ORIGINAL ARTICLE

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

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

Methods: Eighteen adults with SMA 3 (50% SMA 3a, 50% SMA 3b) and 18 age-/sex-matched healthy controls prospectively underwent magnetization transfer contrast imaging in a 3-Tesla magnetic resonance 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 10 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 (CSAs) of the sciatic nerve were analysed. In addition, 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 the SMA 3 ($P < 0.0001$), SMA 3a ($P < 0.0001$) and SMA 3b groups ($P = 0.0020$) than in respective controls. In patients with SMA 3, MTR correlated with all clinical scores, and arm nerve compound motor action potentials (CMAPs). CSA was lower in the SMA 3 ($P < 0.0001$), SMA 3a ($P < 0.0001$) and SMA 3b groups ($P = 0.0006$) than in controls, but did not correlate with clinical scores or electrophysiologic results.

Conclusions: Magnetization transfer ratio 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 adults are limited to two uncontrolled

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observational studies on the use of nusinersen [6,7], but objective biomarkers that can clearly define placebo-free effects of novel disease-modifying medications in adults with SMA are still urgently needed.

Recently, we reported that high-resolution magnetic resonance neurography (MRN) [8–12] detects and quantifies peripheral nerve involvement in adults with SMA with high sensitivity [13]. We concluded that the two quantitative MRN markers apparent T2-relaxation time ($T2_{app}$) and proton spin density (ρ) might serve as novel imaging biomarkers in SMA [13], but 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 magnetic resonance imaging (MRI) sequences [14–16].

In the present exploratory study, we used MTC imaging as a tool with which to quantify sciatic nerve lesions in patients with SMA 3a ('walkers', with onset of first symptoms before the age of 3 years) and those with SMA 3b (symptom onset after the age of 3 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 our 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 men, six women, mean (range) age 34.2 ± 2.5 (18–55) years], and 18 sex-matched healthy volunteers [12 men, six women, mean (range) age 34.1 ± 2.3 (23–55) years] between September 2017 and November 2019. Patients with SMA 1 or 2 were not enrolled in the study because a high percentage of these patients had massive joint contractures that precluded 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 (LL) impairment causing limitations with mobility or walking was identified as having the greatest effect on the lives of adults with SMA [18], the ALSFRS-R 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. the 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 were conducted to assessdistal motor latencies, compound muscle action potentials (CMAPs) and nerve conduction velocities of the right (RPN) and left peroneal nerves (LPN), the right (RTN) and left tibial nerves (LTN), the right median nerve (RMN) and the left ulnar nerve (LUN). Sensory nerve action potentials (SNAPs) and nerve conduction velocities 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 the patients with SMA [21–23]. RULM score was used to achieve a detailed characterization of patients with SMA, even though MTC imaging of the upper extremities was not part of this study.

Magnetic resonance neurography 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 MRI 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 =9984 μs, frequency offset =1200 Hz), were carried out at the exact same slice position and with the following exact same sequence parameters: repetition time =50 ms, echo time =4.92 ms, field of view =160 × 160 mm^2, matrix-size 256 × 256, band-width

=370 Hz/Px, 16 slices, slice thickness =3.5 mm, voxel-size = 0.6 × 0.6 × 3.5 mm^3, flip angle = 7°, and acquisition time = 3:48 min. The total acquisition time, including survey scans, was 8:04 min.

Image analysis

After pseudonymization, one neuroradiologist (J.K.) blinded to clinical data, analysed all generated images in ImageJ (version 1.51; NIH, Bethesda, MD, USA) by manually delineating the sciatic nerve circumference as an intraneural region of interest (ROI) approximately 1 cm proximal to 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 exclude any possible inaccuracy of ROI positions between the two sequences, for example, due to patient motion. Only 10 central slices within each image slab were analysed 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 $MTR = 100 \times (S_0\text{-}S_1) / S_0$. Subsequently, MTR values were extracted from each slice position and averaged over all 10 slice positions for each participant. Calculated MTR mean values of the sciatic nerve were then compared between the different groups (cumulated SMA 3 vs. cumulated healthy controls, SMA 3a vs. $Controls_{SMA3a}$, SMA 3b vs. $Controls_{SMA3b}$).

