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# Quantification of muscle involvement by MRI in familial amyloid polyneuropathy

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## **Abstract**

### ***Objective:***

To quantify using magnetic resonance imaging (MRI) fat infiltration (FF) and the magnetization transfer ratio (MTR) in individual muscles of symptomatic and asymptomatic transthyretin familial amyloid polyneuropathy (TTR-FAP) patients. Secondly, we aimed to assess correlations with clinical and electrophysiologic variables.

### ***Methods:***

39 patients with a confirmed mutation in the TTR gene (25 symptomatic and 14 asymptomatic) and 14 healthy volunteers were included. A total of 16 muscles were manually delineated on the nondominant lower limb from T1-weighted anatomical images. The corresponding masks were propagated on the MTR and FF maps. Detailed neurologic and electrophysiologic examinations were conducted in each group.

### ***Results:***

MTR was decreased (42.6 AU,  $p=0.001$ ) and FF was increased (14%,  $p=0.003$ ) in the lower limb of the symptomatic group with a preferential posterior and lateral involvement. In the asymptomatic group, an increased FF was quantified in the gastrocnemius lateralis muscle (11%,  $p=0.021$ ). FF was significantly correlated with disease duration ( $r=0.49$ ,  $p=0.015$ ), NIS-LL ( $r=0.42$ ,  $p=0.041$ ), ONLS ( $r=0.49$ ,  $p=0.013$ ), PND ( $r=0.57$ ,  $p=0.03$ ) and the sum of CMAP ( $r=0.52$ ,  $p=0.009$ ). MTR was strongly correlated to FF ( $r=0.78$ ,  $p<0.0001$ ), and a few muscles with a FF within the normal range displayed a reduced MTR.

### ***Conclusion:***

These observations suggest that FF and MTR could be interesting biomarkers in TTR-FAP. In asymptomatic patients, the FF in gastrocnemius lateralis muscle could be used as a good indicator of the transition from an asymptomatic to a symptomatic form of the disease. Of interest, MTR could be an early biomarker of muscle alterations.

## Introduction

Transthyretin familial amyloid polyneuropathy (TTR-FAP) is a rare genetic disease with autosomal-dominant inheritance. TTR-FAP rapidly progresses, characterized by axonal, length-dependent polyneuropathy, primarily affecting the small fibers of the autonomic nervous system<sup>1 2</sup>. The onset age, manifestation, and disease progression are highly variable, influenced by the type of TTR gene variant and the patient's ethnicity<sup>3 4</sup>. In the absence of therapy, the progression is rapid and leads to death in about ten years<sup>5</sup>. Early diagnosis is crucial, as various effective therapies are available<sup>6 7</sup> some of which are under trial with promising preliminary results<sup>8 9</sup>.

The diagnosis of hereditary transthyretin amyloidosis is based on the genetic study aiming at detecting amyloidogenic variants in TTR gene. Supplementary tests such as nerve conduction measurements using electroneuromyography (EDX), clinical scoring (Peripheral Neuropathy Disability score, Neuropathy Impairment Score, Medical Research Council testing, Overall Neuropathy Limitations Scale, Rasch-Built Overall Disability Scale) aid in evaluating the disease stage of TTR-FAP, initiating treatment in asymptomatic patients<sup>10</sup> and monitoring therapeutic efficiency<sup>11 12</sup>. Despite their utility, these tools have recognized limitations in their sensitivity<sup>13 14</sup>.

Recent studies have demonstrated the potential of MRI in detecting and quantifying nerve damage in patients<sup>15,16</sup>. Using T2-weighted MRI measurements, alterations in large-caliber<sup>17</sup> (tibial and fibular nerves) and small-caliber<sup>18</sup> (sural nerve) nerves have been reported in asymptomatic TTR subjects indicating a prognostic value of MRI. Similarly, MRI has been used to assess muscle changes in patients with a range of neuromuscular pathologies<sup>19 20 21 22</sup>. These muscle damages due to denervation have been linked to alterations in the magnetization transfer ratio (MTR, reflecting exchanges between water and macromolecules), fatty infiltration, and muscle volume (i.e., atrophy). These MRI quantitative indices of muscle lesions secondary to denervation could provide valuable measures of neuropathic degeneration progress. This quantitative MRI approach's usefulness has also been documented in Charcot Marie Tooth disease where leg fatty infiltration correlated strongly with clinical scores<sup>23,24</sup>. However, a similar muscular quantitative approach has not yet been employed for TTR-FAP to our knowledge.

The main objective of our study was to quantify and characterize potential MRI abnormalities in individual muscles of TTR-FAP patients. We aimed to integrate a MRI quantitative approach and a dedicated segmentation method to quantify both fat fractions and MTR values in symptomatic and asymptomatic TTR-FAP patients and investigate their potential correlations with clinical scores.

## **Patients and Methods**

### ***Clinical assessment***

Thirty-nine patients with a confirmed mutation within the TTR gene were enrolled in the Reference Center for Neuromuscular Disease and ALS (Marseille-France).

Each patient's clinical assessment comprised demographic details, medical history, type of TTR-mutation, disease duration (measured as the time between the first symptoms and the date of the MRI scan), and details of previous and ongoing therapies. A neurologist specializing in neuromuscular diseases (E.D) carried out the neurologic examinations, which included testing of motor and sensory functions in each limb. Peripheral neuropathy was scored using the Overall Neuropathy Limitations Scale (ONLS), the Rasch-Built Overall Disability Scale (RODS), and the neuropathy impairment score for the lower limb (NIS-LL). The severity of the disease was assessed on the basis of the Polyneuropathy Disability Score (PND).

Additionally, patients underwent electrophysiological assessment for each limb, which included measurements of the distal motor latency of the peroneal, tibial, median, and ulnar nerves; nerve conduction velocity in the peroneal, tibial, sural, median, and ulnar nerves; sensory nerve action potential (SNAP) amplitude of the sural, median, and ulnar nerves; and amplitude of the compound muscle action potential (CMAP) of the peroneal, tibial, median, and ulnar nerves. Because of the typically very low or non-assessable motor amplitudes in the lower limbs of symptomatic patients due to axonal loss, we calculated the sum of CMAP amplitudes in both lower limbs and considered it as a reflection of the nervous function. Autonomic dysfunction was assessed by testing the sympathetic skin response, electrochemical skin conductance (sudscan values), and the variation of the R-R interval during deep breathing.

