DR. JENNIFER KOLLMER (Orcid ID : 0000-0002-6254-9192)

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Magnetization transfer ratio – a quantitative imaging biomarker for 5q spinal muscular atrophy

Original research article

Jennifer Kollmer, MD,[1] Tobias Kessler, MD,[2] Georges Sam, PhD,[2] John M. Hayes, BA,[3] Stephen I. Lentz, PhD,[4] Sabine Heiland, PhD,[1,5] Martin Bendszus, MD,[1] Wolfgang Wick, MD,[2] and Markus Weiler, MD[2]

[1] Department of Neuroradiology, Heidelberg University Hospital, Heidelberg, Germany

[2] Department of Neurology, Heidelberg University Hospital, Heidelberg, Germany

Medicine, Heidelberg University Hospital, Heidelberg, Germany

[3] Department of Neurology, University of Michigan, Ann Arbor, MI, USA

[4] Department of Internal Medicine, Division of Metabolism, Endocrinology & Diabetes, University of Michigan, Ann Arbor, MI, USA

[5] Division of Experimental Radiology, Department of Neuroradiology, Heidelberg University Hospital, Heidelberg, Germany

Corresponding Authors:

Markus Weiler, MD

Department of Neurology

Heidelberg University Hospital
Im Neuenheimer Feld 400
D-69120 Heidelberg
Germany
Phone: +49 6221 567504
Fax: +49 6221 565461
Email: markus.weiler@med.uni-heidelberg.de

or

Jennifer Kollmer, MD
Department of Neuroradiology
Heidelberg University Hospital
Im Neuenheimer Feld 400
D-69120 Heidelberg
Germany
Phone: +49 6221 5638305
Fax: +49 6221 564673
Email: jennifer.kollmer@med.uni-heidelberg.de

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Abstract

Background: We quantified peripheral nerve lesions in adult patients with 5q-linked spinal muscular atrophy (SMA) type 3 by analyzing the magnetization transfer ratio (MTR) of the sciatic nerve and tested its potential as a novel biomarker for macromolecular changes.

Methods: Eighteen adult patients with SMA 3 (50% SMA 3a, 50% SMA 3b) and 18 age-/sex-matched healthy controls prospectively underwent magnetization transfer contrast imaging in a 3T MR-scanner. Two axial three-dimensional gradient echo sequences with and without an off-resonance saturation rapid frequency pulse were performed at the right distal thigh. Sciatic nerve regions of interest were manually traced on ten consecutive axial slices in the images generated without off-resonance saturation, and then transferred to corresponding slices generated by the sequence with the off-resonance saturation pulse. Subsequently, MTR and cross-sectional areas (CSA) of the sciatic nerve were analyzed. Besides, detailed neurologic, physiotherapeutic and electrophysiologic examinations were conducted in all patients.

Results: Sciatic nerve MTR and CSA reliably differentiated between healthy controls and SMA 3, 3a or 3b. MTR was lower in SMA 3 ($p<0.0001$), SMA 3a ($p<0.0001$), and SMA 3b ($p=0.0020$) than in respective controls. In SMA 3, MTR correlated with all clinical scores, and arm nerve compound motor action potentials (CMAPs). CSA was lower in SMA 3 ($p<0.0001$), SMA 3a ($p<0.0001$), and SMA 3b ($p=0.0006$) than in controls, but did not correlate with clinical scores or electrophysiologic results.

Conclusions: MTR is a novel imaging marker that quantifies macromolecular nerve changes in SMA 3, and positively correlates with clinical scores and CMAPs.

Introduction

5q-linked spinal muscular atrophy (SMA) is an autosomal-recessive neuromuscular disease characterized by degeneration of anterior horn cells in the spinal cord and progressive muscle wasting. The underlying genetic causes are homozygous deletions or loss-of-function mutations in the *survival-motor-neuron 1 gene (SMN1)* on chromosome 5q13 with retained function of at least one copy of the paralogous gene *SMN2*.[1]

Highly innovative therapies driving SMN expression via distinct molecular mechanisms are now clinically available and results from sham-controlled clinical trials in children are encouraging.[2-5]

Efficacy data in adult patients are limited to two uncontrolled observational studies into the use of nusinersen,[6,7] but objective biomarkers that can clearly define placebo-free effects of novel disease-modifying medications in adult SMA patients are still urgently needed.

Recently, we reported that high-resolution magnetic resonance neurography (MRN)[8-12] detects and quantifies peripheral nerve involvement in adult SMA patients with high sensitivity.[13] We concluded that the quantitative MRN parameters, apparent T2-relaxation time ($T2_{app}$) and proton spin density (ρ), might serve as novel imaging biomarkers in SMA,[13] yet the macromolecular changes underlying alterations in $T2_{app}$ and ρ are still not fully understood. Magnetization transfer contrast (MTC) imaging can provide valuable information on the concentration of protons bound to macromolecules and their interaction with free water molecules that cannot be measured directly by conventional MRI sequences.[14-16]

In this exploratory study, we used MTC imaging as a tool to quantify sciatic nerve lesions in SMA 3a ("walkers" with onset of first symptoms before the age of three years) and SMA 3b patients (symptom onset after the age of three years), in comparison with clinical and electrophysiologic findings, and with healthy controls.