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 10 slice positions per participant and then compared among 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 combined SMA 3 patients and healthy controls as well as differences in clinical scores and nerve conduction studies between patients with SMA 3a and those with SMA 3b were evaluated with the Mann–Whitney test. Differences among the patients with SMA 3a, patients with SMA 3b and respective controls ($Controls_{SMA3a}$, $Controls_{SMA3b}$) were tested using one-way ANOVA for *a priori* assumptions, and subsequent *post hoc* analyses were corrected for multiple comparisons 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 α level of significance was defined at $P < 0.05$. All results are documented as mean values ± SEM.

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 having SMA 3a (mean age 33.6 ± 3.4 years), and 50% as having SMA 3b (34.9 ± 3.8 years, $P = 0.65$). The male:female ratio differed between the two groups: 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, male:female ratio 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 groups ($P = 0.50$). 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 patients with SMA 3a and 0% of patients with SMA 3b. Except for one, all patients with SMA 3a were wheelchair-bound, whereas eight of nine SMA 3b patients (89%) were ambulatory. Mean ALSFRS-R and MRC sum scores ($P = 0.0001$, respectively) as well as 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 patients with SMA (Table 1), and marked differences were found between patients with SMA 3a and those with 3b.

Electrophysiologic data

All patients with SMA underwent detailed electroneurographic examinations (Table 1). Mean amplitudes

Table 1 Summary of clinical, genetic, physiotherapeutic and electrophysiologic results in patients with spinal muscular atrophy types 3, 3a, and 3b

	SMA type 3	SMA type 3a	SMA type 3b	*P*
Patients, *n*	18	9	9	N/A
Age, years	34.2 ± 2.5	33.6 ± 3.4	34.9 ± 3.8	0.65
Male: female ratio	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, years	27.7 ± 2.5	32.3 ± 3.4	23.0 ± 3.2	0.11
ALSFRS-R total score (range 0–48)	35.5 ± 1.5	30.3 ± 1.7	40.7 ± 0.6	0.0001
ALSFRS-R LL subscore (range 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

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. All results are presented as mean values ± SEM. *P* values indicate respective results from statistical tests in patients with SMA 3a versus those with SMA 3b.

of CMAPs for each examined arm and leg nerve were markedly higher in the SMA 3b than in the SMA 3a group (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 the SMA 3a and 3b groups ($P = 0.0172$).

When evaluating the combined SMA 3 group (SMA 3a and 3b), CMAP amplitudes of all examined arm and leg nerves positively correlated with clinical scores, i.e. ALSFRS-R score [from $r = 0.7611$, $P = 0.0006$ (LUN) to $r = 0.5218$, $P = 0.0381$ (RPN)], MRC sum score [from $r = 0.8325$, $P < 0.0001$ (LUN) to $r = 0.5004$, $P = 0.0484$ (LTN)], HFMSE score [from $r = 0.8149$, $P = 0.0001$ (LUN) to $r = 0.5801$, $P = 0.0297$ (LPN)], and RULM score [from $r = 0.7392$, $P = 0.0011$ (RTN) to $r = 0.5038$, $P = 0.0466$ (LTN)], except for the RPN which did not correlate with HFMSE and RULM scores, and the RTN which did not correlate with MRC sum score. In the SMA 3a group, CMAP amplitudes of single nerves positively correlated with MRC sum score (LUN: $r = 0.7077$, $P = 0.0495$; LTN: $r = 0.7547$, $P = 0.0499$), and RULM score (RMN: $r = 0.7841$, $P = 0.0124$), but not with any of the other clinical scores. In the SMA 3b group, not a single positive correlation with any of the clinical scores was observed. Given the clear correlations between CMAPs and clinical scores in the combined SMA 3 group, the lack of correlations in the SMA 3a and 3b subgroups might attributable to 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 combined SMA 3 group (26.2 ± 0.7%) than in the combined control group (32.4 ± 0.6%, $P < 0.0001$; Fig. 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 the SMA 3a group (24.6 ± 1.1%) than in the $Controls_{SMA3a}$ group (32.2 ± 0.9%; $P < 0.0001$), and lower in the SMA 3b (27.8 ± 0.5%) than in the $Controls_{SMA3b}$ group (32.7 ± 0.8%, $P = 0.0020$; Fig. 1B, 2a–c), while relevant differences between the SMA 3a and 3b groups were not observed ($P = 0.07$; Fig. 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 (Fig. 1b), with the lack of statistical significance assumed to be the result of small subgroup sizes.