Following this initial evaluation, patients were categorized as either symptomatic or asymptomatic carriers. Patients were considered asymptomatic if they exhibited a normal clinical examination, scores (ONLS, RODS, and NIS-LL) within the normal range and normal electrophysiological assessment. Symptomatic patients were defined the presence of functional complaints related to the disease and at least one abnormal test result in a complementary exam. The control group was made up of individuals with no previous medical history of neuromuscular disorder.

### ***MRI protocol***

Subjects were positioned supine within the scanner and the non-dominant lower limb was scanned at 1.5T (Avanto Siemens-Healthineers). After a set of localization images, 2D anatomical T1-weighted, 3D gradient echo multi-echo, and 3D gradient echo sequences were performed in the transverse plane over a 20-cm central area at the level of the thigh and leg. The corresponding parameters are summarized in Table 1 in the Appendix.

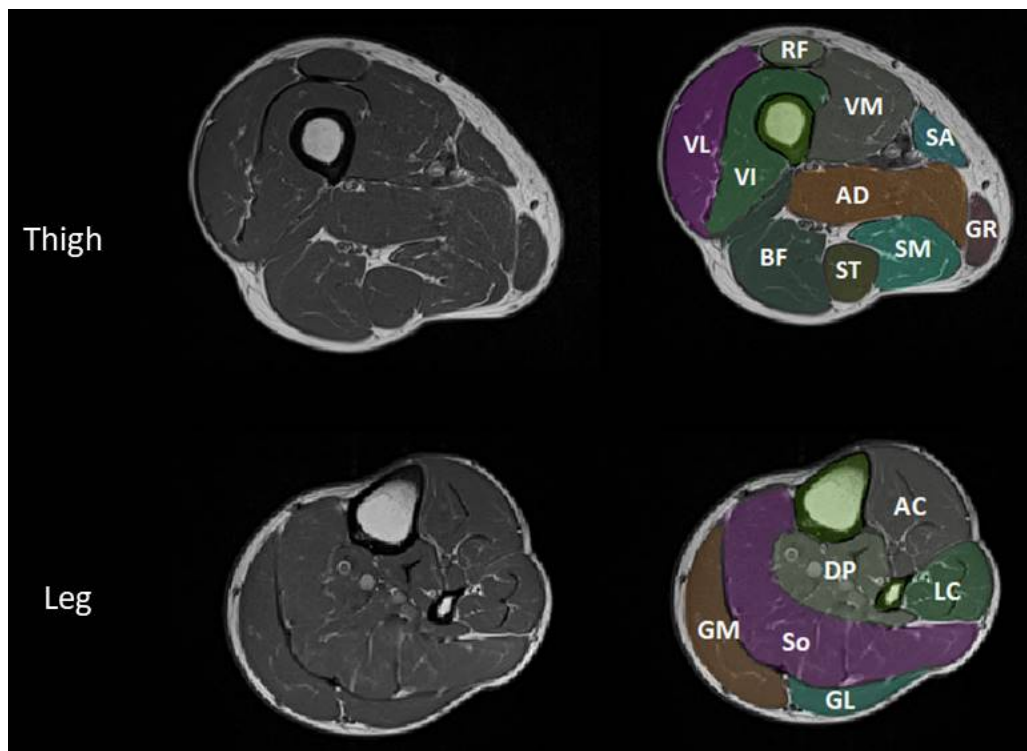
As previously described<sup>22 24</sup>, the 3D gradient echo multi-echo dataset was used in order to generate fat fraction (FF) maps while the magnetization transfer rate (MTR) map was computed from the normalized ratio of an image acquired with and without saturation and after correction for B1 field inhomogeneities<sup>25</sup>.

### ***Segmentation protocol***

Using FSleyes (FSL, FMRIB Software Library, Oxford, UK) 16 muscles (10 at the thigh level and 6 for the lower-leg) were manually delineated in a limited number of slices (3 to 4 on average) from T1-weighted images. Rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM), vastus intermedius (VI), sartorius (SA), gracilis (GR), semitendinous (ST), semimembranous (SM), biceps femoris (BF) and long adductor (AD) were delineated at the thigh level while anterior muscle compartment (AC = tibialis anterior, extensor hallucis longus, extensor digitorum longus, and fibularis tertius), deep posterior compartment (DP = tibialis posterior, flexor hallucis longus, flexor digitorum longus, and popliteus), lateral compartment (LC = fibularis longus and brevis), soleus (So), gastrocnemius lateralis (GL) and medialis (GM) were delineated at the lower-leg level (figure 1). These masks were then automatically propagated to the remaining unsegmented

slices as previously described <sup>26 27</sup>. Based on resampling and registration processes, the resulting masks were propagated to the quantitative maps i.e. FF and MTR.

For anatomical standardization purposes between individuals, 18 slices were eventually selected at the thigh level for the fat infiltration and MTR maps. The proximal limit selected for the thigh was the appearance of the short head of the biceps femoris. At the lower-leg level, 20 slices were selected so that the distal limit of the ROI (region of interest) was the disappearance of the gastrocnemius medialis. Quantitative measurements were computed for each muscle and averaged for each slice over the same area of interest for each subject.



**Figure 1 : T1-weighted axial images and regions of interest in a control subject**

**Thigh:** VL= vastus lateralis, RF= rectus femoris, VI= vastus intermedius, VM= vastus medialis, AD= adductor, BF= biceps femoris, ST= semi tendinous, SM= semi membranous, SA= Sartorius, GR= gracilis

**Leg:** AC= anterior compartment, LC= lateral compartment, DP= deep posterior compartment, So= soleus, GL= gastrocnemius lateralis, GM= gastrocnemius medialis

## **Statistical analyses**

Quantitative values were compared between groups using a one-way analysis of variance (ANOVA) and the T3 Dunnett test for post hoc comparisons. The Spearman rank correlation was used to analyze the correlations between metrics ( $r$ , Spearman). Values are presented as mean  $\pm$  SD, and differences were considered as significantly different for  $p$  values lower than 0.05. Analyses were performed with IBM SPSS Statistic (version 20).

The study was approved by the local ethics committee (No. 2017-A02402-51). Written informed consent was obtained from all participants in the study. The data supporting the findings of this study are available on request from the corresponding author.

## **Results**

Twenty-five (20 males) patients were classified as symptomatic and 14 (7 males) as patients with asymptomatic TTR-FAP. The control group was composed of 14 (8 males) individuals.

Body mass index were similar between symptomatic patients ( $23.9 \pm 4.6$  kg/m<sup>2</sup>), asymptomatic patients ( $24.6 \pm 2.8$  kg/m<sup>2</sup>) and their respective matched controls ( $22.1 \pm 2.9$  kg/m<sup>2</sup>). Symptomatic patients and controls had a similar age (respectively 60 and 54 years old) whereas asymptomatic patients (41 years old) were younger.

