

Clinical and Radiological Profile of patients with Spinal Muscular Atrophy type 4

Running-title: SMA type 4

Paulo Victor Sgobbi Souza^{1}, MD; Wladimir Bocca Vieira de Rezende Pinto¹, MD, MSc; Auro Ricarte², PT; Bruno de Mattos Lombardi Badia¹, MD; Daniel Delgado Seneor¹; Daniel Tadeu Teixeira², PT; Lisandra Caetano², PT; Eduardo Augusto Gonçalves¹, MD; Marco Antônio Troccoli Chieia¹, MD; Igor Braga Farias¹, MD; Enrico Bertini³, MD; Acary Souza Bulle Oliveira¹, MD, PhD*

¹Department of Neurology and Neurosurgery, Federal University of São Paulo (UNIFESP), São Paulo, SP, Brazil.

²Neurotherapy Rehabilitation Center.

³Unit of Neuromuscular and Neurodegenerative Disorders, Bambino Gesù Children's Research Hospital, IRCCS, Rome, Italy.

***Corresponding author:** Paulo Victor Sgobbi de Souza, MD; Department of Neurology and Neurosurgery, Federal University of São Paulo (UNIFESP), São Paulo, Brazil. Embau Street, 67; ZIP CODE: 04039-060. Vila Clementino, São Paulo SP, Brazil. Telephone number: +55 (11) 5571-3324. Fax number: +55 (11) 5083-1051. E-mail: pvgobbi@gmail.com

Character count of the title: 81.

Word count manuscript: 3496 – Total (with references and abstract): 4888.

Number of references: 44.

Number of figures: 03.

Number of tables: 03.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as doi: [10.1111/ENE.14587](https://doi.org/10.1111/ENE.14587)

This article is protected by copyright. All rights reserved

Supplementary Material: 01.

Disclosure of Conflict of interests: The authors declare that they do not have conflict of interest to declare.

Financial disclosure and funding support: We have nothing to disclose.

Ethical statement: Full consent was obtained from the patients described in this original manuscript. This study was approved by our Ethics Institution.

Key words: Spinal Muscular Atrophy; *SMN1*; genetics; neuromuscular diseases; Motor Neuron Disease.

Data Availability Statement: Data is available on request from the authors. The data that support the findings of this study are available from the corresponding author upon reasonable request.

DR. PAULO VICTOR SGOBBI DE SOUZA (Orcid ID : 0000-0002-7416-7108)

DR. WLADIMIR BOCCA VIEIRA DE REZENDE PINTO (Orcid ID : 0000-0002-0150-525X)

DR. ENRICO BERTINI (Orcid ID : 0000-0001-9276-4590)

Article type : Original Article

Abstract:

Objective: Spinal Muscular Atrophy (SMA) is the most important cause of motor neuron disease in childhood and continues to represent the leading genetic cause of infant death. Adulthood-onset SMA (SMA type 4) is rare with few isolated cases reported. The objective of this study is to describe a cohort of patients with SMA type 4.

Methods: A cross-sectional study was conducted to characterize clinical, genetic, radiological and neurophysiological features of patients with adulthood-onset SMA. Correlation of functional assessment with genetic, radiological and neurophysiological data was performed.

Results: Twenty patients with SMA type 4 were identified in a Brazilian cohort of 227 patients with SMA. The most common clinical symptom was limb-girdle muscle weakness observed in 15 (75%) patients. The most frequent neurological findings were absent tendon reflexes in 18 (90%) and fasciculations 9 (45%) of patients. Sixteen patients (80%) had the homozygous deletion of exon 7 in the *SMN1* gene with 12 patients (60%) showing 4 copies of the *SMN2* gene. The functional scales HFMSE, ALSFRS-R, RULM, SMAFRS, 6WT and TUGO presented a correlation with duration of disease. MUNIX index was correlated with duration of disease and with performance in functional assessment. Radiological studies exhibited a typical pattern with involvement of biceps femoris short head and gluteus minimus in all patients.

Conclusion: This study represents the largest cohort of patients with SMA type 4 and provide functional, genetic, radiological and neurophysiological features that can be used as potential biomarkers for the new specific genetic therapies for SMA.

Key words: Spinal Muscular Atrophy; *SMN1*; genetics; neuromuscular diseases; Motor Neuron Disease.

1. Introduction

Spinal Muscular Atrophy (SMA) is an autosomal recessive neuromuscular disease characterized by loss of lower motor neurons leading to progressive muscle weakness and atrophy predominating in proximal limb muscles^{1,2}.

SMA is caused by deletions or disease-causing variants in the survival motor neuron 1 gene (*SMN1*) [OMIM *600354] localized on chromosome 5 (5q13.2) that encodes for *SMN* protein particularly abundant in alpha motor neurons of the spinal cord¹. SMA occurs in approximately 1 in 10,000 live births with a high frequency of carriers depending on the population (1:47 to 1:72 individuals)³.

SMA is classified into five phenotypes based on age of onset and maximal motor abilities reached by patients showing an inverse correlation between the number of *SMN2* gene (OMIM *601627) copies and SMA severity^{1,2,4,5}. The severe congenital phenotype known as SMA type 0 is a very rare presentation with most patients dying in the first weeks of life, and clinically characterized by hypotonia, arthrogryposis, bulbar dysfunction and non-neuromuscular manifestations such as congenital heart defects⁴. SMA type 1 also known as Werdnig-Hoffman disease is the most common form of 5q-SMA, and classically patients start clinical symptoms before 6 months of age and never acquire the ability to sit and dying by 2 years without intervention^{1,2}. SMA type 2 usually presents with onset between 7 and 18 months of age with patients achieve the ability to sit unsupported but they do not acquire the ability to walk independently^{1,2}. SMA type 3, also known as Kugelberg-Welander disease, is a more heterogeneous form with all patients reaching the ability to walking independently, with progressive proximal muscle weakness starting over 18 months of age^{1,2}.

