



Research Paper

A Molecular Signature of Myalgia in Myotonic Dystrophy 2

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ABSTRACT

Background: Chronic muscle pain affects close to 20% of the population and is a major health burden. The underlying mechanisms of muscle pain are difficult to investigate as pain presents in patients with very diverse histories. Treatment options are therefore limited and not tailored to underlying mechanisms. To gain insight into the pathophysiology of myalgia we investigated a homogeneous group of patients suffering from myotonic dystrophy type 2 (DM2), a monogenic disorder presenting with myalgia in at least 50% of affected patients.

Methods: After IRB approval we performed an observational cross-sectional cohort study and recruited 42 patients with genetically confirmed DM2 plus 20 healthy age and gender matched control subjects. All participants were subjected to an extensive sensory-testing protocol. In addition, RNA sequencing was performed from 12 muscle biopsy specimens obtained from DM2 patients.

Findings: Clinical sensory testing as well as RNA sequencing clearly separated DM2 myalgic from non-myalgic patients and also from healthy controls. In particular pressure pain thresholds were significantly lowered for all muscles tested in myalgic DM2 patients but were not significantly different between non-myalgic patients and healthy controls. The expression of fourteen muscle expressed genes in myalgic patients was significantly up or down-regulated in myalgic compared to non-myalgic DM2 patients.

Interpretation: Our data support the idea that molecular changes in the muscles of DM2 patients are associated with muscle pain. Further studies should address whether muscle-specific molecular pathways play a significant role in myalgia in order to facilitate the development of mechanism-based therapeutic strategies to treat musculoskeletal pain.

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1. Introduction

Chronic pain affects 11–24% of the world's population and as such represents a major burden on health services (Breivik et al., 2006). A substantial proportion of pain patients suffer from musculoskeletal pain (McBeth and Jones, 2007). Treatments for muscle pain and myalgia are often ineffective and have not been tailored to treat pain which is associated with a diverse range of pathologies. In order to gain insight into the molecular pathology of pain in a genetically uniform group we have studied patients with myotonic dystrophy type 2 (DM2) (Udd and

Krahe, 2012). DM2 is an autosomal dominant multisystem disorder caused by a CCTG repeat expansion of the cellular nucleic acid-binding protein, CNBP (Liquori et al., 2001). Mutant transcripts lead to a toxic RNA gain of function and miss-splicing of several effector genes (Charlet-B et al., 2002; Tang et al., 2012). Many, but not all DM2 patients complain of chronic muscle pain (George et al., 2004; Suokas et al., 2012). The clinical features are highly variable and include late-onset progressive muscle weakness, myotonia, cardiac conduction defects, early-onset cataracts and insulin resistance (Rhodes et al., 2012; Savkur et al., 2004; Udd and Krahe, 2012; Wahbi et al., 2009). How the CNBP mutation confers risk for muscle pain in some patients but not others is unknown and is the key question addressed in this study.

Quantitative sensory testing (QST) is a standardized technique to assess human somatosensory function and document altered nociceptive signal processing (Backonja et al., 2013). By determining pain and detection threshold to external mechanical and thermal stimuli, sensory

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profiles are generated that can potentially trace underlying pathophysiological mechanisms. QST profiles have been made of patients with muscle-related disorders such as fibromyalgia, chronic back pain and myogenic temporomandibular disease revealing similarities as well as differences that may mirror distinct neurobiological mechanisms (Blumenstiel et al., 2011; Pfau et al., 2009). We used a comprehensive QST assessment to characterize the sensory phenotype of our cohort, a pre-requisite for identifying molecular signatures of muscle pain. Our analysis of the clinical and molecular profile of muscle pain in DM2 has enabled us to identify molecular signals in the affected muscle that segregate with muscle pain.

2. Methods

2.1. Study design and participants

In this cross-sectional study we investigated a cohort of 42 DM2 patients and 20 age and gender-matched healthy controls between March 2013 and January 2015. All DM2 patients were recruited from Muscle Disorders Outpatient Clinic at Charité Campus Buch, Berlin, Germany. The local ethics committee (EA1-127-14) approved the study. All patients and healthy subjects signed the written informed consent forms. Inclusion criteria were age > 18 years and molecularly confirmed diagnosis of DM2. Exclusion criteria were additional neurological disorders that could affect sensory function (e.g. stroke) or treatment with opioid analgesics (Supplementary Fig. 1). Healthy volunteers were excluded if they had diabetes, hypertension, neurological disorders affecting sensory function, took analgesics or had muscle pain in the last 3 months. We also obtained written informed consent from 12 DM2 patients, who underwent muscle biopsies for diagnostic purposes between 2004 and 2014, to subject their stored muscle biopsy specimens to RNA Seq analysis.

2.2. Clinical assessment of DM2 patients

Patients were asked about current unpleasant or painful sensations in their muscles lasting for more than 3 months. They were asked to rate the (1) unpleasant muscle sensation/pain, (2) muscle weakness and (3) muscle stiffness on a visual analogue scale (score of 0 for "no symptom" score of 10 for "worst imaginable intensity of symptom"). Patients with DM2 were allocated to either the myalgia or no myalgia group based on positive history of muscle pain and pain rating on the testing day. Patients indicated on a drawing where the pain was located. Frequency, duration, and modulating factors of pain such as temperature and movement were also recorded. Patients completed the German version of the McGill Pain Questionnaire (MPQ). Pain rating index (MPQ-PRI), number of words chosen (MPQ-NWC) and present pain index (MPQ-PPI) were calculated. Past medical history was obtained including presence of comorbidities, recent laboratory values, current pain medication, smoking status, education and work status. Genetic diagnosis of DM2 was performed at the Institute for Medical Genetics, University of Würzburg, Germany.