Electromyographic and clinical characteristics of the two groups of patients are summarized in Table 1. The percentage of Val30Met mutation was similar in both groups, 57% in the asymptomatic group and 56% in the symptomatic group. In symptomatic individuals, the disease stage as defined by the Polyneuropathy disability (PND), the NIS LL, the RODS and the ONLS scores were  $2 (\pm 0.9)$ ,  $23.2 (\pm 18.7)$ ,  $71.3 (\pm 61.9)$  and  $3 (\pm 2)$  respectively. Clinical scores were normal in the asymptomatic group with ONLS and NISS LL being equal to 0 and RODS score was 100. The CMAP sum in the lower limb was significantly lower in the symptomatic group as compared to the asymptomatic patients ( $p < 0.0001$ ).



	Asymptomatic	Symptomatic
Number	14	25
Mutation Val30Met (%)	57%	56%
Disease duration (months)	NA	47.5 ± 43.3
PND (0 – 4)	0	2 ± 0.9
NIS-LL (0-88)	0	23.2 ± 18.7
ONLS (0-10)	0	3 ± 2
RODS (0-100)	100	71.3 ± 21.9
Sum of CMAP Lower limbs (mV)	35.3 ± 7.4	1.4 ± 10.6

**Table 1: Nerve conduction studies and clinical characteristics of patients**

### Fat Infiltration

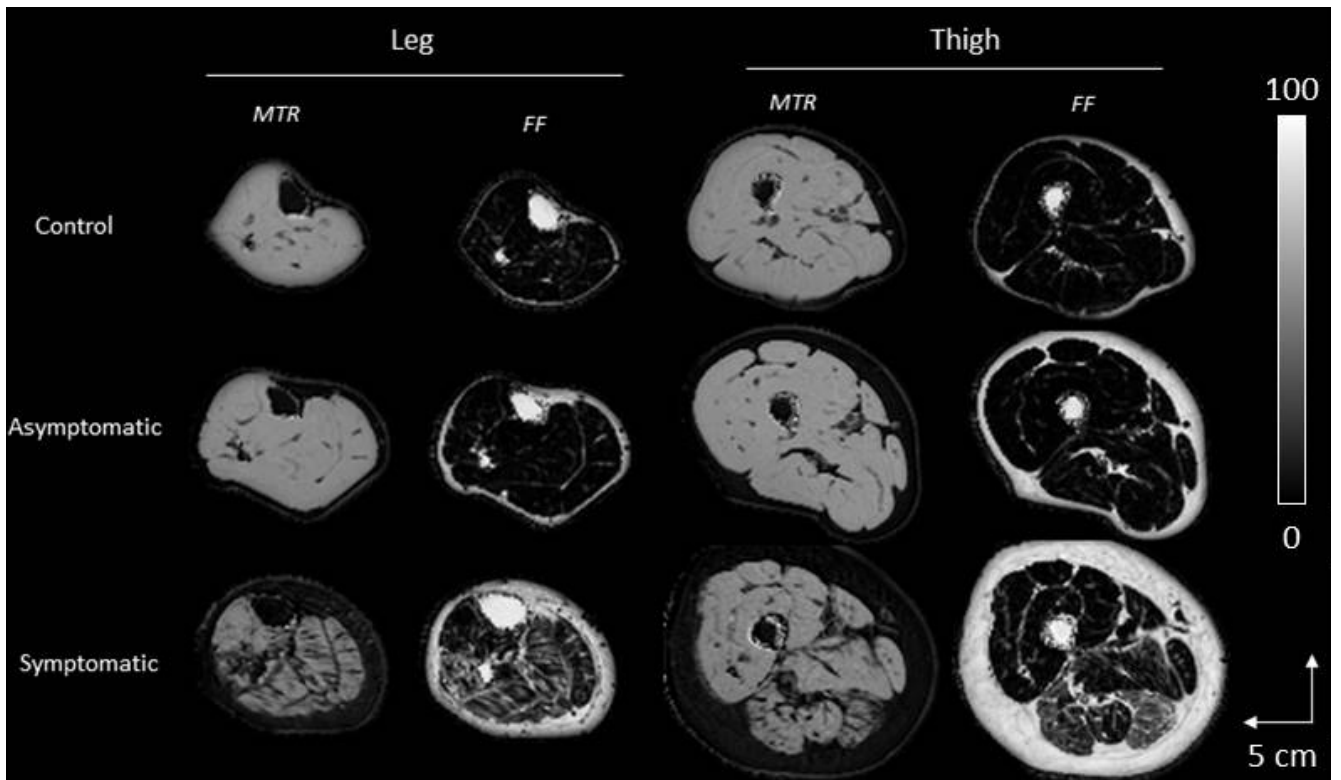
Visual analysis of FF maps illustrated a variable fatty infiltration at the leg and thigh levels (figure 2). In symptomatic patients, FF averaged in the lower limb (14%) was significantly higher than in controls (6.9%  $p=0.003$ ). A similar increase was quantified in the thigh and leg ( respectively 11.3% and 16.8%,  $p<0.05$ , figure 3). For each individual muscle except for some thigh muscles, i.e. sartorius, gracilis, rectus femoris, and vastus medialis, FF was significantly larger in patients (figure 4). The most fat-infiltrated muscles were the lateral compartment (20.5%), the lateral gastrocnemius (20.3%), the medial gastrocnemius (19.3%) in the leg, and the semimembranosus (15.9%), the biceps femoris (13.5%), the semitendinosus (12.3%) in the thigh.

In the asymptomatic group, FF was only significantly increased in the gastrocnemius lateralis muscle (11%,  $p= 0.021$ ).