SMA type 4 is a particularly rare condition with onset of symptoms over 18 years of age and a mild course of disease characterized by slowly progressive muscle weakness^{1,2}. In this study, we present detailed clinical, neurophysiological and radiological features in a cohort of 20 patients with SMA type 4.

2. Material and Methods

2.1 Patients Selection

This study reviewed medical records of a Brazilian cohort of 227 patients with SMA followed at Universidade Federal de São Paulo between 2000 and 2018 with 20 patients classified as SMA type 4 defined by a genetic documentation of 5q-SMA with symptom onset after 18 years of age. All patients gave written informed consent for participation in the study and for this publication, and study procedures were approved by institutional ethics committees. Only for neurophysiological evaluation and comparison, we select 20 gender- and age-matched healthy control and with 15 adult patients with SMA type 3. All data is available on request from the authors.

2.2 Patients Evaluation

All 20 patients are alive and were invited to a single medical interview performed in January of 2019 for reviewing clinical data, neurological examination, referral for muscle MRI and application of functional assessment scales. At the time of the clinical evaluation all patients were untreated with Nusinersen or other genetic modifying therapy.

2.3 Laboratory Analysis

Before the application of functional assessment scales, all patients were submitted to blood tests including complete blood counts, creatinine and urea, serum creatine kinase (CK) and liver enzymes.

2.4 Genetic Tests

Multiplex Ligation-dependent Probe Amplification (MLPA) using the SALSA MLPA kit P060-SMA was performed to analysis the number of copies of exon 7 and 8 of *SMN1* and *SMN2* genes according to manufacturer instructions. *SMN1* and *SMN2* sequencing was performed as previously described in the literature⁶⁻⁸.

2.5 Functional Assessment

All patients were evaluated with the following functional assessment scales: 1) Hammersmith Functional Motor Scale Expanded (HFMSE); 2) ALS Functional Rating Scale Revised (ALSFRS-R); 3) Revised Upper Limb Module (RULM); 4) Spinal Muscular Atrophy Functional Rating Scale (SMAFRS); 5) Fatigue Severity Score (FSS); 6) Six-Minute

Walking Test (6MWT); 7) Time Up and Go Test (TUGO) and 8) MRC Sum Score (MRC Score) derived from the analysis of 20 muscle groups of the upper and lower limbs.

The functional assessment was performed at January of 2019 in a single-visit and the proposed scales were applied by a neurologist with experience in Neuromuscular Disorders (PVSS) in conjunction with a trained physical therapist (DTT) in functional scales according to validated studies and international recommendations.

2.6 Muscle MRI

Muscle MRI of the lower limbs were performed in a 1.5T scanner with 4-channel phased-array coil (Magnetom Avanto; Siemens, Munich, Germany) using a standardized protocol with axial images (T1-weighted spin-echo [repetition time (TR)/echo time (TE) 335/19 ms, slice thickness 7mm, matrix 360-464x512], short tau inversion recovery [TR/TE 3,650/52 ms, inversion time 160 ms, slice thickness 7mm, matrix 480-576x512]).

All patients performed muscle MRI studies between March and June of 2019 and all studies were reviewed independently by two neuromuscular specialists (PVSS and WBVRP) with experience in radiological diagnostic methods in neuromuscular disorders.

Each muscle was examined throughout its whole extension using the Mercuri Classification for evaluation of fatty infiltration^{9,10}. At the thigh level, the muscles were grouped in anterior, posterior and medial compartment for global atrophy classification as previously described¹¹. Total Fatty Infiltration (TFI) score was calculated for each patient and defined as the sum of fatty infiltration in each muscle group of the lower limbs ranging from 0 to 108 and Total Atrophy Score (TAS) defined as the sum of muscle atrophy in the three muscles compartments at thigh level ranging from 0 to 9.

2.7 Neurophysiological Studies

All patients underwent Nerve Conduction Studies (NCS) and electromyography (EMG) between January and March of 2018 with NCS of bilateral median, radial, ulnar, peroneal, tibial and sural nerves and needle EMG in proximal and distal muscles of the upper and lower limbs and paravertebral regions using a standard technique¹². For Motor Unit Number Index (MUNIX) study we use the same methodology as previously described¹³ in the biceps brachii (BB), abductor pollicis brevis (APB), first dorsal interosseous (FDI) and abductor digiti minimi (ADM) in the right hands of 20 patients with SMA type IV. We also obtained additional neurophysiological indexes such as Split Hand Index Munix (SHI) and Preserved

Thenar Index (PTI) as previously described¹⁴ and a new index referred to as Total Hand Index Munix (THMI) defined by the sum of MUNIX score of APB, ADM and FDI muscles.

2.8 Statistical Analysis

The Shapiro-Wilk, D'Agostino and Kolmogorov-Smirnov (with Lilliefors adjustments) tests were performed to evaluate the normal distribution of the sample, and all variables proved to fit the normal distribution by at least one method. Student's *t*-test and the chi-squared test or the Fisher exact test were used for correlation of quantitative and qualitative variables, respectively. Correlations between qualitative variables were performed with Pearson correlation coefficient with a correlation coefficient of $r < 0.3$ considered as weak, $r = 0.3 - 0.59$ a moderate, and $r \geq 0.6$ a strong correlation. All quantitative variables are presented with the mean value ($\pm SD$, standard deviation). The software StatPlus® and Stata® 16.0 were used to statistical analysis, and two- sided $p < 0.05$ was considered statistically significant.

3. Results

3.1 Clinical and Laboratory Results

Twenty patients full-filled diagnostic criteria for SMA type 4, including 11 men and 9 women, and clinical and demographical data are summarized in Table 1. The median age at symptom-onset was 31.4 years (± 8.1), time for definitive genetic diagnosis was 12.4 years (± 7.3) and duration of disease was 16.0 years (± 7.3).

The most common clinical symptoms were proximal lower limb weakness reported by 15 (75%) patients, cramp-fasciculations in 4 (20%) patients and one case (5%) with asymptomatic hiperCKemia. The clinical suspicion, before first evaluation in our center, included Limb-Girdle Muscular Dystrophy in 12 (60%) patients, Amyotrophic Lateral Sclerosis in 4 (20%) patients, Inflammatory Myopathy in 2 (10%) patients and Chronic Inflammatory Demyelinating Polyradiculoneuropathy (10%) in 2 cases.