2.3. Sensory testing protocols

We used a comprehensive, multimodal, QST protocol to generate somatosensory profiles for each DM2 patient and healthy controls (Rolke et al., 2006). We assessed pain thresholds for skin and muscle tissue. Skin thermal and mechanical testing was performed in a unilateral fashion (dominant hand side) over the hand dorsum, shoulder and thigh. Thresholds for pressure pain were obtained over eight muscles on the left and right side of the body: extensor digitorum communis, deltoid, quadriceps and anterior tibialis. The repertoire of pain tests included pressure pain threshold (PPT), mechanical pain threshold (MPT), mechanical pain sensitivity (MPS), dynamic mechanical allodynia and wind-up ratio (WUR).

Thermal detection tasks included warm detection threshold (WDT), cold detection threshold (CDT), heat pain threshold (HPT) (Frenzel et al., 2012) and the paradoxical heat detection (PHS). Pain testing procedures, instruments and methods were used strictly according to those prescribed by the German research network on neuropathic pain (Rolke et al., 2006).

2.4. Transcriptomic analysis of muscle tissue

Human muscle biopsy specimens were obtained from *M. quadriceps femoris* under sterile conditions and frozen in liquid nitrogen. Total RNA from 12 DM2 biopsy specimens (6 patients with and 6 patients without muscle pain) was isolated with RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. RNA quantity and purity were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA) and an Agilent 2100 bioanalyser (Agilent Technologies, USA). The cDNA libraries were prepared using TruSeq Stranded mRNA library preparation kit (Illumina, USA). RNA paired-end sequencing was performed using the Illumina HiSeq platform (Illumina, USA). Differentially expressed genes were validated by qPCR using Sybr green assay at Stratagene Mx3000P cycler (Agilent Technologies, USA) according to manufacturer's instructions. Sequences of the oligonucleotides are provided in Supplementary Table 1.

2.5. Statistical analysis

All statistical calculations were performed using R software. The QST parameters – CDT, WDT, PPT, WUR, MPS, MDT, and MPT – are usually normally distributed in log-space and thus were log-transformed. The QST-profiles of DM2 patients were compared to controls using repeated measure two factorial (for group and tested site) ANOVA. All QST measures from each patient were then standardized by z-transformation with respect to the age- and sex-matched healthy subject group.

$$\text{Z-score} = (\text{Mean}_{\text{individual DM2}} - \text{Mean}_{\text{healthy subjects}}) / \text{SD}_{\text{healthy subjects}}$$

where mean_{individual DM2} is the value of the QST parameter in a DM2 patient, and mean_{healthy subjects} and SD_{healthy subjects} are mean and standard deviation of the corresponding QST parameter in the healthy control group. Z-scores signs were adjusted so that a z-score > 0 indicated means gain of sensory function (lower threshold), and z-score < 0 means loss of function (increase in threshold). The advantage of graphical representation of QST profiles as z-transformed data was to directly compare between sensory modalities of different units and ranges between groups and tested sites.

Gene expression data was analysed with CLC Genomic Workbench v7.0 (Qiagen, Germany) and Qlucore Omics Explorer v3.1 (Qlucore, Denmark). Samples obtained from patients without muscle pain were considered as a reference group. Preprocessed raw sequences were imported and trimmed in CLC Genomics Workbench and all trimmed reads were aligned to the human reference genome (GRCh37) and mapped back to the human transcriptome (v.19). Mapped read counts were normalized using Trimmed mean of M-values (TMM) method implemented in Edge-R package. Normalized read counts were used for analysis of differential gene expression at Qlucore Omics Explorer. P values were calculated by two group comparison T-test. Genes with P-value < 0.05 and fold change > ± 1.8 were considered to be differentially expressed and were presented as a heatmap with hierarchical clustering of the samples. Differentially expressed genes from RNAseq data were additionally confirmed with qPCR using ΔΔCt method with the average of Ct values for GAPDH and cyclophilin A used as a reference. Ct values were calculated by MxPro qPCR software v4.1 (Agilent Technologies, USA). Gene

functions were analysed using Quick GO gene ontology database (<http://www.ebi.ac.uk/QuickGO/>).

3. Results

3.1. Patient characteristics

DM2 patient characteristics, EMG findings, information on myotonia and MRC quantification of muscle weakness are listed in Table 1 and Supplementary Table 2. None of the patients received mexiletine. All 42 patients were ambulatory and attended the clinical and QST assessment session. Data from 7 patients were excluded from the analysis: six patients took opioid analgesics at the testing day and one patient had sensory deficits due to stroke. Twenty three patients reported muscle pain (DM2-myalgia group) and 12 did not (DM2-no-myalgia group). On a scale of 0–10, myalgias were subjectively rated as most burdening in everyday activity (5.7 ± 2.3) compared to muscle weakness (5.1 ± 2.8) and myotonia (3.5 ± 2.8). Seventeen of 23 DM2 patients reporting myalgia had experienced myalgia for more than 5 years. Muscle pain was typically localized symmetrically but the affected body region differed between patients. The character of pain (assessed by the MPQ) was: tugging (14/23), cramping (13/23), tiring (12/23), punishing (10/23), annoying (9/23), radiating (8/23), burning (8/23) and dull (6/23). Myalgias were reported as being worse during cold weather in 10 patients. Of the 10 diabetic patients, one had a diagnosis of diabetic polyneuropathy and trigeminal neuralgia but did not complain of myalgia. Nine (39%) DM2 patients with myalgia had other types of concurrent pain (low back pain, arthralgia, and headaches). A higher proportion of patients with myalgia (18/23, 78%) had clinical evidence of muscle weakness compared to DM2 patients without myalgia (3/12, 25%) ($P < 0.05$, χ^2 -test). Otherwise there was no difference in the frequency of comorbidities (diabetes mellitus, cataracts, and dyslipidemias) between the groups. Twenty healthy subjects (11 females and 9 males) were recruited with a mean \pm SD age of 55 ± 8.3 years ($P > 0.05$ t-test, compared to DM2). The

proportion of males and females between DM2 (54% females) and healthy controls (55% females) was comparable.