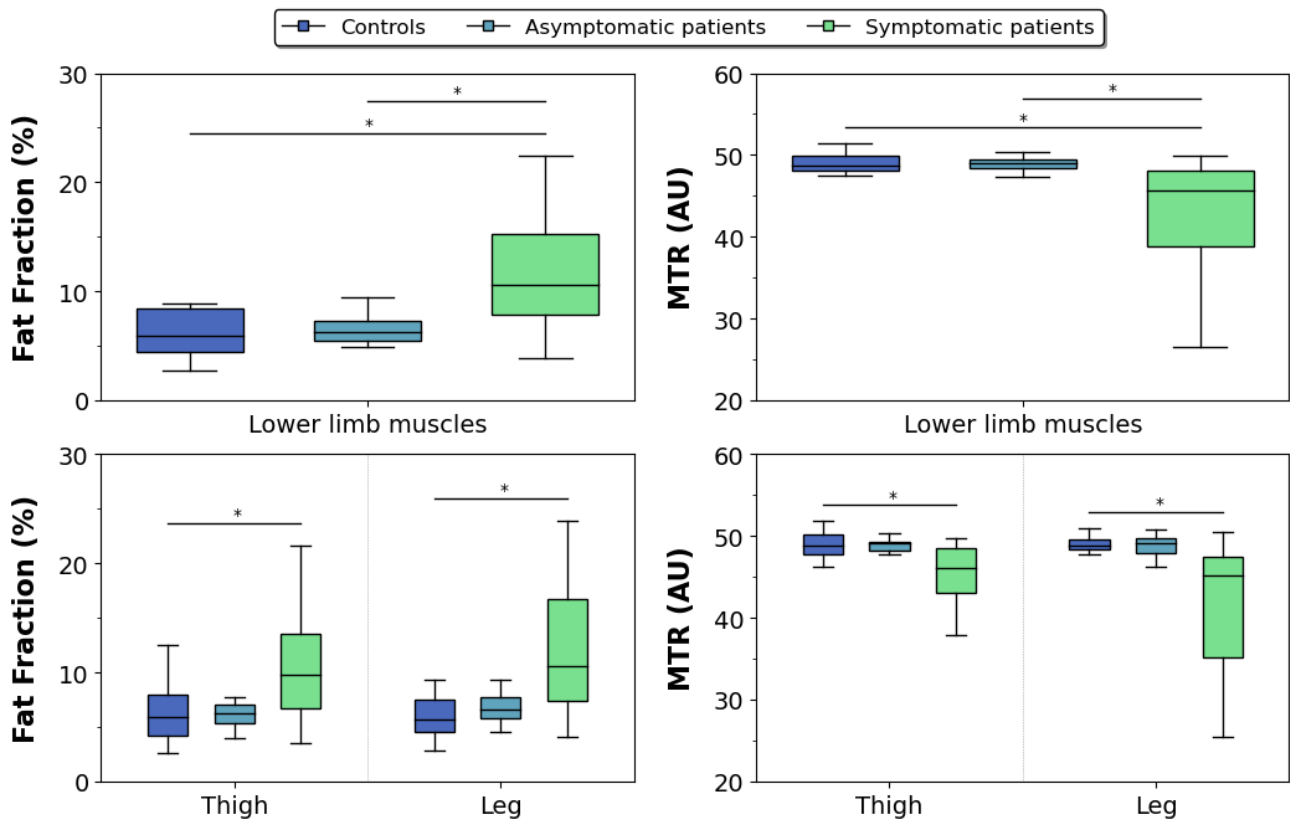
### MTR

Visual analysis of MTR maps illustrated variable MTR values at the leg and thigh levels (figure 2). In symptomatic patients, MTR averaged within the lower limb (42.6 AU) was significantly lower than in controls (49 AU  $p=0.001$ ). A similar reduction was found in the thigh and leg (respectively 44.5 and 40.6 AU  $p<0.05$ , figure 3). At the level of individual muscles, MTR was systematically reduced except for the rectus femoris (figure 4). The lowest values were identified in the lateral gastrocnemius (37.6 AU), medial gastrocnemius (37.8 AU), lateral compartment (40.2 AU) of the lower-leg, Sartorius (40.7 AU), semimembranosus (42.8 AU) and biceps femoris (43 AU) of the thigh muscles.

No MTR abnormality was found in the asymptomatic group.



**Figure 2:** FF and MTR maps in controls, asymptomatic and symptomatic patients (PND 3, NIS-LL 40 and 1 year of disease progress). One can observe a preferential involvement of posterior and lateral compartments in the symptomatic patient.