Methods

Study design, neurologic and electrophysiologic assessments

This prospective case-control study was approved by the institutional ethics board (University of Heidelberg; S-398/2012), and written informed consent was obtained from all participants according to the Declaration of Helsinki.

We enrolled 18 therapy-naïve patients with genetically confirmed SMA 3a or 3b (12 males, 6 females, mean age 34.2±2.5 years, range 18-55), and 18 sex-matched healthy volunteers (12 males, 6 females, mean age 34.1±2.3 years, range 23-55) between September 2017 and November 2019. Patients with SMA 1 or 2 were not included into this study due to a high percentage of patients with massive joint contractures that precluded an adequate positioning and coil usage according to our standard protocols. Pediatric patients and patients with SMA 4 were not available at our center. Exclusion criteria were pregnancy, any MRI contraindications, and any risk factors for peripheral neuropathies such as diabetes mellitus, alcoholism or malignant diseases.

A detailed medical history was taken in all patients including assessments for the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) score.[17] Since lower limb

impairment causing limitations with mobility or walking was identified as having the greatest effect on the lives of adult SMA patients,[18] the ALSFRS-R lower limb (LL) subscore was additionally evaluated.[19] Comprehensive neurologic examinations contained assessments of the Medical Research Council (MRC) sum score (M.W.) for which six muscle groups, i.e., shoulder abductors, elbow flexors, wrist extensors, hip flexors, knee extensors, and foot dorsiflexors, were bilaterally examined for strength, each with a score from 0 to 5 according to the MRC scale.[20] SMA 3a or 3b were classified based on the age of onset and achievement of motor milestones.[1] Motor nerve conduction studies (NCS) assessed distal motor latencies, compound muscle action potentials (CMAPs), and nerve conduction velocities (NCV) of the right (RPN) and left peroneal nerves (LPN), the right (RTN) and left tibial nerves (LTN), right median (RMN), and left ulnar nerve (LUN). Sensory nerve action potentials (SNAP) and NCVs were measured for the right (RSN) and left sural nerves (LSN), RMN, and LUN (G.S.;M.W.). Skin temperature was controlled at a minimum of 32°C.

Physiotherapeutic assessments

The Hammersmith Functional Motor Scale-Expanded (HFMSE) for the evaluation of gross motor function, and the Revised Upper Limb Module (RULM) as the most robust scale for assessment of upper limb function in SMA were assessed by experienced physiotherapists in all SMA patients.[21-23] The RULM score was used to achieve a detailed characterization of SMA patients, even though MTC imaging of the upper extremities was not part of this study.

MRN imaging protocol

A 15-channel Transmit-Receive knee-coil (INVIVO, Gainesville, FL, USA) was positioned at the right distal thigh, and all participants underwent MTC imaging feet first and supine in a 3.0 Tesla MR-scanner (Magnetom PRISMA, Siemens Healthineers, Erlangen, Germany). Two axial three-dimensional, gradient echo sequences with and without an off-resonance saturation pulse (Gaussian envelop, duration=9984µs, frequency offset=1200Hz) were carried out at the exact same slice position and with the following exact same sequence parameters:

Repetition time=50 ms, echo time=4.92ms, field of view=160x160mm², matrix-size 256x256, band-width=370 Hz/Px, 16 slices, slice thickness=3.5mm, voxel-size=0.6x0.6x3.5mm³, flip angle=7°, acquisition time=3:48min.

The total acquisition time including survey scans was 8:04 minutes.

Image analysis

After pseudonymization, one neuroradiologist (J.K.) blinded to clinical data, analyzed all generated images in ImageJ (version 1.51; NIH, Bethesda, Maryland, USA) by manually delineating the sciatic nerve circumference as intraneural region of interest (ROI) approximately 1 cm proximal of the nerve bifurcation. All ROIs were primarily traced on axial slices generated by the sequence without off-resonance saturation, and then transferred to the corresponding axial slices generated by the sequence with off-resonance saturation using the "synchronize windows" tool in ImageJ. Each ROI was visually inspected to rule out any possible inaccuracy of ROI positions between the two sequences, e.g., due to patient motion. Only ten central slices within each image slab were analyzed to avoid any artifacts or systematic errors caused by inhomogeneities of the B1-field of the saturation pulse.