3.2. Sensory changes in patients with DM2

Amongst all the QST measures (Fig. 1, Supplementary Tables 3 and 4) the most prominent abnormality was seen in pressure pain threshold (PPT). Repeated-measures ANOVA indicated a statistically significant decrease in pressure pain threshold in the DM2-myalgia group compared with the control groups. There is a general and strong shift for mean pressure pain thresholds towards hyperalgesia in patients with DM2, with mean z-score values for DM2 patients with myalgia lying beyond the healthy subject range of 2 z-score units (Fig. 1A and Supplementary Table 3). Between-group comparisons (Fig. 1) reveal significant differences, in the gain-of-function direction, between the healthy subject groups and the DM2-no-myalgia and DM2-myalgia groups for all muscle groups ($P < 0.0001$, ANOVA). The number (percentage) of DM2-myalgia patients with z-scores for PPT lying beyond +1.96 (170 KPa for EDC, 200 KPa for deltoid, 265 KPa for anterior tibialis, and 265 KPa for the quadriceps) are 9(39%), 15(65%), 13(57%), and 16(70%) for EDC, deltoid, anterior tibialis, and quadriceps muscles respectively. This contrasts with 1(8%), 1(8%), 2(16%), and 2(16%) for EDC, deltoid, anterior tibialis, and quadriceps muscles respectively in DM2-no-myalgia patients.

The median z-scores for all thermal and mechanical pain parameters in the DM2 group generally fall within the healthy subject ranges except for a few individuals (Fig. 1B & C). Within mechanical pain parameters, all DM2 participants showed a slight loss of MPT function, i.e., increased pain threshold to pinprick stimulation, compared to healthy subjects ($P = 0.03$, ANOVA). When comparing DM2 patients, we found no significant differences in MPT, however, we observed enhanced subjective rating to pinprick stimuli (MPS) and increased windup ratio in DM2-myalgia compared to DM2-no-myalgia group (Supplementary Fig. 2, Supplementary Table 4). Thus DM2 patients with myalgia exhibited increased MPS and windup ratios (+ve z-score, gain of function) typical for patients with generalized central sensitization ($P = 0.03$, ANOVA). Allodynia occurred in one patient with myalgia in the shoulder region. A total of 3 subjects with DM2 (two with myalgia, one without myalgia and one healthy subject) reported paradoxical heat sensations.

Interestingly, mechanical detection threshold was significantly increased in DM2 patients indicating that touch sensation was impaired in all DM2 patients [median/inter quartile range in mN in healthy subject 1.1/0.7 (hand), 2.0/2.0 (shoulder), and 4.5/4.1 (thigh); in DM2 patients 3.2/3.1 (hand), 4.0/8.9 (shoulder), and 8.0/7.8 (thigh)]. The mean z-score values for MDT reveal a loss of function in the DM2 group compared to healthy subjects (Supplementary Table 2). This effect held true after excluding the 10 diabetic patients (data not shown). We observed loss of thermal warming discrimination and therefore a loss of sensory function in four DM2 patients (two with and two without myalgia). Paradoxical heat sensation was observed in two DM2-myalgia patients, one DM2-no-myalgia patient and one healthy control subject.

3.3. Myalgic and non-myalgic DM2 muscle has distinct transcriptome profiles

We analysed transcriptome profiles of muscle biopsy specimens obtained from myalgic and non-myalgic, age-matched DM2 patients ($n = 6$ for each group, Supplementary Table 5). Grouping of muscle biopsies into myalgic and nonmyalgic groups was based on documented chronic myalgia in the medical records. Myopathological findings were typical for DM2 (Meola and Cardani, 2015) and similar in both groups (Supplementary Table 5). Bioinformatic analysis of muscle RNAseq data revealed 14, muscle-specific, differentially expressed (DE) genes based on presence or absence of myalgia (Fig. 2 and Supplementary Table 6). Due to limited muscle tissue material, only

Table 1
Summary of characteristics of DM2 patients.

	Myalgia	No myalgia	Total
N	23	12	35
Age, y	57.3 ± 10.3	48.6 ± 15.2	54.3 ± 12.7
Male/female	9/14	7/5	16/19
BMI, kg/m ²	26.2 ± 4.5	24.4 ± 4.8	25.6 ± 4.6
Muscle features			
Muscle weakness	15 (68%)	5 (42%)	20 (63%)
Myotonia	19 (85%)	7 (58%)	26 (63%)
Systemic features			
Insulin resistance	4 (17%)	1 (8%)	5 (14%)
Hyperlipidemia	11 (48%)	5 (42%)	16 (46%)
Cataract	8 (35%)	3 (25%)	11 (46%)
Hypertension	11 (48%)	5 (42%)	16 (46%)
Cardiac conduction abnormalities	7 (30%)	1 (6%)	8 (23%)
CK	286 ± 196	506 ± 414	
Pain features			
Pain intensity, NAS	4.2 \pm 2.2	NA	
Pain duration, y	7.4 \pm 3.3	NA	
Pain location			
Thigh	16		
Shoulder	14		
Back	8		
Lower arm	9		
Lower leg	8		
Myalgia related to movement	10 (43%)	NA	
Myalgia related to cold	14 (61%)	NA	
MPQ			
PRI	8.3	NA	
MWC	16.1	NA	
PPI	3.3	NA	