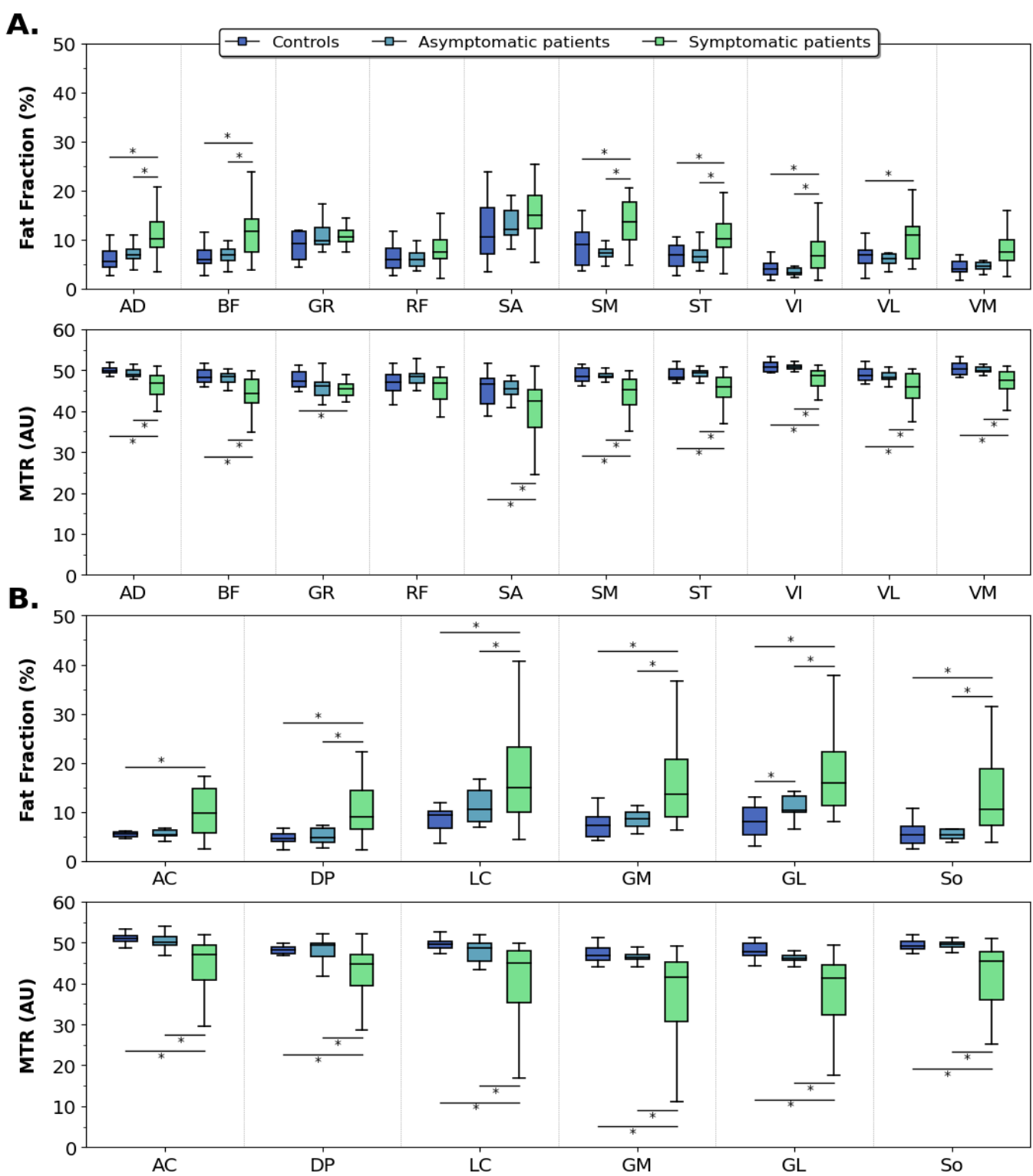


**Figure 3: FF (%) and MTR (AU) in control subjects, asymptomatic and symptomatic patients.**

\*indicates a p value <0.05

FF was significantly increased and MTR was significantly decreased in symptomatic patients compared with controls.

There was no difference between asymptomatic patients and control subjects.



**Figure 4: FF (%) and MTR (AU) for each muscle in thigh (A) and leg (B) in control subjects, asymptomatic and symptomatic patients**

\* indicates a p value < 0.05.

**Thigh:** VL= vastus lateralis, RF= rectus femoris, VI= vastus intermedius, VM= vastus medialis, AD= adductor, BF= biceps femoris, ST= semi tendinous, SM= semi membranous, SA= Sartorius, GR= gracilis

**Leg:** AC= anterior compartment, LC= lateral compartment, DP= deep posterior compartment, So= soleus, GL= gastrocnemius lateralis, GM= gastrocnemius medialis

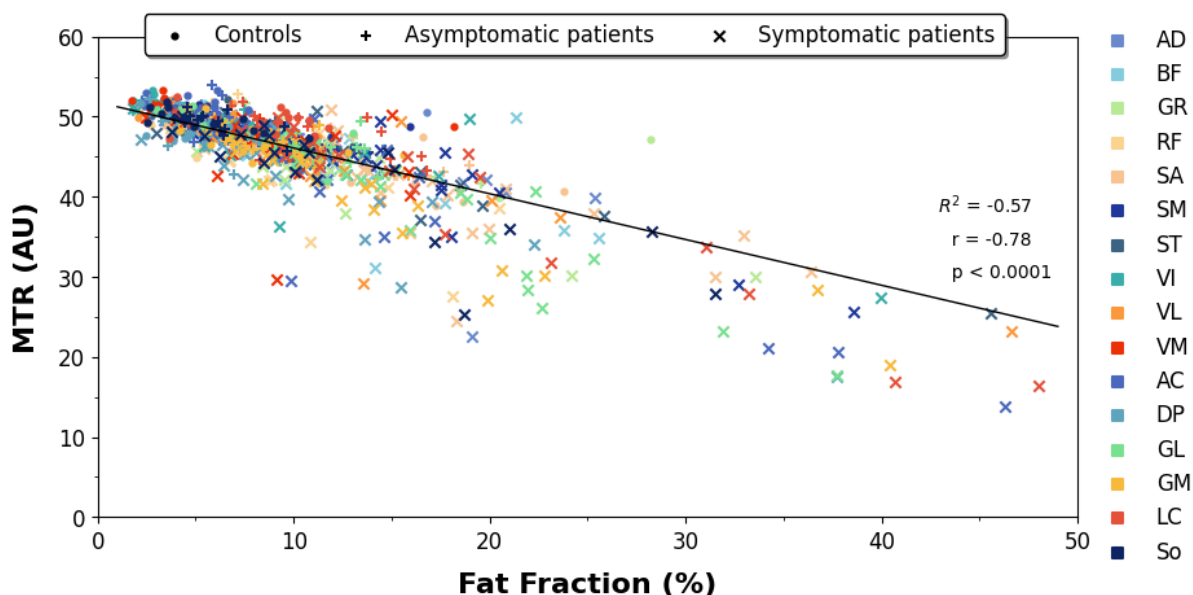
**In the symptomatic group** MTR was decreased and FF was increased in multiple muscles with a preferential posterior and lateral involvement. For SA, GR and VM MTR was decreased with a FF within the normal range. **In the asymptomatic group**, an increased FF was quantified only in the GL

## Correlations

Potential correlations between quantitative fat fraction, MTR, clinical scores and electrophysiological measurements were analyzed. As indicated in table 2, FF and MTR in the lower limb was significantly correlated with age, disease duration, NIS-LL, RODS, ONLS score and the sum of CMAP. Regarding the muscle compartments identified as pathologic, a significant relationship was found between most of muscle fat fractions and MTR values, all scores and electrophysiological measurements.

Of note, muscles without pathological fatty infiltration but with abnormal MTR values (i.e. vastus medialis, sartorius and gracilis) present for the majority significant correlations between MTR values, clinical and electrophysiological data.

Of interest, a very strong correlation was identified between MTR and fat infiltration in patients and controls ( $r=0.78$   $p<0.0001$ , figure 5).



**Figure 5: Correlation between MTR and FF for each muscle in control, asymptomatic and symptomatic patients.**

**Thigh:** VL= vastus lateralis, RF= rectus femoris, VI= vastus intermedius, VM= vastus medialis, AD= adductor, BF= biceps femoris, ST= semi tendinous, SM= semi membranous, SA= Sartorius, GR= gracilis,

**Leg:** AC= anterior compartment, LC= lateral compartment, DP= deep posterior compartment, So= soleus, GL= gastrocnemius lateralis, GM= gastrocnemius medialis