The most common neurological findings observed included absent tendon reflexes in 18 (90%) patients, fasciculations in thighs in 9 (45%), distal upper limb tremor in 8 (40%) and calf pseudohypertrophy in 6 (30%). Main neurological features are presented in Figure 1.

In this cohort, 1 patient (5%) is dependent of non-invasive mechanical ventilation at night, no patient required gastrostomy, and all patients remained ambulatory and able to walk without assistance. The most common laboratory abnormality was high CK levels present in 19/20

(95%) patients and the median of CK was 587.2 (± 239.4).

3.2 Genetic Results

Sixteen patients (80%) had the homozygous deletion of exon 7 in the *SMN1* gene, while 4 (20%) patients were compound heterozygous for a deletion of exon 7 and an intragenic variant in *SMN1* gene. In the group of heterozygous patients, two patients had the variant c.770_780dup (p.Gly261Leufs*8) and the other two patients harbored the variant c.5C>G (p.Ala2Gly).

Regarding *SMN2* allele copy number, 12 (60%) patients had 4 copies of *SMN2* alleles, 5 (25%) had 3 copies and 3 (15%) patients had 2 copies. Compound heterozygous cases were associated with 2 and 3 *SMN2* allele copies in this cohort. Six patients (30%) had the c.859G>C variant in exon 7 of *SMN2* gene that is indicated to be a positive modifier of phenotype¹⁵.

In this cohort, 18/20 (90%) were index patients in the family and 2 (10%) patients had a positive family history for Spinal Muscular Atrophy. Genetic findings for each patient are summarized in the Supplementary Material.

3.3 Functional Assessment

All patients performed the functional assessment included in the study design and the results are summarized in the table 2. The mean scores for functional scales were 44.80 (± 11.56) for HFMSE, 42.40 (± 4.58) for ALSFRS-R, 33.95 (± 6.08) for RULM, 6.03 (± 0.65) for FSS, 41.70 (± 6.85) for SMAFRS and 136.6 (± 18.7) for MRC Score. The mean distance in 6MWT was 478.65 meters (± 200.65) and the median time evaluated by TUGO was 8.39 seconds (± 3.48). Regarding fatigue, the mean score for FSS was 6.03 (± 0.650 with 14/20 (70%) patients had an FSS greater than or equal to 6.0.

There was a strong correlation between TUGO with duration of disease ($r=0.89$) and strong inverse correlation of duration of disease with MRC Score ($r=-0.97$), 6WT ($r=-0.95$), ALSFRS-R ($r=-0.92$), SMAFRS ($r=-0.74$), RULM ($r=-0.73$) and HFMSE ($r=-0.68$).

MRC Score has a strong positive correlation with ALSFRS-R ($r=0.93$), 6WT ($r=0.92$), RULM ($r=0.70$), SMAFRS ($r=0.71$) and HFMSE ($r=0.64$) and an inverse correlation with TUGO ($r=-0.88$).

Evaluation of the average distance covered in the first and sixth minute of the 6WT as a measure of fatigue was performed and applying a paired t-test there was a statistical difference ($t=11.42$, $p=0.00001$) between the median distance covered in the first (85.15m, 95% CI=70.20-100.09) and sixth (74.25m, 95% CI=57.42-91.07) minutes. The $\Delta D1-D6$, defined as the difference between the distance walked in the first and sixth minutes, has a strong positive correlation with duration of disease ($r=0.95$), an inverse correlation with MRC Score ($r=-0.92$), 6WT ($r=-0.94$), ALSFRS-R ($r=-0.85$), HFMSE ($r=-0.66$) and SMAFRS ($r=-0.65$) and did not correlate with FSS score.

3.4 Neurophysiological Study

In this study, 20/20 (100%) patients disclosed chronic denervation findings characterized by large-amplitude, long-duration and polyphasic motor unit action potentials in three spinal segments (cervical, thoracic and lumbosacral), 12/20 (60%) patients presented chronic denervation signals in bulbar segments (evaluated by electromyography of genioglossus muscle) and 3/20 (15%) patients had also acute denervation findings (fibrillation potentials, positive waves and fasciculations) in at least one segment. Neurophysiological results are summarized in table 2.

MUNIX score demonstrated an inverse strong correlation with duration of disease for FDI ($r=-0.97$), APB ($r=-0.97$), ADM ($r=-0.95$) e BB ($r=-0.94$) muscles. PTI had a positive correlation ($r=0.85$) and THMI an inverse correlation ($r=-0.99$) with duration of the disease.

PTI had an inverse correlation with 6WT ($r=-0.81$), ALSFRS-R ($r=-0.78$) and a positive correlation with TUGO ($r=0.65$). THMI exhibited strong positive correlation with 6WT ($r=0.94$), ALSFRS-R ($r=0.92$), SMAFRS ($r=0.72$), RULM ($r=0.72$), HFMSE ($r=0.66$) and an inverse correlation with TUGO ($r=-0.88$). BB MUNIX has positive correlation with 6WT ($r=0.92$), ALSFRS-R ($r=0.86$), RULM ($r=0.73$), SMAFRS ($r=0.66$), HFMSE ($r=0.64$) and inverse correlation with TUGO ($r=-0.80$).

MUNIX of BB, APB, ADM and FDI were significantly reduced in SMA patients compared to healthy controls and PTI in patients with SMA type 4 (1.06 ± 0.15) showed a highly significant difference to controls (0.52 ± 0.11 , $p<0.0001$) but did not differ from a series of 15 patients with SMA type 3.

3.5 Muscle MRI

Heatmap representing pattern and severity of thigh and leg muscle MRI involvement is

shown in Figure 2. All patients exhibited fatty degeneration of biceps femoris short head and gluteus minimus and preservation of posterior tibial, fibular and extensor digitorum longus muscles and 5/20 (25%) patients showed STIR (short tau inversion recovery) hyperintensity suggestive of muscle edema. Some examples of muscle MRI findings of patients with different degrees of severity are presented in Figure 3.