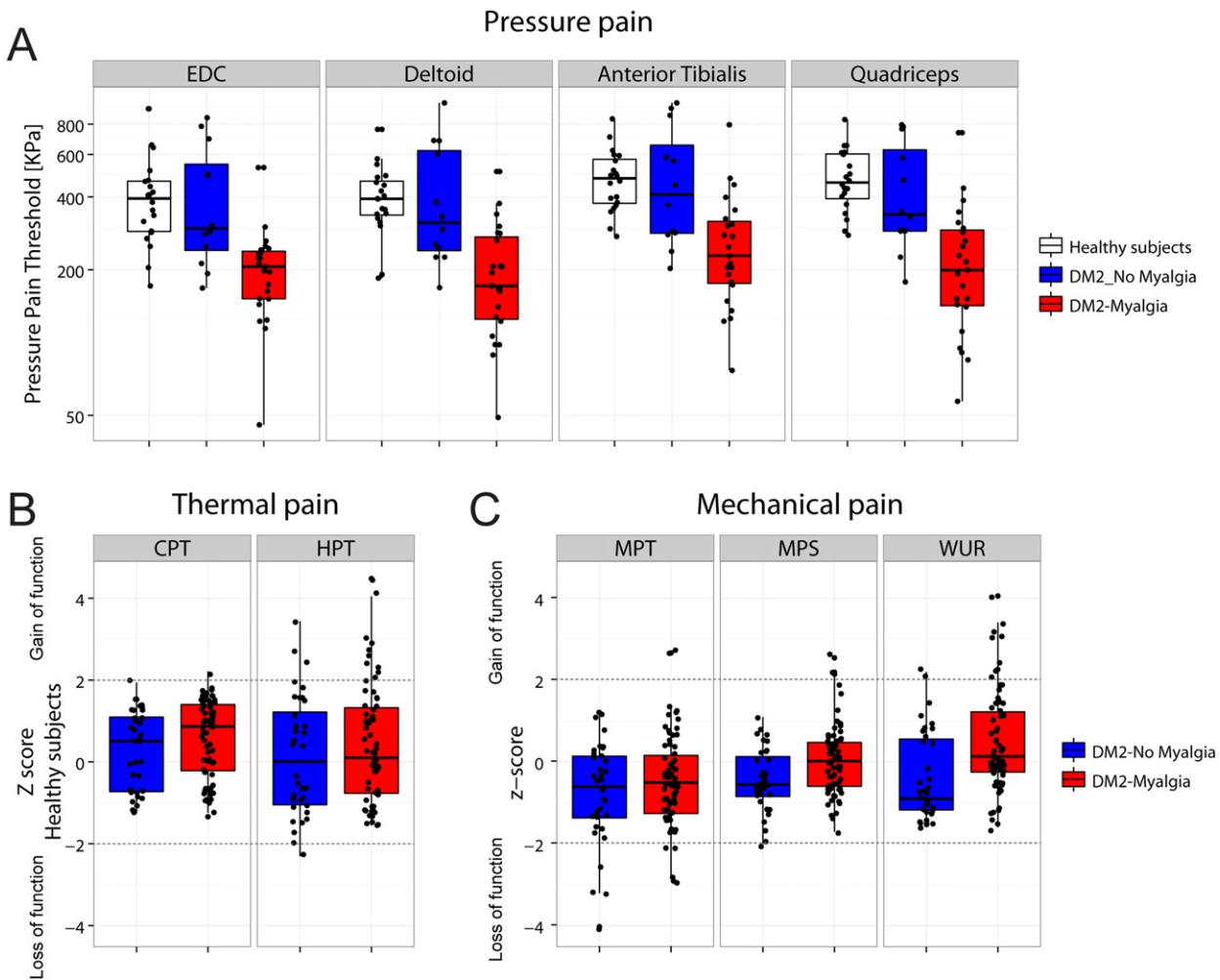


Fig. 1. Plots of QST parameters for pressure, thermal and mechanical pain. (A) Scatter and box and whisker plots of pressure pain threshold across the 4 muscle groups in healthy subjects, DM2-myalgia and DM2-no-myalgia groups. (B) Thermal and (C) mechanical pain QST parameters represented as scatter and box and whisker plots of z-scores over the 3 tested areas (hand, shoulder, and thigh). Mean of the healthy subject z-score is represented by "0" and dotted lines designating ± 1.96 SD. EDC, M. extensor digitorum communis; CPT, cold pain threshold; HPT, heat pain threshold; PPT, pressure pain threshold; MPT, mechanical pain threshold; MPS, mechanical pain sensitivity; WUR, wind-up ratio; MPT, mechanical pain threshold.

five of these genes were confirmed by qPCR; however, all five confirmed the RNASeq analysis (Supplementary Table 6). The highest differential expression found was a decrease in the levels of monoamine oxidase A (*MAOA*) in myalgic DM2 patients. *MAOA* encodes an enzyme that degrades amine neurotransmitters, such as dopamine, norepinephrine, and serotonin. Another gene with decreased expression in myalgic DM2 was activity-regulated cytoskeleton-associated protein (ARC). Significantly increased expression in myalgic DM2 muscle were found for *CYB5D1*, *GSTCD*, *GRB14*, *PANK1*, *ZNF711*, *FAM26E*, *PFKFB2*, *ZNF841*, *HECW2*, *SLC16A12*, *FRMPD1*, *NR4A3* and *SLC16A12*. Analysis of gene functions showed that these genes are involved in transcriptional regulation (*NR4A3*, *ZNF711* and *ZNF841*), metabolic regulation (*PANK1*, *PFKFB2*), ubiquitin ligation (*HECW2*), signal transduction (*GRB14*), iron binding (*CYB5D1*), creatine transmembrane transport (*SLC16A12*) or predicted cation channel activity (*FAM26E*).