		Age		Disease duration		PND		RODS		ONLS		NIS-LL		Sum of CMAP	
		r	pvalue	r	pvalue	r	pvalue	r	pvalue	r	pvalue	r	pvalue	r	pvalue
<b>Mean Lower Limb</b>	FF	0.64	0.001	0.49	0.015	0.57	0.003	-0.48	0.020	0.49	0.013	0.42	0.041	-0.52	0.009
	MTR	-0.70	0.0001	-0.47	0.02	-0.80	0.0001	0.7	0.0001	-0.76	0.0001	-0.70	0.0001	0.78	0.00001
<b>Thigh</b>															
AD	FF	0.63	0.001	0.37	0.07	0.70	0.0009	-0.68	0.003	0.60	0.001	0.51	0.011	-0.46	0.024
	MTR	-0.64	0.001	-0.46	0.025	-0.73	0.0001	0.77	0.0001	-0.66	0.0001	-0.54	0.006	0.61	0.001
BF	FF	0.60	0.001	0.26	0.02	0.59	0.002	-0.55	0.007	0.52	0.008	0.45	0.028	-0.31	0.13
	MTR	-0.68	0.0001	-0.36	0.07	-0.79	0.0001	0.72	0.0001	-0.76	0.0001	-0.69	0.0001	0.77	0.0001
SM	FF	0.70	0.0009	0.30	0.05	0.58	0.002	-0.59	0.003	0.58	0.002	0.47	0.02	-0.35	0.09
	MTR	-0.70	0.0001	-0.35	0.09	-0.72	0.0001	0.72	0.0001	-0.72	0.0001	-0.66	0.0001	0.72	0.0001
ST	FF	0.56	0.004	0.29	0.06	0.61	0.001	-0.52	0.011	0.48	0.014	0.45	0.026	-0.28	0.18
	MTR	-0.52	0.007	-0.3	0.04	-0.68	0.0001	0.59	0.003	-0.58	0.002	-0.46	0.024	0.52	0.009
VI	FF	0.57	0.003	0.43	0.03	0.52	0.008	-0.49	0.019	0.43	0.031	0.30	0.15	-0.38	0.07
	MTR	-0.71	0.0001	-0.38	0.06	-0.73	0.0001	0.72	0.0001	-0.68	0.0001	-0.60	0.0001	0.70	0.0002
VL	FF	0.56	0.003	0.40	0.053	0.57	0.003	-0.52	0.011	0.45	0.025	0.33	0.12	-0.29	0.17
	MTR	-0.59	0.002	-0.32	0.01	-0.66	0.0001	0.56	0.005	-0.61	0.001	-0.47	0.01	0.56	0.005
VM	FF	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	MTR	-0.56	0.004	-0.52	0.09	-0.76	0.0008	0.69	0.002	-0.71	0.0006	-0.66	0.003	0.78	0.00008
SA	FF	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	MTR	-0.42	0.03	-0.48	0.01	-0.6	0.002	0.63	0.01	-0.58	0.02	-0.46	0.02	0.46	0.02
GR	FF	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	MTR	0.07	0.7	-0.19	0.3	-0.2	0.2	0.55	0.007	-0.35	0.08	-0.2	0.3	0.1	0.52

		Age		Disease duration		PND		RODS		ONLS		NIS-LL		Sum of CMAP		
		r	pvalue	r	pvalue	r	pvalue	r	pvalue	r	pvalue	r	pvalue	r	pvalue	
<b>Leg</b>																
LC	FF	<b>0.52</b>	<b>0.007</b>	<b>0.44</b>	<b>0.030</b>	<b>0.51</b>	<b>0.008</b>	-0.28	0.195	<b>0.47</b>	<b>0.018</b>	<b>0.41</b>	<b>0.043</b>	-	<b>0.56</b>	<b>0.004</b>
	MTR	<b>-0.63</b>	<b>0.001</b>	<b>-0.46</b>	<b>0.02</b>	<b>-0.66</b>	<b>0.003</b>	<b>0.58</b>	<b>0.03</b>	<b>-0.69</b>	<b>0.0001</b>	-	<b>0.58</b>	<b>0.003</b>	<b>0.74</b>	<b>0.0003</b>
AC	FF	<b>0.70</b>	<b>0.0008</b>	<b>0.42</b>	<b>0.037</b>	<b>0.47</b>	<b>0.016</b>	-0.30	0.167	0.36	0.072	0.31	0.130	-	<b>0.44</b>	<b>0.031</b>
	MTR	<b>-0.73</b>	<b>0.0003</b>	<b>-0.42</b>	<b>0.04</b>	<b>-0.70</b>	<b>0.002</b>	<b>0.64</b>	<b>0.001</b>	<b>-0.69</b>	<b>0.0002</b>	-	<b>0.61</b>	<b>0.001</b>	<b>0.86</b>	<b>0.0001</b>
DP	FF	<b>0.60</b>	<b>0.002</b>	<b>0.61</b>	<b>0.001</b>	<b>0.65</b>	<b>0.004</b>	<b>-0.44</b>	<b>0.033</b>	<b>0.53</b>	<b>0.006</b>	<b>0.47</b>	<b>0.018</b>	-	<b>0.62</b>	<b>0.001</b>
	MTR	<b>-0.67</b>	<b>0.0002</b>	<b>-0.5</b>	<b>0.01</b>	<b>-0.90</b>	<b>0.001</b>	<b>0.75</b>	<b>0.001</b>	<b>-0.82</b>	<b>0.0001</b>	-	<b>0.65</b>	<b>0.001</b>	<b>0.86</b>	<b>0.00005</b>
So	FF	<b>0.57</b>	<b>0.003</b>	<b>0.50</b>	<b>0.012</b>	<b>0.65</b>	<b>0.004</b>	<b>-0.42</b>	<b>0.047</b>	<b>0.53</b>	<b>0.006</b>	<b>0.51</b>	<b>0.010</b>	-	<b>0.53</b>	<b>0.002</b>
	MTR	<b>-0.67</b>	<b>0.0001</b>	<b>-0.49</b>	<b>0.01</b>	<b>-0.86</b>	<b>0.001</b>	<b>0.70</b>	<b>0.002</b>	<b>-0.80</b>	<b>0.0001</b>	-	<b>0.78</b>	<b>0.0001</b>	<b>0.84</b>	<b>0.00002</b>
GM	FF	<b>0.62</b>	<b>0.001</b>	<b>0.59</b>	<b>0.002</b>	<b>0.59</b>	<b>0.002</b>	<b>-0.50</b>	<b>0.015</b>	<b>0.60</b>	<b>0.001</b>	<b>0.47</b>	<b>0.020</b>	-	<b>0.61</b>	<b>0.001</b>
	MTR	<b>-0.61</b>	<b>0.001</b>	<b>-0.55</b>	<b>0.005</b>	<b>-0.80</b>	<b>0.001</b>	<b>0.67</b>	<b>0.001</b>	<b>-0.82</b>	<b>0.0001</b>	-	<b>0.74</b>	<b>0.0001</b>	<b>0.86</b>	<b>0.00009</b>
GL	FF	<b>0.55</b>	<b>0.004</b>	<b>0.41</b>	<b>0.044</b>	<b>0.52</b>	<b>0.007</b>	-0.40	0.06	<b>0.40</b>	<b>0.046</b>	0.33	0.109	-	<b>0.41</b>	<b>0.044</b>
	MTR	<b>-0.62</b>	<b>0.001</b>	<b>-0.44</b>	<b>0.03</b>	<b>-0.80</b>	<b>0.003</b>	<b>0.75</b>	<b>0.0003</b>	<b>-0.68</b>	<b>0.0001</b>	-	<b>0.65</b>	<b>0.001</b>	<b>0.71</b>	<b>0.00009</b>

**Table 2 : Spearman correlation values (r) between mean FF and MTR in lower limb muscles, clinical scores and electrophysiological data.**

significant correlations are indicated in bold.