Notably, consistently positive correlations were found between sciatic nerve MTR and all clinical scores in the combined 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 score (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 ($r = 0.793$, $P = 0.0109$). With the exception that the MTR positively correlated with CMAP amplitudes of the RMN ($r = 0.549$, $P = 0.0183$) and the LUN ($r = 0.6982$, $P = 0.0026$) in the combined SMA 3 group, no further correlations between the MTR and CMAP or SNAP amplitudes were identified.

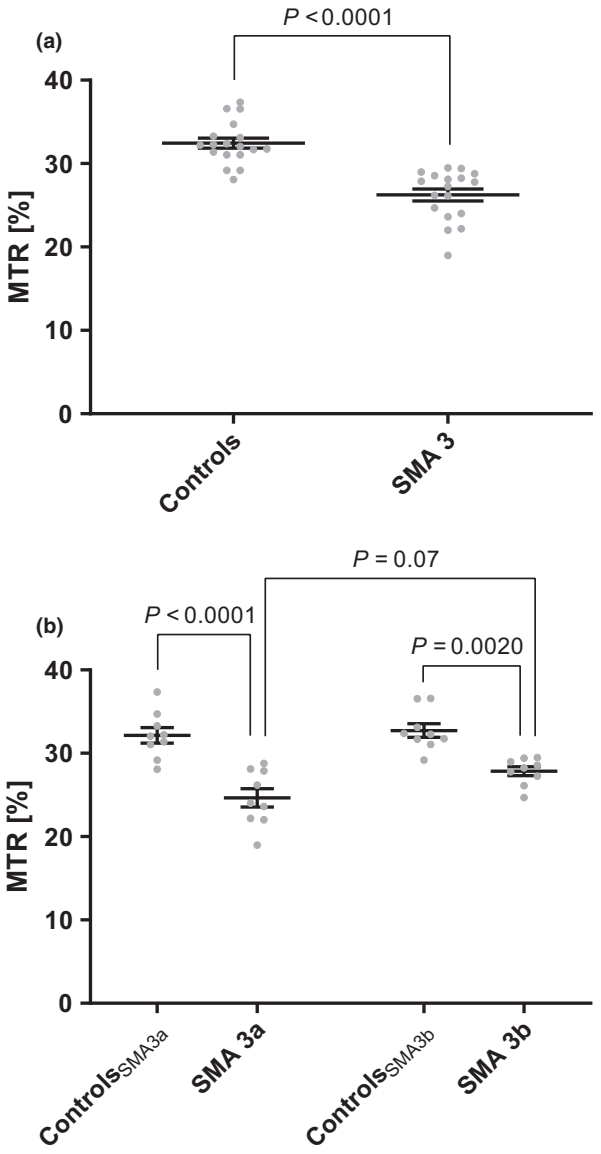

Figure 1 Magnetization transfer ratio (MTR). Mean values of sciatic nerve MTR are plotted for combined controls and combined patients with spinal muscular atrophy (SMA) type 3 (a), and for patients with SMA types 3a and 3b, together with their respective control groups (b). Sciatic nerve MTR was markedly decreased in the combined SMA 3 group as well as in the SMA 3a and SMA 3b subgroups when compared to their respective healthy control groups. Higher MTR values were seen in the SMA 3b than in the SMA 3a group, but were not statistically significant. Error bars represent SEM. Significant differences are indicated by *P* values.