Magnetization Transfer Ratio

The MTR was calculated separately for each participant, and each evaluated axial imaging slice according to the following equation, in which S_0 is the signal without and S_1 with off-resonance saturation:

$$MTR = 100 \times \frac{(S_0 - S_1)}{S_0}$$

Subsequently, MTR values were extracted from each slice position and averaged over all ten slice positions for each participant. Calculated MTR mean values of the sciatic nerve were then compared between the different groups (cumulated SMA 3 versus cumulated healthy controls, SMA 3a versus $Controls_{SMA3a}$, SMA 3b versus $Controls_{SMA3b}$).

Cross sectional area

Morphometric quantification was additionally performed by measuring the cross-sectional area (CSA) of the sciatic nerve per participant and per slice position. Subsequently, CSAs were averaged over all ten slice positions per participant, and then compared between the three groups.

Statistical analyses

Statistical data analyses were performed with GraphPad Prism 7.03 (J.K.;J.M.H.). Differences in MTR and CSA between cumulative SMA 3 patients and healthy controls as well as differences in

clinical scores and NCS between SMA 3a and SMA 3b were evaluated with the Mann-Whitney test. Differences between SMA 3a, SMA 3b, and respective controls ($Controls_{SMA3a}$, $Controls_{SMA3b}$) were tested with a one-way ANOVA for *a priori* assumptions, and subsequent post hoc analyses were corrected for multiple comparisons by using the Tukey-Kramer test. Pearson's correlation coefficients were calculated for further correlation analyses. Additional data simulation and visualization of the MTR was performed using qMTLab within MatLab 9.6.[24] Statistical tests were two-tailed and an alpha level of significance was defined at $p<0.05$. All results are documented as mean values±SEM.

Data availability statement

All data used to conduct this study are documented in the "Methods" section. Additional anonymized datasets that support the findings of this study are available from the corresponding author upon reasonable request.

Results

Patient demographics, genetic and clinical data

Table 1 summarizes mean values±SEM of important clinical, genetic and electrophysiologic data. Fifty percent of the 18 patients with SMA 3 were classified as SMA 3a (mean age 33.6±3.4 years), and 50% as SMA 3b (34.9±3.8 years, p=0.65). The male:female ratio differed between the two groups and was 4:5 in SMA 3a, and 8:1 in SMA 3b. For this reason, we used individual age- and sex-matched controls for each individual SMA group ($Controls_{SMA3a}$: mean age 33.4±3.3 years, male:female ratio 4:5; $Controls_{SMA3b}$ 34.7±3.5 years, 8:1).

The homozygous deletion of exons 7 and 8 of the *SMN1* gene was found in 15 of 18 patients (83%). Three patients (17%) were diagnosed with a compound heterozygous mutation of the *SMN1* gene (two SMA 3a patients (c*3+6T>G; c.90_91insT) and one SMA 3b patient (c.283G>C)). The mean *SMN2* copy number did not differ between SMA 3a and 3b (p=0.50). The mean duration of clinical symptoms prior to the study examinations was also not different (p=0.11). Spinal fusion had been performed in 22% of SMA 3a and 0% of SMA 3b patients. Except for one, all SMA 3a patients were wheelchair-bound, whereas 8 of 9 SMA 3b patients (89%) were ambulatory. Mean ALSFRS-R and MRC sum scores (p=0.0001, respectively) as well as the ALSFRS-R LL subscore (p=0.0004) discriminated well between the two patient groups.

For physiotherapeutic assessments, HFMSE and RULM scores were determined in all SMA patients (Table 1), and marked differences were found between SMA 3a and 3b.

Electrophysiologic data

All SMA patients received detailed electroneurographic examinations (Table 1). Mean amplitudes of CMAPs for each examined arm and leg nerve were markedly higher in SMA 3b than in SMA 3a (RMN p=0.0078; LUN p=0.0006; RPN p=0.0332; LPN p=0.0047; RTN p=0.0003; LTN p=0.0022). Amplitudes of SNAPs were only different for the LSN between SMA 3a and 3b (p=0.0172).

When evaluating the cumulative SMA 3 group (SMA 3a & 3b), CMAP amplitudes of all examined arm and leg nerves positively correlated with clinical scores, i.e. the ALSFRS-R (from r=0.7611, p=0.0006 (LUN) to r=0.5218, p=0.0381 (RPN)), the MRC sum score (from r=0.8325, p<0.0001 (LUN) to r=0.5004, p=0.0484 (LTN)), the HFMSE (from r=0.8149, p=0.0001 (LUN) to r=0.5801, p=0.0297 (HFMSE)), and the RULM (from r=0.7392, p=0.0011 (RTN) to r=0.5038, p=0.0466 (LTN)), except for the RPN that did not correlate with HFMSE and RULM scores, and the RTN that did not correlate with the MRC sum score. In SMA 3a, CMAP amplitudes of single nerves positively correlated with the MRC sum score (LUN: r=0.7077, p=0.0495; LTN: r=0.7547, p=0.0499), and the RULM score (RMN: r=0.7841, p=0.0124), but not with any of the other clinical scores. In SMA 3b, not a single positive correlation with any of the clinical scores was observed. Given the clear correlations between CMAPs and clinical scores in the cumulative SMA 3 group, lacking correlations in the SMA 3a and 3b subgroups might be a consequence of the small sample sizes. In accordance with the motor neuronal symptoms of the disease, SNAP amplitudes and clinical scores did not consistently correlate in any of the investigated groups.