At the pelvis level, the median of fatty infiltration (FI) per patient was 4.95 (± 3.44) ranging between 2-14.5, and the more common fatty degenerated muscle was gluteus minimus with a median FI of 1.72 (± 0.93) and showing a score of 2 or higher in 9/20 (45%) patients. At the thigh level, the median of FI was 9.6 (± 6.12) ranging between 3-22.5, with biceps femoral short head exhibiting a median FI of 1.4 (± 0.68) and a score of 2 or higher in 6/20 (30%) patients. At the leg level, median FI was 1.85 (± 1.78) ranging from 0-6, with more common involvement of gastrocnemius medialis showing a median FI of 0.8 (± 0.69) and a score of 2 or higher in 3/20 (15%) patients.

The median of TFI scores was 16.4 (± 10.4 , ranged between 6 and 43) and for TAS measured at thigh level ranged between 0 and 7 (2.30, ± 2.34), atrophy score was higher in posterior compartment with a median of 1.2 (± 1.0) and less pronounced in medial compartment (mean 0.3 ± 0.57).

There was a positive correlation between duration of disease with TAS ($r=0.64$) and TFI ($r=0.60$), an inverse correlation of TFI with 6WT ($r=-0.67$), RULM ($r=-0.57$), ALSFRS-R ($r=-0.55$), SMAFRS ($r=-0.53$) and HFMSE ($r=-0.51$); and, a strong inverse correlation of TAS with 6WT ($r=-0.68$), SMAFRS ($r=-0.67$) and ALSFRS-R ($r=-0.66$). All correlations are summarized in table 3.TFI had no correlation with the patient's age.

4. Discussion

SMA type 4 still represents an underdiagnosed presentation of Motor Neuron Disease in adults and the rarest clinical subtype of 5q-SMA, being even frequently questioned by some authors about its diagnostic criteria and definition and only rarely included in international patient database and registries^{1,16-19}. SMA type 4 is a complex neurodegenerative disorder that can be overlooked and has a wide differential diagnosis with others adult-onset neurogenic and myopathic disorders²⁰⁻²². In our sample, most cases had been previously misdiagnosed in other centers as myopathic disorders (Limb-Girdle Muscular Dystrophy) and neurogenic conditions (like sporadic young-onset or juvenile ALS), reflecting a poor

awareness of most neurologists about adult-onset SMA, with the wrong current belief that 5q-SMA is a disease that generally affects subjects in the pediatric age. It is not surprising to find a mean value of 12.4 years for definitive diagnosis of SMA type 4 in our cohort with an average consultation of 7 different medical specialists before the final diagnosis was reached, largely far from much shorter delay that seems to occur in other subtypes²³.

There are no specific natural history studies regarding SMA type 4 in the literature with most current knowledge obtained by small case series²⁴⁻²⁸. In this cohort, the median age at onset of the symptoms was 31.4 years, which is similar to previous series described in the literature that indicate the onset of the disease occurring between 30-40 years of age^{1,24,26,27}. The most common initial clinical complaint was slowly progressive symmetrical proximal weakness in the lower limbs reported by 75% of patients with preferential involvement of quadriceps, iliopsoas and adductors muscles according to other cases reported²⁴⁻²⁷. In one patient the first symptom was hiperCKemia with two-year duration preceding muscle weakness and this finding has never been described in the literature, representing an expansion of the phenotype for 5q-SMA. The neurological pattern of arreflexia, calf pseudohypertrophy and hand tremor has been observed with similar incidence to that reported in other cohorts of SMA type 4 patients^{1,24-28}.

Bulbar dysfunction and chronic respiratory insufficiency were not prominent features in this cohort with tongue atrophy and fasciculations occurring in less than a quarter of patients but with preserved tongue movements. We had only one patient exhibiting significant reduction in FVC (forced vital capacity) requiring intermittent non-invasive positive pressure ventilation due to respiratory muscle weakness as similar described in the literature that respiratory involvement is very rare in SMA type 4²⁸.

Previous descriptions of SMA type 4 in the literature were associated with increased copy number of *SMN2* gene, mainly with four to six copies resulting from gene conversion and linked to homozygous deletion of *SMN1* gene^{18,30}. In this study, we describe four patients with compound heterozygous variants in *SMN1*, presenting with deletion of exon 7 in one allele and other pathogenic variant in the second allele, the known pathogenic variants c.5C>G (p.Ala2Gly) and c.770_780dup (p.Gly261Leufs*8)] were associated with reduced *SMN2* copy number than in classical descriptions for SMA type 4^{18,27,28,30}, and carrying the c.859G>C variant at *SMN2* that has been associated with milder phenotypes¹⁵. From this experience, we can speculate that these previously reported *SMN1* variants seem to act as

hypomorphic alleles, together with the known c.859G>C modifier in *SMN2*, and should be added to the array of additional genetic and molecular factors that modulate clinical course and severity in SMA patients^{15,18,31,32}.

The natural history of motor impairment characterized by progressive proximal weakness in the lower limbs with a preferential pattern of involvement of adductor and quadriceps sparing distal muscles in the upper and lower limbs, with most patients able to walk without support resembles the features of adult patients with SMA described in other studies^{27,33}.

Fatigue is a major problem in patients with SMA with recent studies showing that endurance shuttle tests are good instruments for access fatigue in this population³⁴; we demonstrated that the $\Delta D1-D6$ is an interesting index for evaluate fatigue in SMA type 4 with a good correlation with duration of disease and functional scales specific for SMA patients like HFMSE as previously described for ambulatory patients with SMA type 3^{35,36}.

Our results, which are similar to those of other studies supports the use of MUNIX as an important neurophysiological biomarker for the evaluation of motor neuron diseases and reinforce the data that SMA patients present with predominant involvement of ADM and FDI muscles (“reverse split hand”), in a different pattern that occurs in Amyotrophic Lateral Sclerosis that is invariable mentioned as the main differential diagnosis for motor neuron disease in adulthood³⁷.