Cross-sectional area

Sciatic nerve CSA was determined for additional morphologic quantification of nerve calibers. Mean CSA was markedly decreased in the combined SMA 3 group (14.3 ± 0.6 mm^2) when compared to the combined control group (20.0 ± 0.5 mm^2, $P < 0.0001$; Fig. 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 the SMA 3a group (13.9 ± 1.0 mm^2) versus the Controls$_{SMA3a}$ (20.2 ± 0.9 mm^2, $P < 0.0001$), as well as lower CSA in the SMA 3b (14.7 ± 0.8 mm^2) versus the Controls$_{SMA3b}$ (19.9 ± 0.6 mm^2, $P = 0.0006$; Fig. 3b), indicating severe generalized peripheral nerve atrophy in SMA. However, CSA differences between SMA 3a and SMA 3b were not observed ($P = 0.90$; Fig. 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 antisense oligonucleotide drug nusinersen (Spinraza®) was approved by the US Food and Drug Administration (FDA) and by the European Medicines Agency (EMA) in mid 2017 as the first drug for patients of all ages with SMA, and with different types and disease stages of SMA. 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]. Notably, 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 that 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

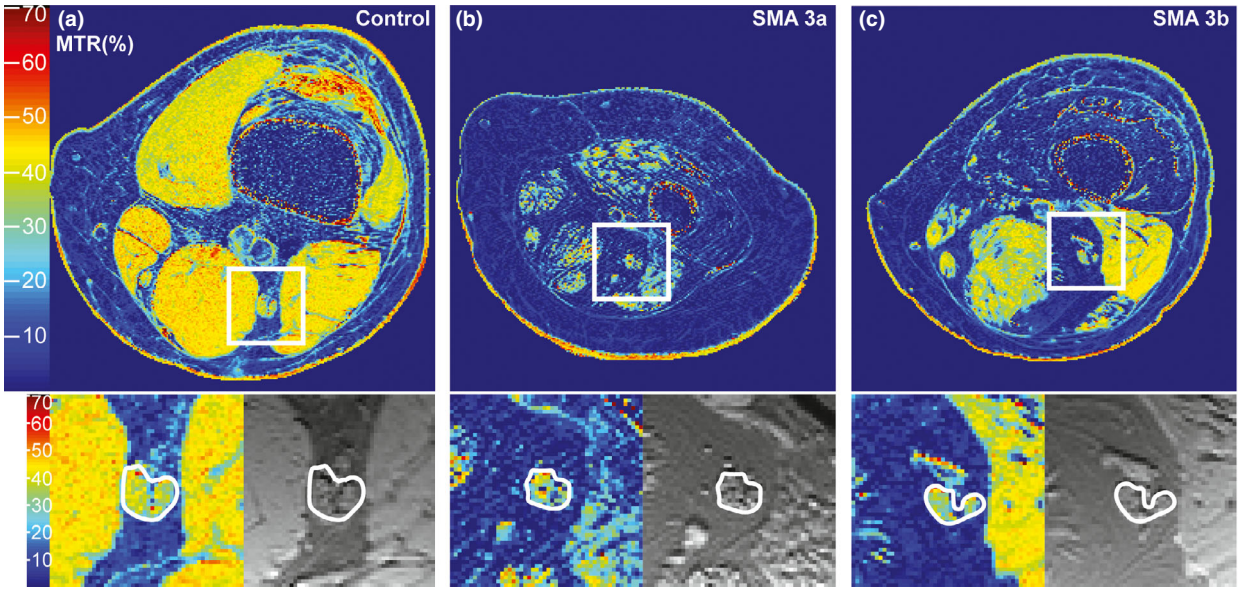

Figure 2 Magnetization transfer ratio (MTR) map. Representative MTR pseudo-colorized (%) maps are shown for a healthy control (a), a patient with spinal muscular atrophy (SMA) type 3a (b), and a patient with SMA type 3b (c). The white boxes in parts (a) to (c) are magnified and displayed below to show 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 groups compared to the healthy control group.

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 adults with SMA.

The present study is the first to apply MTC imaging in patients with SMA. Our results show that the sciatic nerve MTR in therapy-naïve patients with SMA 3a and 3b is markedly lower than in healthy controls (Figs 1 and 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 (Fig. 1b; Table 1). Most importantly, unlike CSA, MTR correlated well with all examined clinical scores.