Magnetization transfer ratio

Sciatic nerve MTR was markedly lower in the cumulative SMA 3 group (26.2±0.7%) than in the cumulative control group (32.4±0.6%, p<0.0001; Figure 1A). For the SMA 3a and 3b subgroups and their respective control groups one-way ANOVA revealed marked differences in sciatic nerve MTR (p<0.0001, F=19.17). In detail, mean MTR was lower in SMA 3a (24.6±1.1%) than in $Controls_{SMA3a}$ (32.2±0.9%, p<0.0001), and lower in SMA 3b (27.8±0.5%) than in $Controls_{SMA3b}$ (32.7±0.8%, p=0.0020; Figure 1B, 2A-C), while relevant differences between SMA 3a and 3b were not observed (p=0.07; Figure 1B, 2B/C). However, a tendency towards lower MTR values

was seen in the more severely affected SMA 3a patients than in the less affected SMA 3b patients (Figure 1B), supposing that missing statistical significance might be the result of small subgroup sizes.

Notably, consistently positive correlations were found between sciatic nerve MTR and all clinical scores in the cumulative SMA 3 group (ALSFRS-R: r=0.770, p=0.0002; ALSFRS-R LL subscore: r=0.775, p=0.0002; MRC sum score: r=0.544, p=0.0197; HFMSE: r=0.838, p<0.0001; RULM: r=0.749, p=0.0004). Regarding the two SMA 3 subtypes, such a correlation was only found for the ALSFRS-R LL subscore (SMA 3a: r=0.855, p=0.0033; SMA 3b: r=0.844, p=0.0042) and the HFMSE (SMA 3a: r=0.853, p=0.0034; SMA 3b: r=0.876, p=0.0020). Further positive correlations were found between MTR values and MRC sum scores in SMA 3a (r=0.773, p=0.0193), and the ALSFRS-R in SMA 3b (r=0.793, p=0.0109). Besides positive correlations between the MTR and CMAP amplitudes of the RMN (r=0.549, p=0.0183) and the LUN (r=0.6982, p=0.0026) in the cumulative SMA 3 group, MTR did not correlate with CMAP or SNAP amplitudes.

Cross-sectional area

Sciatic nerve CSA was determined for additional morphologic quantification of nerve calibers. Mean CSA was markedly decreased in the cumulative SMA 3 group (14.3±0.6mm²) when compared to cumulative controls (20.0±0.5mm², p<0.0001; Figure 3A). Distinct differences were observed when evaluating the subgroups (ANOVA p<0.0001, F=15.92). *Post hoc* analyses revealed lower sciatic nerve CSA in SMA 3a (13.9±1.0mm²) versus $Controls_{SMA3a}$ (20.2±0.9mm², p<0.0001) as well as lower CSA in SMA 3b (14.7±0.8mm²) versus $Controls_{SMA3b}$ (19.9±0.6mm², p=0.0006; Figure 3B), indicating severe generalized peripheral nerve atrophy in SMA. However, CSA differences between SMA 3a and SMA 3b were not observed (p=0.90; Figure 3B).

Unlike MTR, CSA correlated neither with any of the clinical scores nor with CMAP amplitudes of any arm or leg nerve in any SMA group.

Discussion

Recently, highly innovative pharmacotherapies driving SMN expression via distinct genetic mechanisms developed for SMA have become clinically available. Based on the results of two pivotal studies in children with SMA 1 or 2,[2,3] the ASO drug nusinersen (Spinraza®) was

approved by the United States Food and Drug Administration (FDA) and by the European Medicines Agency (EMA) in mid-2017 as the first drug for SMA patients of all ages, types, and disease stages. However, data on nusinersen treatment in adults with SMA were generated only after its clinical approval, and could thus not be controlled by sham treatments.[6,7] Of note, a recent multicenter observational study provides evidence for the safety and efficacy of nusinersen in a large real-world cohort of adult patients with SMA 2 and 3. Numerous patients in this study showed clinically meaningful improvements in motor function or disease stabilization, independent of age.[7] Despite these encouraging findings, the lack of controlled data for nusinersen in adults makes it likely that results of patient-reported questionnaires and outcome scores of purely clinical tests for motor functions are biased by placebo effects to some degree. Facing cost-intensive therapies, there is an urgent need to establish objective biomarkers indicating a potential early therapeutic response in adult SMA patients.