- - correlation not realized for muscles without pathological fatty infiltration

**Thigh:** AD= adductor, BF= biceps femoris, SM= semi membranous, ST= semi tendinous, VI= vastus intermedius, VL= vastus lateralis, VM= vastus medialis, SA=Sartorius, GR=gracilis

**Leg:** LC= lateral compartment, AC= anterior compartment, DP= deep posterior compartment, So= soleus, GM= gastrocnemius medialis, GL= gastrocnemius lateralis

**PND=** Peripheral Neuropathy Disability score, **RODS=** Rasch-Built Overall Disability Scale, **ONLS=** Overall Neuropathy Limitations Scale, **NIS-LL=** Neuropathy Impairment Score Lower-Limb

## Discussion

The use of specific imaging sequences and a semi-automatic image segmentation technique allowed us to study exhaustively, several muscles of the thigh and leg in 39 patients with TTR-FAP. We detected almost systematic changes in FF and MTR with preferential involvement of posterior and lateral compartments in the symptomatic group. Fat fraction abnormalities were also found in asymptomatic patients.

Fatty infiltration in TTR-FAP patients was previously reported by *Primiano et al*<sup>28</sup>. Their observations were based on a visual analysis of T1-weighted images and on a single slice on 10 patients without control subjects. This is a descriptive and semi-quantitative approach, as it does not allow an exact quantification of the anomalies. In this project, we used quantitative imaging sequences that allow a finer and more sensitive analysis of muscular alterations on multiple slices based on FF and MTR values. We also utilized a dedicated and semi-automatic segmentation technique to reliably analyze multiple individual muscles in the thigh and leg.

Quantitative MRI has been previously used to assess muscle fatty infiltration in Charcot Marie Tooth disease (CMT), a genetically inherited length-dependent neuropathy. Using a similar quantitative approach, *Bas et al.*<sup>24</sup> reported a preferential involvement of the anterior and lateral compartments of the lower leg in CMT1A patients, a demyelinating neuropathic form. Interestingly, in CMT2A, an axonal neuropathic form, a preferential posterior involvement was reported<sup>29</sup>. This pattern is similar to what we report in the present study in TTR-FAP patients.

Two quantitative MRI parameters i.e MTR and FF were computed in several muscles and several slices of the lower limb. This approach allowed us to identify muscles that could be of particular interest for follow-up investigations. The GM in the leg, was most notably affected according to both FF and MTR values. Furthermore, this muscle displayed the strongest correlations with clinical scores and the CMAP sum. However, it should be remembered that this muscle is easily identifiable on MRI, which eases the segmentation process. Because of its preferential involvement and the length-dependent character of the amyloid neuropathy,, it is plausible to hypothesize that the GM would be the first to be affected in the natural course of TTR-FAP. Therefore, GM could be a good muscle candidate for assessing disease progression from the onset of clinical symptoms.

For asymptomatic patients, the GL muscle in the leg, could be a more interesting candidate, as abnormal fat infiltration was observed in this muscle. Interestingly, this muscle exhibited



fewer correlations with clinical scores, particularly with the NIS-LL impairment score, which includes neurological deficits. This is not surprising as these muscle abnormalities were quantified in asymptomatic patients with a normal clinical examination and EDX. These results could suggest that the fatty infiltration of the GL might be more sensitive than the currently available clinical tools for assessing neurological involvement.

In the symptomatic group, we identified decreased MTR and increased FF values in multiple muscles. MTR measurements reflect the rate of protons exchange between water and macromolecules<sup>30</sup>. Similar decreases in MTR have been reported in several neuromuscular diseases<sup>31</sup>. *Sinclair et al*<sup>32</sup> reported a similar reduction in CMT and chronic demyelinating polyradiculoneuropathy (CIDP). This reduction has been suggested as indicative of a decrease in the macromolecular content associated with the neuropathic process. In fact, muscle degeneration due to denervation results in fiber loss and, eventually, chronic replacement of muscle by adipose tissue. These processes are likely to decrease the macromolecular structure and thereby affect the magnetization transfer properties of the muscle tissue.

In our study, we found a strong inverse relationship ( $r=-0.78$ ,  $p<0.0001$ ) between MTR and FF values, suggesting that fat infiltration might be related to the MTR decrease. Therefore, both parameters might reflect the same pathological process. However, considering individual muscles, our results demonstrate some peculiarities for a few muscles (i.e. Sartorius, Gracilis and Vastus medialis), where FF values were within the normal range, but MTR was reduced. Furthermore, for these muscles, significant correlations between MTR values and clinical and electrophysiological data were observed. For these specific muscles, MTR could be sensitive to subtle changes in macromolecular content occurring before the onset of the fatty infiltration process. In other words, the MTR could be considered as an early marker of the neuropathic process. However, in the asymptomatic group, the GL muscle was abnormally infiltrated by fat while MTR values only tended to be reduced. This nonsignificant difference might be explained by the small number of asymptomatic carriers. Hence, the utility of MTR as an early marker of the neuropathic process should be confirmed by additional studies conducted with a larger number of subjects. It may be interesting to conduct a specific study investigating MTR in asymptomatic carriers and controls to identify potential significant differences.

Significant correlations between muscle abnormalities quantified by MRI, clinical scores, and the sum of the CMAP suggest that FF and MTR quantified in individual

muscles using MRI might serve as potential biomarkers of TTR-FAP. It is worth noting that we found a significant correlation between age, MTR, and FF. This finding becomes more important considering that asymptomatic subjects, who are younger, still present a pathological fatty infiltration in the GL compared to the older control subjects.