Magnetization transfer contrast imaging is an MRI technique that provides indirect information about the macromolecular composition of different tissues, that is, 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 from being 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 have been two studies that applied MTC imaging in patients with peripheral neuropathies, but their 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

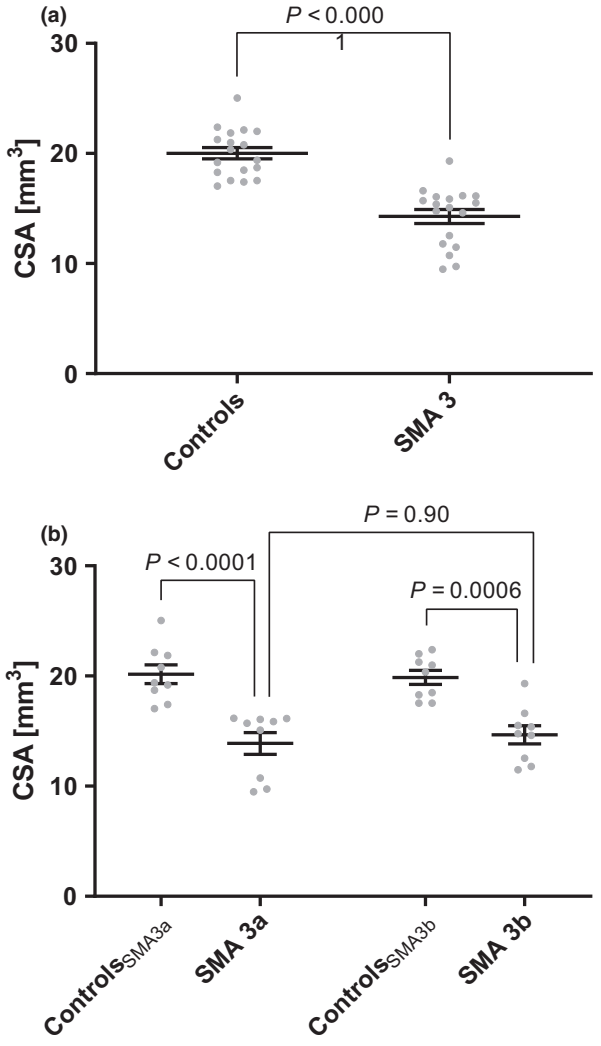

Figure 3 Cross-sectional area (CSA). Mean values of sciatic nerve CSA are plotted for combined controls and the combined spinal muscular atrophy (SMA) type 3 group (a), and for patients with SMA types 3a and 3b, together with their respective control groups (b). Sciatic nerve CSA was lower in the combined SMA 3 group than in the combined control group 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.

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, in which we found evidence that sciatic nerve MTR is decreased in patients with hereditary transthyretin amyloidosis with polyneuropathy and correlates well with electrophysiologic results and the Neuropathy Impairment Score of the Lower Limb [40].

Results from the present study are in line with these findings and extend them: 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), which, 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 variables, such as quantitative MRN markers. By correlating with the CMAP amplitudes of the arm nerves, it can be speculated that MTR might be a good reflection of the severity of the disease. Like MTR, CSA (an MRN measure for nerve caliber) was also decreased in patients with SMA 3a and 3b compared to controls (Fig. 3). However, only MTR correlated with all established clinical scores, favoring this MRN variable as a more promising imaging marker than CSA, even though both MTR and CSA almost equally differentiate between patients with 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 in 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 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 abeparvovecxioi (Zolgensma®) only for the treatment of children aged <2 years, the EMA recently recommended this therapy for patients with SMA 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 abeparvovecxioi, although they were as severely affected as patients with 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.

The relatively low number of patients in the present 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 adults with SMA 2 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 MRI 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. MTR therefore 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], intra-individual longitudinal comparisons are needed and are already the subject of ongoing investigations. MTC imaging might then help to better monitor SMA patients on causal pharmacotherapies because of its ability to give a direct inside view into nerve tissue integrity *in vivo*.

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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.

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