Here, we report the first study to apply MTC imaging in SMA patients. Our results show that the sciatic nerve MTR in therapy-naïve patients with SMA 3a and 3b is markedly lower than in healthy controls (Figures 1&2). Moreover, we found a clear, yet not significant, tendency towards lower MTRs in more severely affected patients with SMA 3a compared to patients with SMA 3b who tend to have milder symptoms (Figure 1B; Table 1). Most importantly, unlike CSA, MTR correlated well with all examined clinical scores.

MTC imaging is an MRI technique that provides indirect information about the macromolecular composition of different tissues, i.e., about protons bound to macromolecular structures, such as myelin lipids or collagen.[14] These bound protons have very short T2 relaxation times preventing their signal to be directly measured by conventional MRI sequences. At the same time, they are physically characterized by an increased bandwidth of the resonance compared to protons bound to small water molecules, allowing their selective excitation or saturation.[14] MTC imaging uses an off-resonance pulse to saturate macromolecular bound protons inducing their exchange with free water protons. The resulting decrease in the signal intensity of free water protons consequently enables the visualization of the macromolecular bound pool, which can then be measured and quantified by computing the MTR from two almost identical sequences (one with and one without the off-resonance saturation pulse).[25] Compared with not yet established biomarkers derived from body fluids such as the cerebrospinal fluid,[26] blood and others, imaging

markers like MTR are advantageous in that they can provide information on the macromolecular composition of the primarily injured target tissue (nerve) in SMA, in addition to important further morphometric data that can be gathered within the same imaging session.

While results from MTC studies conducted in the central nervous system (CNS) are promising,[27-37] data on the potential of MTC imaging in the peripheral nervous system are limited. To date, there are two studies that applied MTC imaging in patients with peripheral neuropathies, but results were controversial: while one study found that MTR does not differentiate between patients with hereditary neuropathy with liability to pressure palsies (HNPP) and controls, the other study demonstrated a strong correlation between decreasing sciatic nerve MTR values and higher grades of disability in patients with Charcot-Marie-Tooth disease.[38,39] The latter finding is supported by a recent study from our group, where we found evidence that sciatic nerve MTR is decreased in patients with hereditary transthyretin amyloidosis with PNP and correlates well with electrophysiologic results and the Neuropathy Impairment Score of the Lower Limb (NIS-LL).[40]

Results from the study at hand are in line with these findings and go beyond: a decrease in sciatic nerve MTR clearly correlated not only with axonal degeneration of lower motor neurons but also with patient-reported activities of daily living (ALSFRS-R, ALSFRS-R LL), muscle weakness (MRC sum score), and physiotherapeutic assessment scores (HFMSE, RULM), that taken together comprehensively reflect the patient's physical state (Table 1). Notably, sciatic nerve MTR correlated with the CMAP amplitudes of arm nerves, while correlations with the CMAPs of leg nerves were absent. At first sight, this might appear confusing, but may be explained by the fact that SMA affects motor neurons in the lower extremities more severely than in the upper extremities. The more advanced stage of neurodegeneration leading to highly reduced or even extinguished CMAPs of the leg nerves might hamper meaningful correlations with functional parameters such as quantitative MRN markers. By correlating with the CMAP amplitudes of the arm nerves, MTR, as a matter of speculation, might thus well reflect the severity of the disease. Like MTR, CSA (a MRN measure for nerve caliber) was also decreased in SMA 3a and 3b compared to controls (Figure 3). However, only MTR correlated with all established clinical scores, favoring this MRN parameter as a more promising imaging marker than CSA, even though both, MTR and CSA, almost equally differentiate between SMA 3 and healthy controls.

These findings are supported by results from a recent CNS imaging study, where a decrease in spinal cord grey matter CSA did not correlate with most functional scores.[41] Furthermore, a change in MTR represents a change in the pool of macromolecular bound protons in nerve tissue, and might therewith identify a therapeutic response earlier than CSA when applied for therapy monitoring in the future. In addition, MTR complements the two previously established MRN markers derived from T2 relaxometry sequences, i.e. $T2_{app}$ and ρ, as MTR and T2 reflect changes in different proton pools.[12,13,42,43] Collectively, these markers may contribute to a comprehensive understanding of macromolecular changes in nerve tissue *in vivo*. Longitudinal studies are now needed to evaluate whether individual MRN markers might be particularly sensitive to certain disease stages or stages of therapeutic response (e.g., one marker might potentially indicate an early response, while another marker might give information on long-term effects), or whether they provide the highest informational value when considered altogether.