4. Discussion

We provide compelling evidence that myalgia in DM2 patients could be initiated and maintained by molecular changes within the muscle. This interpretation is supported by our discovery that distinct transcriptome profiles segregate myalgic from non-myalgic patients. In principle miss-splicing of CNBP targets in the nervous system or other tissues could contribute to myalgia. However, if central

nervous system mechanisms were primary a molecular signature for the presence of pain would not be expected in the muscle. Decreased pressure pain thresholds (PPT) in myalgic DM2 patients indicate that the myalgia could be initiated by peripheral mechanisms within the muscle (Graven-Nielsen and Arendt-Nielsen, 2010). Our data is supported by an earlier study that found lowered PPT in DM2 patients distinct from pain associated with other muscle disease (George et al., 2004). Peripheral sensitization mechanisms are prominent in muscle and are known to be a trigger for central sensitization (Graven-Nielsen and Mense, 2001; Lewin et al., 2014; Mense, 2003). Central sensitization was likely present in myalgic DM2 patients as evidenced by a slightly elevated wind-up compared to non-myalgic DM2 patients but not in healthy subjects. We further observed that sensation to non-painful stimuli in DM2 patients was suppressed, independent of the presence of muscle pain. There are various conditions associated with chronic muscle pain, fibromyalgia, low back and neck pain, and these disease entities show different sensory profiles as measured with psychometric methods. However, presentation of myalgia in DM2 patients may be easily misdiagnosed as fibromyalgia (Auvinen et al., 2008). In fibromyalgia there is a generalized mechanical and thermal hypersensitivity and touch modalities are usually unaffected (Blumenstiel et al., 2011). Wind-up ratio elevation has been observed in fibromyalgia syndrome (Staud et al., 2001). Thus there are differences but also similarities

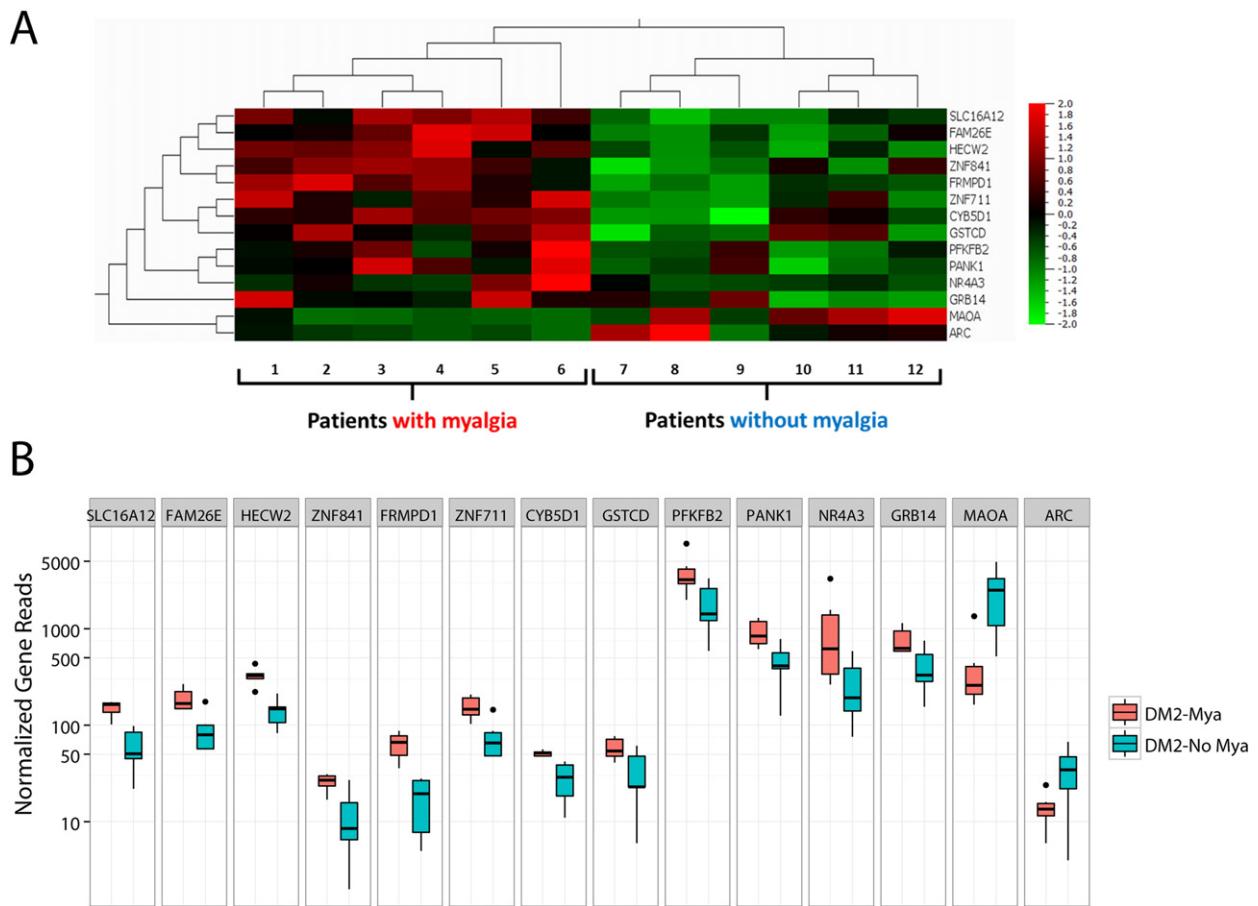


Fig. 2. Transcriptome profile differences between muscle tissues derived from DM2 patients with myalgia and without myalgia. (A) Heat map showing expression patterns of differentially expressed genes (DEG) based on the presence or absence of myalgias. Each row in the heat map indicates gene expression values for one of 14 differentially expressed genes while every column shows gene expression profile for one of the 12 tested DM2 patients. Areas in green correlate with low and areas in red correlate with high gene expression (see scale). (B) Normalized reads per gene for the DEGs.

between the sensory profiles of myalgia due to DM2 disease and fibromyalgia. In contrast, QST profiles in chronic low back pain patients reveal localized alterations such as lowered PPT (Blumenstiel et al., 2011; Gerhardt et al., 2015). We noted however that few patients with DM2 with myalgia have generalized lowered mechanical and thermal (cold and heat) thresholds. Whether such spatial and modality spread in sensory profiles represents a separate pain processing mechanisms or related to disease progression remains to be clarified.