This study also proves that MUNIX is a reliable biomarker for disease severity in patients with SMA type 4 since the main neurophysiological indexes correlate with the duration of disease, and the scores on the functional scales as well, following what has been recently described for SMA and other motor neuron diseases³⁷⁻³⁹.

Muscle MRI is now established as a diagnostic tool and biomarker for neuromuscular disorders, with only few studies performed in patients with SMA^{11,40,41}. In this cohort, the anterior compartment at the thigh level was commonly involved, and the medial compartment was frequently spared, which is similar to a large cohort of patients with SMA type 2 and 3 described in a previous study but showing a different signature in the posterior compartment with common involvement of biceps femoris (short head) and adductor magnus, in contrast to semitendinosus involvement of patients with SMA type 2 and 3¹¹. At the leg level, the soleus involvement was similar to what is described in patients with other types of SMA, and the gastrocnemius (in particular medial gastrocnemius) was more affected than previously described for SMA patients, and similar to what observed in patients with primary muscle

disorders¹¹. The texture appearance is similar to what is described for other patients with SMA and neurogenic disorders as an abnormal signal distributed in “small islands” or “reticular pattern” and in this study the patients exhibited more proximal than distal involvement. This is similar to other descriptions in ambulatory patients with SMA type 3, and the fatty replacement correlates with disease duration instead of patient’s age and maybe it can be a good biomarker for disease progression like in primary muscle disorders¹¹ and in addition with structural measures of spinal cord and brain MRI that have been recently reported as helpful tools for evaluate adult SMA patients⁴².

5. Conclusions

This article sheds light on a little-recognized and questionable phenotype of SMA expanding the idea that the disease recognized as SMA is nowadays a broad and non-homogenous condition which is constantly changing. Our study reinforces the concept of a disease as a spectrum of neuromuscular manifestations that are not restricted to pediatric age, and that are potentially treatable disorders also in adult patients, affected by a neuromuscular disorder that frequently lacks early detection.

We have also systematically assessed the motor function of SMA type 4 patients, using several functional scales, some of which we have adopted and validated for patients with SMA to better characterize the degree of motor impairment, and to apply for them for natural course studies in this rare subgroup of SMA, providing several new information for future studies about measuring efficacy of recently approved specific therapies replacing SMN^{43,44}.

Disclosure of conflict of interest: none.

Acknowledgements: this research has received no funding sources to report.

Accepted Article

Legend to Figures

Figure 1. Examination findings in patients with SMA type 4: (A,B; Pt.9) mild involvement of the upper limbs and marked bilateral calf pseudohypertrophy; (C; Pt.17) mild amyotrophy involving the upper limbs; (D; Pt.5) marked amyotrophy involving the shoulder girdle; (E,F; Pt.18) proximal involvement of the proximal portions of the upper and lower limbs and mild winged scapula; (G,H,I; Pt.4) moderate amyotrophy involving anterior and posterior compartment of the lower limbs; (J; Pt.9) severe amyotrophy (mildly asymmetric) involving the thighs.

Figure 2. Heatmap muscle MRI of the lower limbs showing fatty replacement according to Mercuri score (represented by colors) for each muscle group (R: right; L: left) and patient.

Figure 3. Muscle MRI studies from patients with SMA type 4. Patients with different degrees of severity of fatty replacement: (A-E) mild, (F-K) and (L-O) mild to moderate, and (P-T) severe and end-stage muscle involvement. Example 1 (Pt.4): Axial muscle MRI study of the lower limbs showing mild diffuse and mild amyotrophy and fatty replacement in T1-weighted imaging (A-C) and without muscle edema (D) in STIR sequence. Example 2 (Pt.5):

Axial muscle MRI study of the lower limbs showing mild to moderate amyotrophy and fatty replacement in T1-weighted imaging (F,H,I,K) and without muscle edema (G,J) in STIR sequence. Example 3 (Pt.10): A similar pattern is also seen in T1-weighted imaging (L,M,O) and without muscle edema in STIR sequence (N). Example 4 (Pt.9): Axial muscle MRI study of the lower limbs showing diffuse and severe fatty replacement and amyotrophy in T1-weighted sequences (P,Q,S) and without muscle edema in STIR sequences (R). Coronal muscle MRI studies showing mild diffuse involvement (E; Pt.4) and severe fatty replacement (T; Pt.9) in T1-weighted imaging.

References

1. D'Amico A, Mercuri E, Tizano FD, et al. Spinal muscular atrophy. *Orphanet J Rare Dis* 2011;6:71.
2. Talbot K, Tizzano EF. The clinical landscape for SMA in a new therapeutic area. *Gene Ther* 2017;24(9):529-533.
3. Sugarman EA, Nagan N, Zhu H, et al. Pan-ethnic carrier screening and prenatal diagnosis for spinal muscular atrophy: clinical laboratory analysis of >72,400 specimens. *Eur J Hum Genet* 2012;20(1):27-32.
4. Grotto S, Cuisset JM, Marret S, et al. Type 0 Spinal Muscular Atrophy: Further Delineation of Prenatal and Postnatal Features in 16 patients. *J Neuromuscul Dis* 2016;3(4):487-495.
5. Calucho M, Bernal S, Alías L, et al. Correlation between SMA type and SMN2 copy number revisited: An analysis of 625 unrelated Spanish patients and a compilation of 2834 reported cases. *Neuromuscul Disord* 2018;28(3):208-215.
6. Clermont O, Burlet P, Benit P, et al. Molecular analysis of SMA patients without homozygous SMN1 deletion using a new strategy for identification of SMN1 subtle mutations. *Hum Mutat* 2004;24(5):417-427.
7. Alías L, Bernal S, Fuentes-Prior P, et al. Mutation update of spinal muscular atrophy in Spain: molecular characterization of 745 unrelated patients and identification of four novel mutations in the SMN1 gene. *Hum Genet* 2009;125(1):29-39.
8. Ruhno C, McGovern VL, Avenarius MR, et al. Complete sequencing of the SMN2 gene in SMA patients detects SMN gene deletion junctions and variants in SMN2 that modify the SMA phenotype. *Hum Genet* 2019;138(3):241-256.
9. Mercure E, Lampe A, Allsop J, et al. Muscle MRI in Ulrich congenital muscular dystrophy and Bethlem myopathy. *Neuromuscul Disord* 2005;15:303-310.
10. Mercuri E, Pichiecchio A, Counsell S, et al. A short protocol for muscle MRI in children with muscular dystrophies. *Eur J Paediatr Neurol* 2002;6:305-307.
11. Brogna C, Cristiano L, Verdolotti T, et al. MRI patterns of muscle involvement in type 2 and 3 spinal muscular atrophy patients. *J Neurol* 2020;267(4):898-912.
12. Pinto WBVR, Naylor FGM, Chieia MAT, et al. New findings in facial-onset sensory and motor neuronopathy (FOSMN) syndrome. *Rev Neurol (Paris)* 2019;175(4):238-246.