While the FDA approved the one-time administered gene replacement therapy onasemnogene abeparvovec-xioi (Zolgensma®) only for the treatment of children less than two years of age, the EMA recently recommended this therapy for SMA patients with up to three *SMN2* gene copies independent of age. This restriction clearly concerns patients with SMA 2, 3 or 4 who are diagnosed with four *SMN2* copies or more. In our type 3 SMA cohort for instance, 10 of 18 patients (56%) were diagnosed with four *SMN2* copies (Table 1) and would thus not be eligible to receive onasemnogene abeparvovec-xioi, although they were as severely affected as patients of the same SMA 3 subtype with only two or three *SMN2* copies. Alternatively, SMN-driving pharmacotherapies could be administered to SMA patients irrespective of their *SMN2* copy number, with decision-making on the continuation of further treatment depending on individual clinical and biomarker responses.[44] In this context, quantitative imaging biomarkers such as MTR, $T2_{app}$ or ρ,[13] could be a valuable contribution.

Constrictively, the relatively low number of patients in our study limited our ability to significantly delineate differences in MTR (and/or CSA) between SMA 3a and 3b, even though a tendency towards lower MTR values was observed in the more severely affected SMA 3a patients. Moreover, MTC imaging might also be of use in adult SMA 2 patients who were not included in the present study. However, in this more severely affected SMA type, both metal

implants and/or painful joint contractures can preclude adequate positioning of patients in the MR scanner.

We propose MTR as a novel imaging biomarker that can quantify macromolecular nerve changes in SMA 3, and correlates with clinical scores and CMAPs. Hence, it has the potential to indicate regenerative processes inside motor neurons, possibly earlier than clinical, electrophysiologic and even biochemical diagnostic methods. To prove the validity of MTR as a robust imaging biomarker compared with the other two recently established quantitative MRN markers, $T2_{app}$ and ρ,[13] intraindividual longitudinal comparisons are needed and are already subject of ongoing investigations. MTC imaging might then help to better monitor SMA patients on causal pharmacotherapies due to its ability to give a direct inside view into nerve tissue integrity *in vivo*.

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Figure legends

Figure 1.

Magnetization transfer ratio

Mean values of sciatic nerve magnetization transfer ratio (MTR) are plotted for cumulative controls and cumulative patients with SMA type 3 (A), and for patients with SMA types 3a and 3b, together with their respective control groups (B). Sciatic nerve MTR was markedly decreased in cumulative SMA 3 as well as in the SMA 3a and SMA 3b subgroups when compared to their respective healthy control group. Higher MTR values were seen in SMA 3b than in SMA 3a, although not statistically significant. Error bars represent SEM. Significant differences are indicated by p-values.

Figure 2.

Magnetization transfer ratio map

Representative MTR pseudo-colorized (%) maps are shown for a healthy control (A), a patient with SMA type 3a (B), and a patient with SMA type 3b (C). The white boxes in A-C are zoomed-in and displayed below to show detailed views of the MTR (%) map (left) and the MTC sequence without the off-resonance pulse (right) with the sciatic nerve encircled in white. Note the marked decrease of sciatic nerve MTR (%) in the SMA 3a and 3b compared to the healthy control.

Figure 3.

Cross sectional area

Mean values of sciatic nerve cross sectional area (CSA) are plotted for cumulative controls and cumulative patients with SMA type 3 (A), and for patients with SMA types 3a and 3b, together with their respective control groups (B). Sciatic nerve CSA was lower in cumulative SMA 3 than in cumulative controls and also lower in SMA 3a and SMA 3b than in the respective control groups. Error bars represent SEM. Significant differences are indicated by p-values.

