancer Ther. 2014;13:2436-49. https://doi.org/10.1158/1535-7163.MCT-14-0087.

[79] Nozaki K, Das A, Ray SK, Banik NL. Calpeptin attenuated apoptosis and intracellular inflammatory changes in muscle cell. J Neurosci Res. 2011;89:536-43. https://doi.org/10.1002/jnr.22585.

[80] Liang Z, Brown RC, Fletcher JC, Opsahl-Sorteberg HG. Calpain-mediated positional information directs cell wall orientation to sustain plant stem cell activity, growth and development. Plant Cell Physiol. 2015;56:1855-66. https://doi.org/10.1093/pcp/pcv110.

[81] Qin QY, Liao GH, Michel B, Bi XN. Role of calpain-mediated p53 truncation in semaphorin 3A-induced axonal growth regulation. Proc Natl Acad Sci U S A. 2010;107:13883-87. https://doi.org/10.1073/pnas.1008652107.

[82] Lynch G. Memory and the brain: Unexpected chemistries and a new pharmacology. Neurobiol Learn Mem. 1998;70:82-100. https://doi.org/10.1006/nlme.1998.3840.

[83] Jin N, Yin X, Yu D, Cao M, Gong CX, Iqbal K, et al. Truncation and activation of GSK-3β by calpain I: A molecular mechanism links to tau hyperphosphorylation in Alzheimer's disease. Sci Rep. 2015;5:8187. https://doi.org/10.1038/srep08187.

[84] Aweida D, Rudesky I, Volodin A, Shimko E, Cohen S. GSK3-β promotes calpain-1–mediated desmin filament depolymerization and myofibril loss in atrophy. J Cell Biol. 2018;217(10):3698-714. https://doi.org/10.1083/jcb.201802018.

[85] Nelson WB, Smuder AJ, Hudson MB, Talbert EE, Powers SK. Cross-talk between the calpain and caspase-3 proteolytic systems in the diaphragm during prolonged mechanical ventilation. Crit Care Med. 2012;40:1857-63. https://doi.org/10.1097/CCM.0b013e318246bb5d.

[86] Matsumoto A, Fujita N, Arakawa T, Fujino H, Miki A. Influence of electrical stimulation on calpain and ubiquitin-proteasome systems in the denervated and unloaded rat tibialis anterior muscles. Acta Histochem. 2014;116:936-42. https://doi.org/10.1016/j.acthis.2014.03.006.

[87] Fareed MU, Evenson AR, Wei W, Menconi M, Poylin V, Petkova V, et al. Treatment of rats with calpain inhibitors prevents sepsis-induced muscle proteolysis independent of atrogin-1/MAFbx and MuRF1 expression. Am J Physiol Regul Integr Comp Physiol. 2006;290:R1589-R1597. https://doi.org/10.1152/ajpregu.00668.2005.

[88] Spencer MJ, Croall DE, Tidball JG. Calpains are activated in necrotic fibers from mdx dystrophic mice. J Biol Chem. 1995;270(18):10,909-10,914. https://doi.org/10.1074/jbc.270.18.10909.

[89] Hathout Y, Liang C, Ogundele M, Xu G, Tawalbeh SM, Dang UJ, et al. Disease-specific and glucocorticoid-responsive serum biomarkers for Duchenne Muscular Dystrophy. Sci Rep. 2019;9(1):12167. https://doi.org/10.1038/s41598-019-48548-9.

[90] Spencer MJ, Mellgren RL. Overexpression of a calpastatin transgene in mdx muscle reduces dystrophic pathology. Hum Mol Genet. 2002;11:2645-55. https://doi.org/10.1093/hmg/11.21.2645.

[91] Evans DR, Guy HI. Mammalian pyrimidine biosynthesis: Fresh insights into an ancient pathway. J Biol Chem. 2004;279:33035-8. http://dx.doi.org/10.1074/jbc.R400007200.

[92] Ostrander DB, O'Brien DJ, Gorman JA, Carman GM. Effect of CTP synthetase regulation by CTP on phospholipid synthesis in Saccharomyces cerevisiae. J Biol Chem. 1998;273:18992-9001. http://dx.doi.org/10.1074/jbc.273.30.18992.

[93] Martin E, Palmic N, Sanquer S, Lenoir C, Hauck F, Mongellaz C, et al. CTP synthase 1 deficiency in humans reveals its central role in lymphocyte proliferation. Nature. 2014;510(7504):288-92. http://dx.doi.org/10.1038/nature13386.

[94] Higgins MJ, Graves PR, Graves LM. Regulation of Human Cytidine Triphosphate Synthetase 1 by Glycogen Synthase Kinase 3. J Biol Chem. 2007;282(40):29493-503. http://dx.doi.org/10.1074/jbc.m703948200.

[95] Saini-Chohan HK, Mitchell RW, Vaz FM, Zelinski T, Hatch GM. Delineating the role of alterations in lipid metabolism to the pathogenesis of inherited skeletal and cardiac muscle disorders. J Lipid Res. 2011;53(1):4-27. http://dx.doi.org/10.1194/jlr.R012120.

[96] Llagostera E, Catalucci D, Marti L, Liesa M, Camps M, Ciaraldi TP et al. Role of Myotonic Dystrophy Protein Kinase (DMPK) in Glucose Homeostasis and Muscle Insulin Action. PLoS ONE. 2007;2(11):e1134. http://dx.doi.org/10.1371/journal.pone.0001134.