- Accepted Article
13. Nandedkar SD, Barkhaus PE, Stålberg EV, et al. Motor unit number index: guidelines for recording signals and their analysis. *Muscle Nerve* 2018;58(3):374-380.
 14. Günther R, Neuwirth C, Koch JC, et al. Motor Unit Number Index (MUNIX) of hand muscles is a disease biomarker for adult spinal muscular atrophy. *Clin Neurophysiol* 2019;130(2):315-319.
 15. Bernal S, Alías L, Barceló MJ, et al. The c.859G>C variant in the *SMN2* gene is associated with types II and III SMA and originates from a common ancestor. *J Med Genet* 2010;47(9):640-642.
 16. Arnold WD, Kassar D, Kissel JT. Spinal muscular atrophy: diagnosis and management in a new therapeutic era. *Muscle Nerve* 2015;51(2):157-167.
 17. Dubowitz V. Spinal Muscular Atrophy revisited. *Neuromusc Disord* 2019;29(6):413-414.
 18. Wirth B, Brichta L, Schrank B, Lochmuller H, Blick S, Baasner A, et al. Mildly affected patients with spinal muscular atrophy are partially protected by an increased *SMN2* copy number. *Hum Genet* 2006;119(4):422-428.
 19. Belter L, Cook SF, Crawford TO, et al. An overview of the Cure SMA membership database: highlights of key demographic and clinical characteristics of SMA members. *J Neuromuscul Dis* 2018;5(2):167-176.
 20. Juntas Morales R, Pageot N, Taieb G, Camu W. Adult-onset spinal muscular atrophy: an update. *Rev Neurol (Paris)* 2017;173(5):308-319.
 21. Peeters K, Chamova T, Jordanova A. Clinical and genetic diversity of SMN1-negative proximal spinal muscular atrophies. *Brain* 2014;137:2879-2896.
 22. Rossor AM, Kalmar B, Greensmith L, et al. The distal hereditary motor neuropathies. *J Neurol Neurosurg Psychiatry* 2012;83(1):6-14.
 23. Lin CW, Kalb SJ, Yeh WS. Delay in diagnosis of Spinal Muscular Atrophy: a systematic literature review. *Pediatr Neurol* 2015;53:293-300.
 24. Pearn JH, Hudgson P, Walton JN. A clinical and genetic study of spinal muscular atrophy of adult onset: the autosomal recessive form as a discrete disease entity. *Brain* 1978;101(4):591-606.
 25. Brahe C, Servidei S, Zappata S, et al. Genetic homogeneity between childhood-onset

- and adult-onset autosomal recessive spinal muscular atrophy. Lancet 1995;346(8977):741-742.
26. Clermont O, Burlet P, Lefebvre S, et al. SMN gene deletions in adult-onset spinal muscular atrophy. Lancet 1995;346(8991-8992):1712-1713.
27. Wadman RI, Wijngaarde CA, Stam M, et al. Muscle strength and motor function throughout life in a cross-sectional cohort of 180 patients with spinal muscular atrophy types 1c-4. Eur J Neurol 2018;25(3):512-518.
28. Piepers S, van den Berg LH, Brugman F, et al. A natural history study of late onset spinal muscular atrophy types 3b and 4. J Neurol 2008;255(9):1400-1404.
29. Chabanon A, Seferian AM, Daron A, et al. Prospective and longitudinal natural history study of patients with Type 2 and 3 spinal muscular atrophy: Baseline data NatHis-SMA study. PLoS One 2018;13(7):e0201004.
30. Mazzei R, Gambardella A, Conforti FL, et al. Gene conversion events in adult-onset spinal muscular atrophy. Acta Neurol Scand 2004;109(2):151-154.
31. Maretina MA, Zheleznyakova GY, Lanko KM, et al. Molecular factors involved in Spinal Muscular Atrophy pathways as possible disease-modifying candidates. Curr Genomics 2018;19(5):339-355.
32. Zheleznyakova GY, Voisin S, Kiselev AV, et al. Genome-wide analysis shows association of epigenetic changes in regulators of Rab and Rho GTPases with spinal muscular atrophy severity. Eur J Hum Genet 2013;21(9):988-993.
33. Wadman RI, Stam M, Gijzen M, et al. Association of motor milestones, SMN2 copy and outcome in spinal muscular atrophy types 0-4. J Neurol Neurosurg Psychiatry 2017;88(4):365-367.
34. Bartels B, de Groot JF, Habets LE, et al. Fatigability in Spinal Muscular Atrophy: validity and reliability of Endurance Shuttle Tests. Orphanet J Rare Dis 2020;15(1):75.
35. Montes J, McDermott MP, Martens WB, et al. Six-minute walking test demonstrates motor fatigue in Spinal Muscular Atrophy. Neurology 2010;74(10):833-838.
36. Montes J, Young SD, Mazzone ES, et al. Nusinersen improves walking distance and

reduces fatigue in later-onset Spinal Muscular Atrophy. *Muscle Nerve* 2019;60(4):409-414.