Table legend

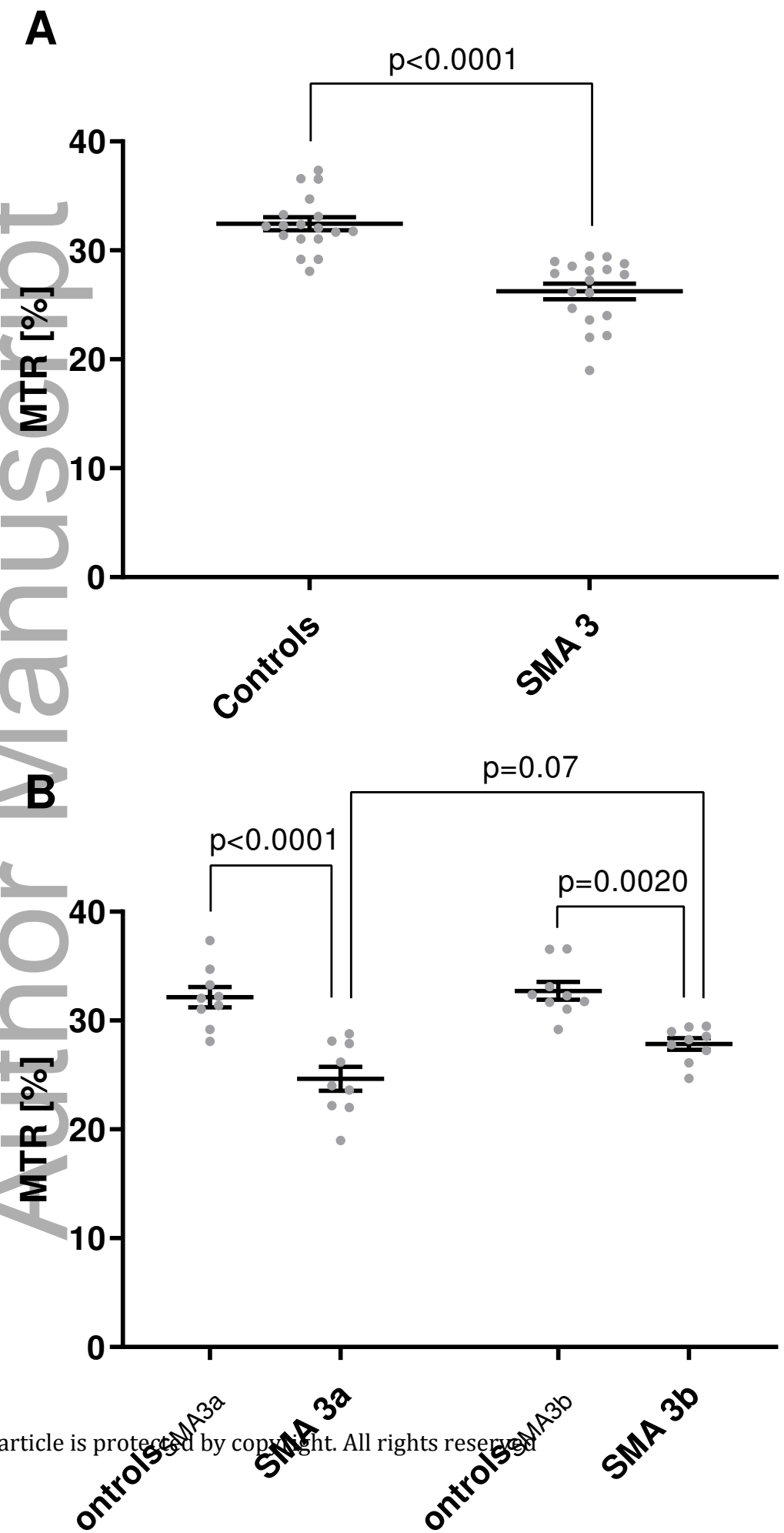
Table 1.

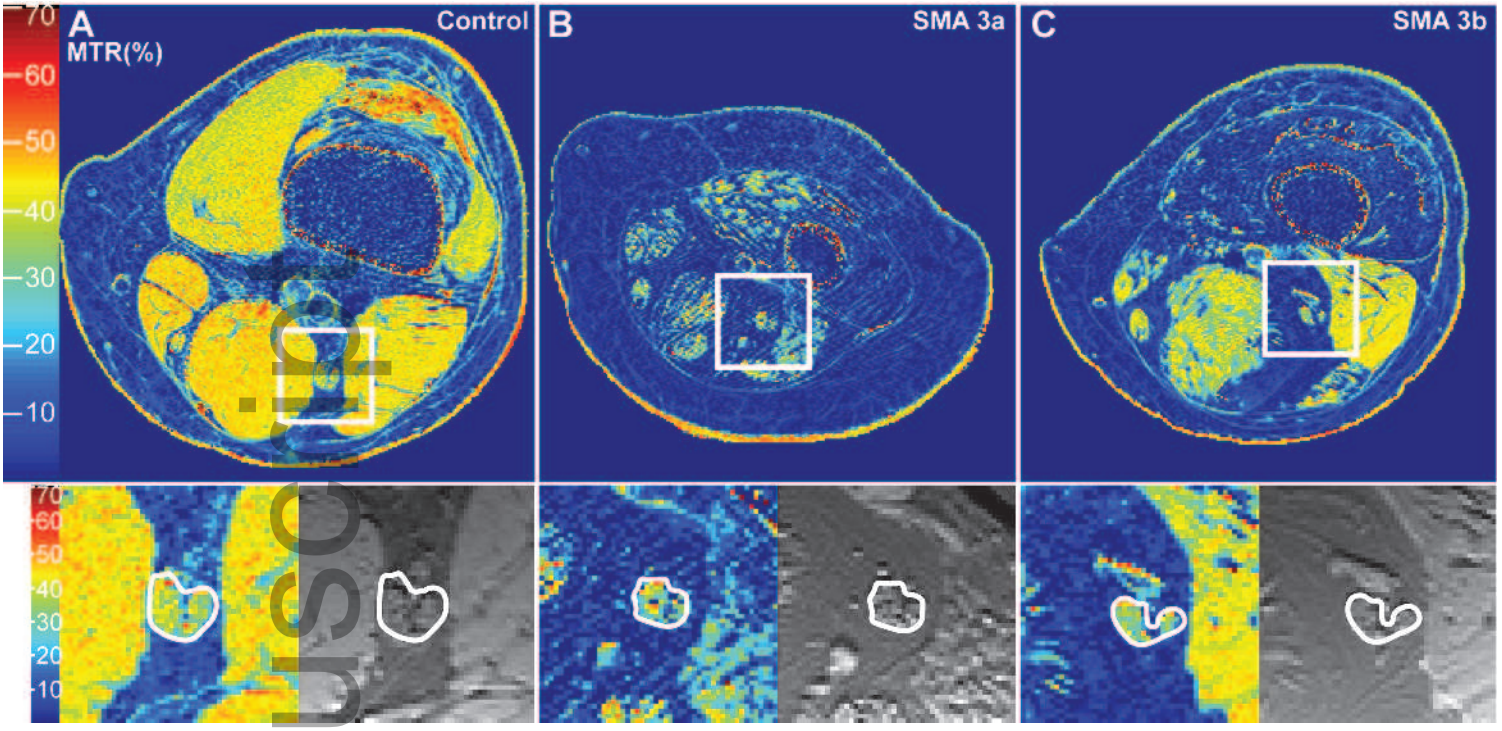
Summary of clinical, genetic, physiotherapeutic, and electrophysiologic results in patients with SMA types 3, 3a, and 3b