[97] Schoneveld OJLM, Gaemers IC, Lamers WH. Mechanisms of glucocorticoid signalling. Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression. 2004;1680(2):114-28. http://dx.doi.org/10.1016/j.bbaexp.2004.09.004.

[98] Rubio-Patiño C, Palmeri CM, Pérez-Perarnau A, Cosialls AM, Moncunill-Massaguer C, González-Gironès DM, et al. Glycogen Synthase Kinase-3β Is Involved in Ligand-Dependent Activation of Transcription and Cellular Localization of the Glucocorticoid Receptor. MOL ENDOCRINOL. 2012;26(9):1508-20. http://dx.doi.org/10.1210/me.2011-1366.

[99] Johansson Å, Andrew R, Forsberg H, Cederquist K, Walker BR, Olsson T. Glucocorticoid Metabolism and Adrenocortical Reactivity to ACTH in Myotonic Dystrophy. J Clin Endocrinol Metab. 2001;86:4276-83. http://dx.doi.org/10.1210/jcem.86.9.7865.

[100] Ruzzin J, Wagman AS, Jensen J. Glucocorticoid-induced insulin resistance in skeletal muscles: Defects in insulin signalling and the effects of a selective glycogen synthase kinase-3 inhibitor. Diabetologia. 2005;48:2119-30. http://dx.doi.org/10.1007/s00125-005-1886-0.

[101] Nieuwenhuis S, Okkersen K, Widomska J, Blom P, 't Hoen PAC, van Engelen B, Glennon JC. Insulin Signaling as a Key Moderator in Myotonic Dystrophy Type 1. Front Neurol. 2019;10:1229. https://doi.org/10.3389/fneur.2019.01229.

[102] Glozak MA, Seto E. Histone deacetylases and cancer. Oncogene. 2007;26(37):5420-32. http://dx.doi.org/10.1038/sj.onc.1210610.

[103] Wang T, Zhang J, Xiao A, Liu W, Shang Y, An J. Melittin ameliorates CVB3-induced myocarditis via activation of the HDAC2-mediated GSK-3β/Nrf2/ARE signaling pathway. BIOCHEM BIOPH RESCO. 2016;480(1):126-31. http://dx.doi.org/10.1016/j.bbrc.2016.09.135.

[104] Ngkelo A, Hoffmann RF, Durham AL, Marwick JA, Brandenburg SM, de Bruin HG, et al. Glycogen synthase kinase-3β modulation of glucocorticoid responsiveness in COPD. AM J PHYSIOL-LUNG C. 2015;309(10):L1112-L1123. https://doi.org/10.1152/ajplung.00077.2015.

[105] Zhang F, Bodycombe NE, Haskell KM, Sun YL, Wang ET, Morris CA, et al. A Flow Cytometry-Based Screen Identifies MBNL1 Modulators That Rescue Splicing Defects in Myotonic Dystrophy Type I. Hum Mol Genet. 2017;26(16):3056-68. http://dx.doi.org/10.1093/hmg/ddx190.

[106] Roskoski R. MEK1/2 dual-specificity protein kinases: Structure and regulation. Biochem Biophys Res Commun. 2012;417(1):5-10. http://dx.doi.org/10.1016/j.bbrc.2011.11.14.

[107] Perry RL, Parker MH, Rudnicki MA. Activated MEK1 binds the nuclear MyoD transcriptional complex to repress transactivation. Mol Cell. 2001;8:291-301. http://dx.doi.org/10.1016/s1097-2765(01)00302-1.

[108] Beffy P, Del Carratore R, Masini M, Furling D, Puymirat J, Masiello P, Simili M. Altered signal transduction pathways and induction of autophagy in human myotonic dystrophy type 1 myoblasts. Int J Biochem Cell Biol. 2010;42(12):1973-83. http://dx.doi.org/10.1016/j.biocel.2010.08.010.

[109] Weg-Remers S, Ponta H, Herrlich P, König H. Regulation of alternative premRNA splicing by the ERK MAP-kinase pathway. EMBO J. 2001;20:4194-203. http://dx.doi.org/10.1093/emboj/20.15.4194.

[110] Mankodi A, Takahashi MP, Jiang H, Beck CL, Bowers WJ, Moxley RT. Expanded CUG repeats trigger aberrant splicing of ClC-1 chloride channel pre-mRNA and hyperexcitability of skeletal muscle in myotonic dystrophy. Mol Cell. 2002;10:35-44. http://dx.doi.org/10.1016/s1097-2765(02)00563-4.

[111] Charlet-B N, Savkur RS, Singh G, Philips AV, Grice EA, Cooper TA. Loss of the muscle-specific chloride channel in type 1 myotonic dystrophy due to misregulated alternative splicing. Mol Cell. 2002;10:45-53. http://dx.doi.org/10.1016/s1097-2765(02)00572-5.

[112] Oana K, Oma Y, Suo S, Takahashi MP, Nishino I, Takeda S, Ishiura S. Manumycin A corrects aberrant splicing of Clcn1 in myotonic dystrophy type 1 (DM1) mice. Sci Rep. 2013;3:2142. http://dx.doi.org/10.1038/srep02142.

[113] Horrigan J, Gomes TB, Snape M, Nikolenko N, McMorn A, Evans S, et al. Phase 2 Study of AMO-02 (Tideglusib) in Congenital and Childhood-Onset Myotonic Dystrophy Type 1 (DM1). Pediatr Neurol. 2020;112:84-93. http://dx.doi.org/10.1016/j.pediatrneurol.2020.08.001. Epub 2020 Aug 5. PMID: 3294208.