37. Günther R, Neuwirth C, Koch JC, et al. Motor Unit Number Index (MUNIX) of hand muscles is a disease biomarker for adult spinal muscular atrophy. *Clin Neurophysiol* 2019;130(2):315-319.
38. Querin G, Lenglet T, Debs R, et al. The motor unit number index (MUNIX) profile of patients with adult spinal muscular atrophy. *Clin Neurophysiol* 2018;129(11):2333-2340.
39. Fatehi F, Grapperon AM, Fathi D, et al. The utility of motor unit number index: a systematic review. *Clin Neurophysiol* 2018;48(5):251-259.
40. Liu GC, Jong YJ, Chiang CH, et al. Spinal muscular atrophy: MR evaluation. *Pediatr Radiol* 1992;22(8):584-586.
41. Mercuri E, Pichiecchio A, Allsop J, et al. Muscle MRI in inherited neuromuscular disorders: past, present and future. *J Magn Reson Imaging* 2007;25:433-440.
42. Querin G, El Mendili M, Lenglet T, et al. The spinal and cerebral profile of adult spinal-muscular atrophy: a multimodal imaging study. *Neuroimage Clin* 2019;21:101618.
43. Ramdas S, Servais L. New treatments in spinal muscular atrophy: an overview of currently available data. *Expert Opin Pharmacother* 2020;21(3):307-315.
44. Wirth B, Karakaya M, Kye MJ, et al. Twenty-five years of spinal muscular atrophy research: from phenotype to genotype to therapy, and what comes next. *Annu Rev Genomics Hum Genet* 2020 [Epub ahead of print]; doi:10.1146/annurev-genom-102319-103602.

Table 1. Clinical Features of patients with SMA type 4.

Patients	General Data		Onset of symptoms			Clinical Features								
	Gender	Current Age (years)	Age at Onset (years)	First symptom	First Clinical Diagnosis	Cervical weakness	Proximal weakness	Distal weakness	Distal Tremor	Fasciculations	Arreflexia	CPH	Tongue Atrophy	Other
Pt#01	M	67	36	W	LGMD	No	LL / UL	LL / UL	Yes	Yes	Yes	No	No	Respiratory Insufficiency
Pt#02	M	59	51	CF	ALS	No	LL / UL	UL	No	Yes	Yes	No	Yes	Dysphagia
Pt#03	F	48	35	W	CIDP	No	LL / UL	No	Yes	No	Yes	No	No	Pes cavus
Pt#04	F	51	36	W	LGMD	No	LL / UL	No	No	No	Yes	No	No	Scoliosis
Pt#05	M	47	24	W	LGMD	Yes	LL / UL	No	No	Yes	Yes	Yes	No	Scoliosis
Pt#06	F	39	26	W	IM	No	LL / UL	No	Yes	No	Yes	No	No	Myalgia
Pt#07	F	38	30	W	LGMD	No	LL	No	No	No	Yes	No	No	--
Pt#08	F	45	24	W	LGMD	No	LL / UL	UL	Yes	No	Yes	No	No	Myalgia
Pt#09	F	43	25	W	LGMD	Yes	LL / UL	No	No	Yes	Yes	Yes	No	--
Pt#10	F	54	36	W	LGMD	No	LL / UL	No	No	Yes	Yes	No	No	Myalgia
Pt#11	M	52	38	W	LGMD	No	LL / UL	No	No	No	Yes	Yes	No	--
Pt#12	M	39	23	CF	ALS	No	LL / UL	No	Yes	Yes	Yes	No	Yes	Dysphagia
Pt#13	F	49	30	W	LGMD	No	LL / UL	No	Yes	No	Yes	No	No	--
Pt#14	M	69	40	W	ALS	No	LL / UL	LL	No	No	No	No	Yes	Scoliosis
Pt#15	M	53	40	W	LGMD	No	LL / UL	No	No	No	Yes	Yes	No	--
Pt#16	M	57	40	W	CIDP	Yes	LL / UL	No	Yes	Yes	Yes	No	No	Pes cavus
Pt#17	M	31	23	CF	ALS	No	LL	No	No	No	Yes	No	No	Dysphagia
Pt#18	M	33	22	CF	LGMD	No	LL	No	No	Yes	Yes	Yes	No	--
Pt#19	F	55	29	W	IM	Yes	LL / UL	LL	Yes	Yes	Yes	No	No	Scoliosis

Pt#20	M	24	21	Elevated CPK	LGMD	No	LL	No	No	No	Yes	No	Myalgia
-------	---	----	----	-----------------	------	----	----	----	----	----	-----	----	---------

Legends to the Table 1. ALS: Amyotrophic Lateral Sclerosis. CF: Cramp-Fasciculations. CIDP: Chronic Inflammatory Demyelinating Polyneuropathy. CPH: calf pseudohypertrophy. CPK: serum creatine kinase. F: Female. IM: Inflammatory Myopathy. LGMD: Limb-Girdle Muscular Dystrophy. LL: lower limb. M: Male. UL: upper limb. W: weakness

Table 2. Summary of Functional Scales and Neurophysiological Results

Functional Scale	Median (range)		
HFMSE	44.8 (22-63)		
ALSFRS-R	42.4 (31-48)		
RULM	33.9 (21-43)		
SMAFRS	41.7 (31-50)		
FSS	6.0 (4.5-6.8)		
6WT	478.6m (105-804)		
ΔD1-D6	10.9m (2-18)		
TUGO	8.3s (4.5-16.3)		
MRC Sum Score	136.6 (96-160)		
Neurophysiological Studies			
Neurophysiological Parameters	SMA type 4	SMA type 3	Controls
BB CMAP	8.2mV (± 1.7)	8.3mV (1.0)	9.6mV (± 2.1)
BB MUNIX	67.5 (± 29.4)	72.8 (± 23.2)	247.9 (± 62.9)
APB CMAP	9.5mV (± 1.08)	9.4mV (± 0.7)	10.7 (± 2.0)
APB MUNIX	164.9 (± 38.3)	168.9 (± 30.4)	224.0 (± 38.1)
ADM CMAP	7.6mV (± 1.7)	7.5mV (± 1.7)	10.4mV (± 1.1)
ADM MUNIX	70.1 (± 28.7)	71.7 (± 20.7)	213.4 (± 39.9)
FDI CMAP	8.1mV (± 2.6)	8.0mV (± 0.8)	12.2mV (± 1.4)
FDI MUNIX	91.1 (± 27.9)	89.6 (± 18.5)	221.0 (± 30.9)
SHI	222.8 (± 54.2)	235.5 (± 52.1)	234.3 (± 44.1)