The DM2 patients studied here showed no differences in age, current medication or histopathology of muscle tissue depending on whether they were myalgic or non-myalgic. Thus we have no evidence that the differences observed in the transcriptome of myalgic and non-myalgic DM2 muscle was based on anything else besides the presence of pain. It is known that certain types of ion channels are miss-spliced in myotonic dystrophies (Charlet-B et al., 2002; Tang et al., 2012). Abnormal splicing and downregulation of ubiquitin ligase, NEDD4, in DM2 patients prescribed HMG Co-A reductase inhibitors (statins) has been associated with adverse reactions (Screen et al., 2014). Also, hyperlipidemia and insulin resistance are common in DM2 patients, suggesting that elevated endogenous lipid levels could conceivably contribute to muscle pain in DM2 (Piomelli and Sasso, 2014; Heatwole et al., 2011).

DM2 transcriptomic profiles identified 14 differentially expressed genes, which possibly contribute to the onset of myalgias in DM2 patients. One of the identified differentially expressed genes encodes monoamine oxidase A (MAOA). Polymorphisms in the MAOA gene have been proposed to segregate with low and high pain responders, however, the genetic evidence is weak (Di Lorenzo et al., 2014; Kim et al., 2006). MAOA has been proposed as a therapeutic

target in fibromyalgia and treatment with a specific MAOA inhibitor, moclobemide, was administered to female patients with fibromyalgia in an earlier study (Hannonen et al., 1998). Moclobemide significantly decreased pain in fibromyalgia patients at the end of a 12 weeks intervention period by 1.2 points on the VAS (5.7 to 4.5) compared to placebo group. Variations in MAOA have been associated with different perceptions of pain and pain-related evoked potentials (Di Lorenzo et al., 2014; Treister et al., 2011). Two recent studies also quantified MAOA gene expression in human muscle biopsies and have associated high expression with insulin resistance (Elgzyri et al., 2012; Mason et al., 2011). In the context of insulin resistance, common in DM2, one of the up-regulated genes in myalgic patients was growth factor receptor-bound protein 14 (GRB14) which is a known negative regulator of insulin receptor signalling (Depetris et al., 2005). Another interesting finding was elevated expression of creatine transporter SLC16A12 in myalgic DM2 which might be related to increased muscle wasting and inflammatory pain (Abplanalp et al., 2013). Interestingly, there is already a small molecule inhibitor identified for the 6-phosphofructo-2-kinase (PFKFB2) enzyme (Telang et al., 2012) the expression of which was up-regulated in the muscle of DM2 patient with myalgia. Thus, our approach may open new avenues to explore the link between novel genes associated with pain and therapeutic interventions at least in animal models.

Our study has several limitations. The time points of molecular and sensory phenotyping were different. The reasons are mainly the invasiveness of the muscle biopsy procedure which requires strict indications. In the light of disease progression with time, and the small sample size, there might have been some unaccounted confounders

affecting gene expression other than myalgia despite similar muscle histopathology. Future research should consider concomitant phenotyping to make direct correlations between molecular changes in the muscle and changes in sensory phenotype in patients with myalgia. As to QST profiling, the group size was small with 12–23 subjects. QST profiles vary with age and sex and our DM2 cohort was matched with a healthy control group. Furthermore, the QST protocol relies on subjective rating of the participant and might reflect behaviour instead of pain thresholds. Finally, we applied the same testing protocol to all patients although they presented with different spatial patterns of pain; however, our results are controlled for by test site using ANOVA analysis.

High variability in pain character and pathophysiology in patients with chronic pain has made it difficult to identify tailored pain medication that show selective efficacy in sub-populations of patients. However, emerging genomic strategies might facilitate the development of new and more effective pain therapeutics (Dib-Hajj and Waxman, 2014). The molecular signature of myalgia shown in this study may also help to identify pathways that are associated with pain in other conditions affecting skeletal muscle. Identifying the molecular source of pain pathophysiology should greatly facilitate focussing effective treatments, although major hurdles remain.

Contributions

G.R.L. and S.S. designed the study. R.M., V.P. performed experiments. U.G. recruited patients. R.M., V.P. and S.G. analysed data. R.M., V.P., G.R.L. and S.S. wrote the manuscript.

Declaration of interests

There are no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ebiom.2016.03.017>.