All results are presented as mean values ± SEM. P-values indicate respective results from statistical tests between SMA 3a and 3b patients.

ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; CMAP = compound muscle action potential; HFMSE = Hammersmith Functional Motor Scale-Expanded for SMA; LL = lower limb; LPN = left peroneal nerve; LSN = left sural nerve; LTN = left tibial nerve; LUN = left ulnar nerve; MRC sum score = Medical Research Council sum score; N/A = not applicable; RMN = right median nerve; RPN = right peroneal nerve; RSN = right sural nerve; RTN = right tibial nerve; RULM = Revised Upper Limb Module for SMA; SMA = spinal muscular atrophy; *SMN2* = survival motor neuron gene 2; SNAP = sensory nerve action potential.

Parameter	SMA type 3	SMA type 3a	SMA type 3b	P value
Patients [n]	18	9	9	N/A
Age [y]	34.2 ± 2.5	33.6 ± 3.4	34.9 ± 3.8	0.65
Sex [m:f]	12:6	4:5	8:1	N/A
SMN2 gene copies [n]	3.4 ± 0.2	3.2 ± 0.3	3.6 ± 0.2	0.50
Patients [n] with				
- **2 SMN2 copies**	3	2	1	N/A
- **3 SMN2 copies**	5	3	2	N/A
- **4 SMN2 copies**	10	4	6	N/A
Duration of symptoms [y]	27.7 ± 2.5	32.3 ± 3.4	23.0 ± 3.2	0.11
ALSFRS-R total score [0-48]	35.5 ± 1.5	30.3 ± 1.7	40.7 ± 0.6	0.0001
ALSFRS-R LL subscore [0-8]	2.1 ± 0.4	0.6 ± 0.2	3.6 ± 0.4	0.0004
MRC sum score [0-60]	35.4 ± 2.5	27.1 ± 2.0	43.7 ± 2.2	0.0001
HFMSE score [0-66]	31.6 ± 5.0	14.1 ± 4.4	49.1 ± 3.4	0.0002
RULM score [0-37]	28.6 ± 2.2	21.1 ± 2.6	36.0 ± 0.9	0.0006
CMAP [mV]				
RMN	8.8 ± 1.0	6.1 ± 0.7	11.4 ± 1.4	0.0078
LUN	6.7 ± 1.3	2.8 ± 0.6	10.7 ± 1.5	0.0006
RPN	6.7 ± 1.1	3.9 ± 0.8	8.9 ± 1.6	0.0332
LPN	7.2 ± 1.8	2.0 ± 0.9	11.0 ± 2.2	0.0047
RTN	8.8 ± 2.3	1.7 ± 0.4	14.3 ± 3.0	0.0003
LTN	7.7 ± 2.0	1.3 ± 0.3	12.7 ± 2.6	0.0022
SNAP [µV]				
RMN	34.4 ± 3.5	41.2 ± 5.3	27.5 ± 3.7	0.07
LUN	28.1 ± 3.5	35.2 ± 6.4	21.7 ± 2.2	0.13
RSN	12.0 ± 2.0	10.2 ± 3.1	13.8 ± 2.4	0.20
LSN	11.5 ± 1.7	7.0 ± 1.2	14.9 ± 2.4	0.0172





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