PTI	1.06 (± 0.15)	1.07 (± 0.11)	0.52 (± 0.1)
-----	---------------------	---------------------	--------------------

Legends to the Table 2. ADM: abductor digiti minimi. APB: abductor pollicis brevis. ALSFRS-R: Amyotrophic Lateral Sclerosis Functional Rating Scale Revised. BB: biceps brachii. FDI: first dorsal interosseous. FSS: Fatigue Severity Scale. HFMSE: Hammersmith Functional Motor Scale Expanded. MUNIX: Motor Unit Number Index. PTI: Preserved Thenar Index. RULM: Revised Upper Limb Module. SHI: Split Hand Index Munix. SMAFRS: Spinal Muscular Atrophy Functional Rating Scale. 6WT: Six-Minute Walking Test.

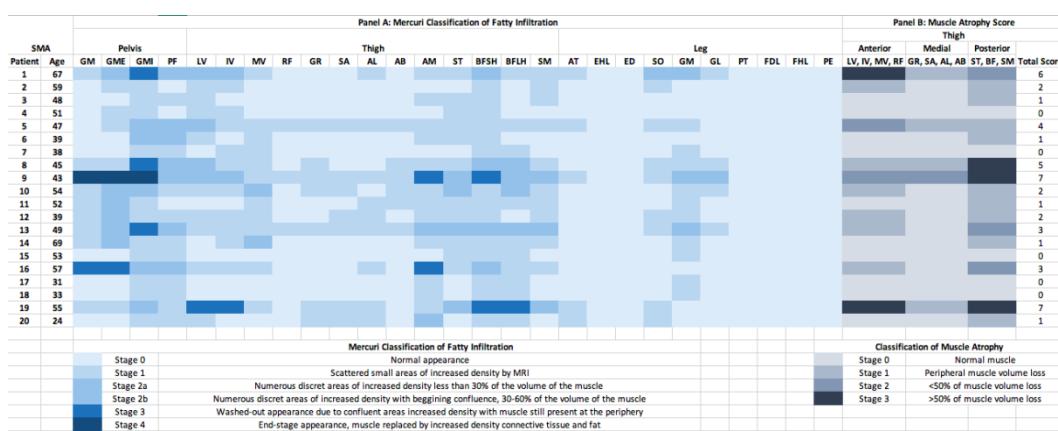
Table 3. MRI Measures and Correlations

MRI Measures	Correlation Coefficient (r)
TFI vs Duration of Disease	0.60 (p=0.005)
TAS vs Duration of Disease	0.64 (p=0.001)
TFI vs 6WT	-0.67 (p=0.001)
TFI vs RULM	-0.57 (p=0.007)
TFI vs ALSFRS-R	-0.55 (p=0.01)
TFI vs HFMSE	-0.51 (p=0.01)
TFI vs SMAFRS	-0.53 (p=0.01)
TFI vs TUGO	0.48 (p=0.03)
TFI vs ΔD1-D6	0.65 (p=0.001)
TFI vs MRC Sum Score	-0.60 (p=0.004)
TAS vs 6WT	-0.68 (p=0.0009)
TAS vs SMAFRS	-0.67 (p=0.001)
TAS vs ALSFRS-R	-0.66 (p=0.001)
TAS vs MRC Sum Score	-0.64 (p=0.002)
TAS vs HFMSE	-0.50 (p=0.02)
TAS vs RULM	-0.47 (p=0.03)
TAS vs ΔD1-D6	0.61 (p=0.003)
TAS vs TUGO	0.54 (p=0.01)

Legends to the Table 3. ALSFRS-R: Amyotrophic Lateral Sclerosis Functional Rating Scale Revised. HFMSE: Hammersmith Functional Motor Scale Expanded. RULM: Revised Upper Limb Module. SMAFRS: Spinal Muscular Atrophy Functional Rating Scale. TAS: Total Atrophy Score. TFI: Total Infiltration Score. TUGO: Time Up and Go Test. 6WT: Six-Minute Walking Test.

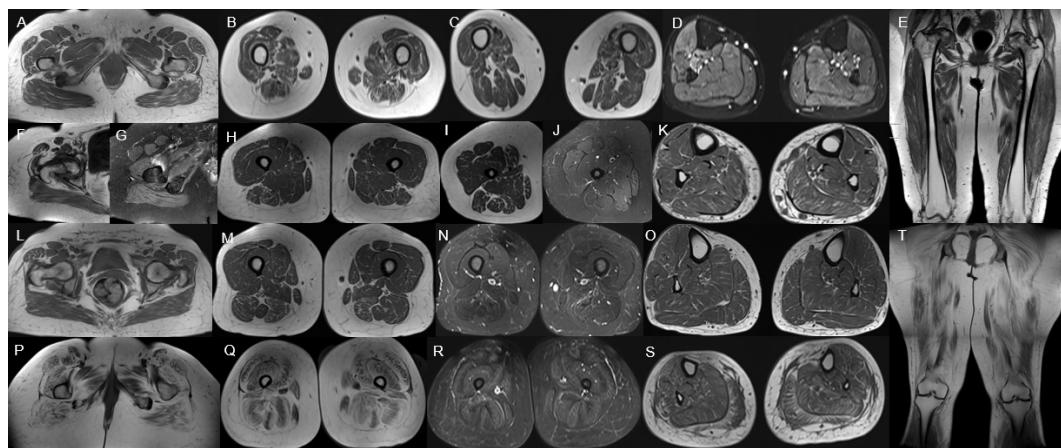


ene_14587_f1.tif



ene_14587_f2.tif

Accepted Article



ene_14587_f3.tif