References

- Applanalp, J., Laczko, E., Philp, N.J., Neidhardt, J., Zuercher, J., Braun, P., Schorderet, D.F., Munier, F.L., Verrey, F., Berger, W., Camargo, S.M.R., Kloekener-Gruissem, B., 2013. The catenact and glucosuria associated monocarboxylate transporter MCT12 is a new creatine transporter. *Hum. Mol. Genet.* 22, 3218–3226. <http://dx.doi.org/10.1093/hmg/ddt175>.
- Auvinen, S., Suominen, T., Hannonen, P., Bachinski, L.L., Krahe, R., Udd, B., 2008. Myotonic dystrophy type 2 found in two of sixty-three persons diagnosed as having fibromyalgia. *Arthritis Rheum.* 58, 3627–3631. <http://dx.doi.org/10.1002/art.24037>.
- Backonja, M.M., Attal, N., Baron, R., Bouhassira, D., Drangholt, M., Dyck, P.J., Edwards, R.R., Freeman, R., Gracely, R., Haanpaa, M.H., Hansson, P., Hatem, S.M., Krumova, E.K., Jensen, T.S., Maier, C., Mick, G., Rice, A.S., Rolke, R., Treede, R.-D., Serra, J., Toelle, T., Tugnoli, V., Walk, D., Walace, M.S., Ware, M., Yarnitsky, D., Ziegler, D., 2013. Value of quantitative sensory testing in neurological and pain disorders: NeuPSIG consensus. *Pain* 154, 1807–1819. <http://dx.doi.org/10.1016/j.pain.2013.05.047>.
- Blumenstiel, K., Gerhardt, A., Rolke, R., Bieber, C., Tesarz, J., Friederich, H.-C., Eich, W., Treede, R.-D., 2011. Quantitative sensory testing profiles in chronic back pain are distinct from those in fibromyalgia. *Clin. J. Pain* 27, 682–690. <http://dx.doi.org/10.1097/AJP.0b013e3182177654>.
- Breivik, H., Collett, B., Ventafridda, V., Cohen, R., Gallacher, D., 2006. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur. J. Pain* 10, 287–333 (doi:10.1016/j.ejpain.2005.06.009).
- Charlet-B., N., Savkur, R.S., Singh, G., Philips, A.V., Grice, E.A., Cooper, T.A., Charlet-B., N., Savkur, R.S., Singh, G., Philips, A.V., Grice, E.A., Cooper, T.A., 2002. Loss of the muscle-specific chloride channel in type 1 myotonic dystrophy due to misregulated alternative splicing. *Mol. Cell* 10, 45–53. [http://dx.doi.org/10.1016/S1097-2765\(02\)00572-5](http://dx.doi.org/10.1016/S1097-2765(02)00572-5).
- Depetris, R.S., Hu, J., Gimpelevich, I., Holt, L.J., Daly, R.J., Hubbard, S.R., 2005. Structural basis for inhibition of the insulin receptor by the adaptor protein Grb14. *Mol. Cell* 20, 325–333. <http://dx.doi.org/10.1016/j.molcel.2005.09.001>.
- Di Lorenzo, C., Daverio, A., Pasqualetti, P., Coppola, G., Giannoudas, I., Barone, Y., Grieco, G.S., Niolu, C., Pascale, E., Santorelli, F.M., Nicoletti, F., Pierelli, F., Siracusano, A., Seri, S., Di Lorenzo, G., 2014. The upstream variable number tandem repeat polymorphism of the monoamine oxidase type A gene influences trigeminal pain-related evoked responses. *Eur. J. Neurosci.* 39, 501–507. <http://dx.doi.org/10.1111/ejn.12458>.
- Dib-Hajj, S.D., Waxman, S.G., 2014. Translational pain research: lessons from genetics and genomics. *Sci. Transl. Med.* 6, 249sr4. <http://dx.doi.org/10.1126/scitranslmed.3007017>.
- Elgzyri, T., Parikh, H., Zhou, Y., Dekker Nitert, M., Rönn, T., Segerström, Å.B., Ling, C., Franks, P.W., Wollmer, P., Eriksson, K.F., Groop, L., Hansson, O., 2012. First-degree relatives of type 2 diabetic patients have reduced expression of genes involved in fatty acid metabolism in skeletal muscle – jc.2011-3037 [WWW document]. *J. Clin. Endocrinol. Metab.* <http://dx.doi.org/10.1210/jc.2011-3037>.
- Frenzel, H., Bohlander, J., Pinsker, K., Wohlleben, B., Tank, J., Lechner, S.G., Schiska, D., Jaijo, T., Rüschendorf, F.F., Saar, K.K., Jordan, J., Millán, J.M., Gross, M., Lewin, G.R., Millan, J.M., Gross, M., Lewin, G.R., Millán, J.M., Gross, M., Lewin, G.R., 2012. A genetic basis for mechanosensory traits in humans. *PLoS Biol.* 10, e1001318. <http://dx.doi.org/10.1371/journal.pbio.1001318>.
- George, A., Schneider-Gold, C., Zier, S., Reiners, K., Sommer, C., 2004. Musculoskeletal pain in patients with myotonic dystrophy type 2. *Arch. Neurol.* 61, 1938–1942. <http://dx.doi.org/10.1001/archneurol.61.12.1938>.
- Gerhardt, A., Eich, W., Janke, S., Leisner, S., Treede, R.-D., Tesarz, J., 2015. Chronic widespread back pain is distinct from chronic local back pain: evidence from quantitative sensory testing, pain drawings, and psychometrics. *Clin. J. Pain* <http://dx.doi.org/10.1097/AJP.00000000000000300>.
- Graven-Nielsen, T., Arendt-Nielsen, L., 2010. Assessment of mechanisms in localized and widespread musculoskeletal pain. *Nat. Rev. Rheumatol.* 6, 599–606. <http://dx.doi.org/10.1038/nrrheum.2010.107>.
- Graven-Nielsen, T., Mense, S., 2001. The peripheral apparatus of muscle pain: evidence from animal and human studies. *Clin. J. Pain* 17, 2–10.
- Hannonen, P., Malminniemi, K., Yli-Kerttula, U., Isomeric, R., Roponen, P., 1998. A randomized, double-blind, placebo-controlled study of moclobemide and amitriptyline in the treatment of fibromyalgia in females without psychiatric disorder. *Br. J. Rheumatol.* 37, 1279–1286.
- Heatwole, C., Johnson, N., Goldberg, B., Martens, W., Moxley, R., 2011. Laboratory abnormalities in patients with myotonic dystrophy type 2. *Arch. Neurol.* 68, 1180–1184. <http://dx.doi.org/10.1001/archneurol.2011.191>.
- Kim, H., Lee, H., Rowan, J., Brahim, J., Dionne, R.A., 2006. Genetic polymorphisms in monoamine neurotransmitter systems show only weak association with acute post-surgical pain in humans. *Mol. Pain* 2, 24. <http://dx.doi.org/10.1186/1744-8069-2-24>.
- Lewin, G.R., Lechner, S.G., Smith, E.S.J., 2014. Nerve growth factor and nociception: from experimental embryology to new analgesic therapy. *Handb. Exp. Pharmacol.* 220, 251–282. http://dx.doi.org/10.1007/978-3-642-45106-5_10.
- Liquori, C.L., Ricker, K., Moseley, M.L., Jacobsen, J.F., Kress, W., Naylor, S.L., Day, J.W., Ranum, L.P., 2001. Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. *Science* 293, 864–867. <http://dx.doi.org/10.1126/science.1062125>.
- Mason, C.C., Hanson, R.L., Ossowski, V., Bian, L., Baier, L.J., Krakoff, J., Bogardus, C., 2011. Bimodal distribution of RNA expression levels in human skeletal muscle tissue. *BMC Genomics* 12, 98. <http://dx.doi.org/10.1186/1471-2164-12-98>.
- McBeth, J., Jones, K., 2007. Epidemiology of chronic musculoskeletal pain. *Best Pract. Res. Clin. Rheumatol.* 21, 403–425. <http://dx.doi.org/10.1016/j.berh.2007.03.003>.
- Mense, S., 2003. The pathogenesis of muscle pain. *Curr. Pain Headache Rep.* 7, 419–425.
- Meola, G., Cardani, R., 2015. Myotonic dystrophies: an update on clinical aspects, genetic, pathology, and molecular pathomechanisms. *Biochim. Biophys. Acta* 1852, 594–606. <http://dx.doi.org/10.1016/j.bbadi.2014.05.019>.
- Pfau, D.B., Rolke, R., Nickel, R., Treede, R.-D., Daublaender, M., 2009. Somatosensory profiles in subgroups of patients with myogenic temporomandibular disorders and fibromyalgia syndrome. *Pain* 147, 72–83. <http://dx.doi.org/10.1016/j.pain.2009.08.010>.
- Piomelli, D., Sasso, O., 2014. Peripheral gating of pain signals by endogenous lipid mediators. *Nat. Neurosci.* 17, 164–174. <http://dx.doi.org/10.1038/nn.3612>.
- Rhodes, J.D., Lott, M.C., Russell, S.L., Moulton, V., Sanderson, J., Wormstone, I.M., Broadway, D.C., 2012. Activation of the innate immune response and interferon signalling in myotonic dystrophy type 1 and type 2 cataracts. *Hum. Mol. Genet.* 21, 852–862. <http://dx.doi.org/10.1093/hmg/ddr515>.
- Rolke, R., Baron, R., Maier, C., Tölle, T.R., Treede, R.-D., Beyer, A., Binder, A., Birbaumer, N., Birklein, F., Bötefür, I.C., Braune, S., Flor, H., Huge, V., Klug, R., Landwehrmeyer, G.B., Magerl, W., Maihöfner, C., Rolko, C., Schaub, C., Scherens, A., Sprenger, T., Valet, M., Wasserkirch, B., 2006. Quantitative sensory testing in the German research network on neuropathic pain (DFNS): standardized protocol and reference values. *Pain* 123, 231–243. <http://dx.doi.org/10.1016/j.pain.2006.01.041>.
- Savkur, R.S., Philips, A.V., Cooper, T.A., Dalton, J.C., Moseley, M.L., Ranum, L.P.W., Day, J.W., 2004. Insulin receptor splicing alteration in myotonic dystrophy type 2. *Am. J. Hum. Genet.* 74, 1309–1313. <http://dx.doi.org/10.1086/421528>.
- Screen, M., Jonson, P.H., Raheem, O., Palmio, J., Laaksonen, R., Lehtimäki, T., Sirito, M., Krahe, R., Hackman, P., Udd, B., 2014. Abnormal splicing of NEDD4 in myotonic dystrophy type 2: possible link to statin adverse reactions. *Am. J. Pathol.* 184, 2322–2332.
- Staud, R., Vierck, C.J., Cannon, R.L., Mauderli, A.P., Price, D.D., 2001. Abnormal sensitization and temporal summation of second pain (wind-up) in patients with fibromyalgia syndrome. *Pain* 91, 165–175.
- Suokas, K.I., Haanpää, M., Kautiainen, H., Udd, B., Hietaharju, A.J., 2012. Pain in patients with myotonic dystrophy type 2: a postal survey in Finland. *Muscle Nerve* 45, 70–74. <http://dx.doi.org/10.1002/mus.22249>.

- Tang, Z.Z., Yarotskyy, V., Wei, L., Sobczak, K., Nakamori, M., Eichinger, K., Moxley, R.T., Dirksen, R.T., Thornton, C.A., 2012. Muscle weakness in myotonic dystrophy associated with misregulated splicing and altered gating of Ca(V)1.1 calcium channel. *Hum. Mol. Genet.* 21, 1312–1324. <http://dx.doi.org/10.1093/hmg/ddr568>.
- Telang, S., Clem, B.F., Klarer, A.C., Clem, A.L., Trent, J.O., Bucala, R., Chesney, J., 2012. Small molecule inhibition of 6-phosphofructo-2-kinase suppresses t cell activation. *J. Transl. Med.* 10, 95. <http://dx.doi.org/10.1186/1479-5876-10-95>.
- Treister, R., Pud, D., Epstein, R.P., Laiba, E., Raz, Y., Gershon, E., Haddad, M., Eisenberg, E., 2011. Association between polymorphisms in serotonin and dopamine-related genes and endogenous pain modulation. *J. Pain* 12 (8), 875–883. <http://dx.doi.org/10.1016/j.jpain.2011.02.348> (Aug).
- Udd, B., Krahe, R., 2012. The myotonic dystrophies: molecular, clinical, and therapeutic challenges. *Lancet Neurol.* 11, 891–905. [http://dx.doi.org/10.1016/S1474-4422\(12\)70204-1](http://dx.doi.org/10.1016/S1474-4422(12)70204-1).
- Wahbi, K., Meune, C., Bécane, H.M., Laforêt, P., Bassez, G., Lazarus, A., Radvanyi-Hoffman, H., Eymard, B., Duboc, D., 2009. Left ventricular dysfunction and cardiac arrhythmias are frequent in type 2 myotonic dystrophy: a case control study. *Neuromuscul. Disord.* 19, 468–472. <http://dx.doi.org/10.1016/j.nmd.2009.04.012>.