The present study is the first in which muscle abnormalities have been identified and quantified in symptomatic patients, while subclinical impairment has been detected in asymptomatic subjects. A limitation related to the number of subjects must be acknowledged. This reduced number is related to the rarity of the disease, a factor that has similarly limited patient numbers in previous studies utilizing MR neurography<sup>17 18</sup>. Based on the identification of potential candidates, the next logical step would be to assess these metrics as part of a longitudinal study and determine their sensitivity with respect to disease progression. Longitudinal analyses of FF and MTR changes could also be helpful in clinical trials.

In conclusion, both MTR and FF were abnormal in several muscles of the thigh and leg, and the corresponding biomarkers were largely correlated with clinical and electrophysiological scores. These findings suggest that, as previously described for other neuromuscular disorders, quantitative muscle MRI metrics could be considered as potential interesting biomarkers in familial amyloid polyneuropathy. Interestingly, MTR was abnormally decreased for muscles with a FF within the normal range, suggesting that MTR could be an early biomarker of muscle alterations. Moreover, in asymptomatic patients, the fat infiltration of Gastrocnemius Lateralis muscle could be considered as an early biomarker of TTR-FAP and specific monitoring of this muscle using MRI could be indicative of the transition from an asymptomatic to a symptomatic form of the disease. A longitudinal evaluation of these parameters would be of high interest to determine their sensitivity to disease progression.

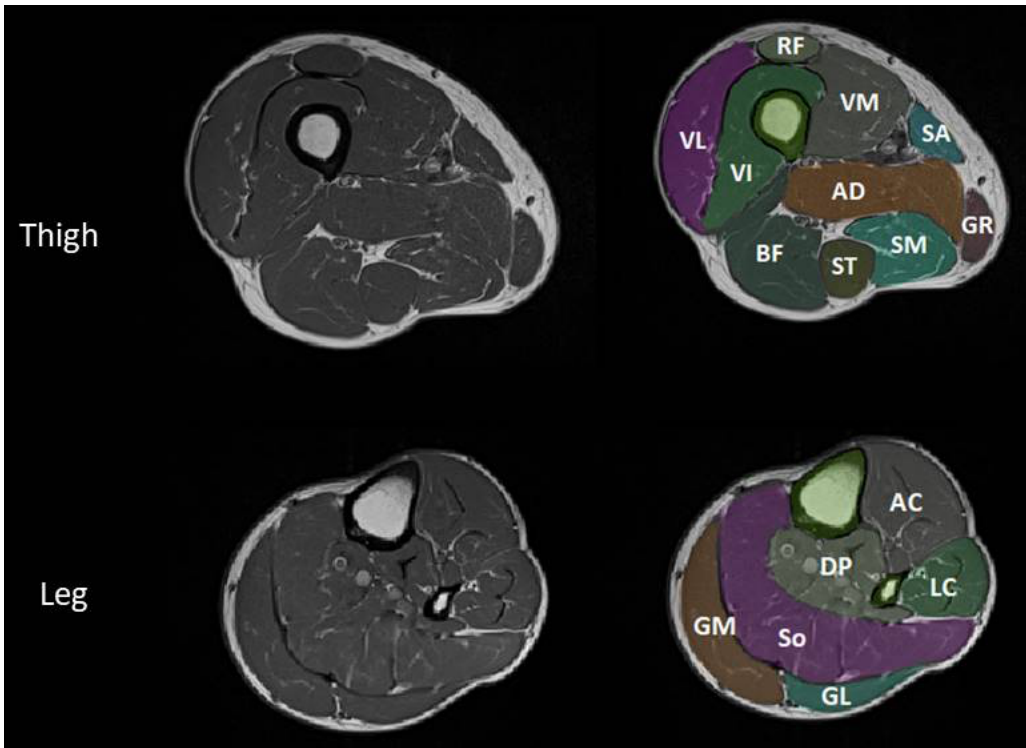
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## REFERENCES

1. Benson MD, Kincaid JC. The molecular biology and clinical features of amyloid neuropathy. *Muscle Nerve*. 2007;36(4):411-423. doi:10.1002/mus.20821
2. Said G, Ropert A, Faux N. Length-dependent degeneration of fibers in Portuguese amyloid polyneuropathy: a clinicopathologic study. *Neurology*. 1984;34(8):1025-1032. doi:10.1212/wnl.34.8.1025
3. Planté-Bordeneuve V, Said G. Familial amyloid polyneuropathy. *The Lancet Neurology*. 2011;10(12):1086-1097. doi:10.1016/S1474-4422(11)70246-0
4. Adams D, Ando Y, Beirão JM, et al. Expert consensus recommendations to improve diagnosis of ATTR amyloidosis with polyneuropathy. *J Neurol*. Published online January 6, 2020. doi:10.1007/s00415-019-09688-0
5. Adams D, Koike H, Slama M, Coelho T. Hereditary transthyretin amyloidosis: a model of medical progress for a fatal disease. *Nat Rev Neurol*. 2019;15(7):387-404. doi:10.1038/s41582-019-0210-4
6. Adams D, Gonzalez-Duarte A, O’Riordan WD, et al. Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis. *N Engl J Med*. 2018;379(1):11-21. doi:10.1056/NEJMoa1716153
7. Benson MD, Waddington-Cruz M, Berk JL, et al. Inotersen Treatment for Patients with Hereditary Transthyretin Amyloidosis. *N Engl J Med*. 2018;379(1):22-31. doi:10.1056/NEJMoa1716793
8. Ferreira N, Saraiva MJ, Almeida MR. Epigallocatechin-3-Gallate as a Potential Therapeutic Drug for TTR-Related Amyloidosis: “In Vivo” Evidence from FAP Mice Models. Ferreira ST, ed. *PLoS ONE*. 2012;7(1):e29933. doi:10.1371/journal.pone.0029933
9. Higaki JN, Chakrabartty A, Galant NJ, et al. Novel conformation-specific monoclonal antibodies against amyloidogenic forms of transthyretin. *Amyloid*. 2016;23(2):86-97. doi:10.3109/13506129.2016.1148025
10. Obici L, Kuks JB, Buades J, et al. Recommendations for presymptomatic genetic testing and management of individuals at risk for hereditary transthyretin amyloidosis. *Current Opinion in Neurology*. 2016;29(Supplement 1):S27-S35. doi:10.1097/WCO.0000000000000290
11. Ando Y, Coelho T, Berk JL, et al. Guideline of transthyretin-related hereditary amyloidosis for clinicians. *Orphanet J Rare Dis*. 2013;8:31. doi:10.1186/1750-1172-8-31
12. Adams D, Suhr OB, Hund E, et al. First European consensus for diagnosis, management, and treatment of transthyretin familial amyloid polyneuropathy: *Current Opinion in Neurology*. 2016;29:S14-S26. doi:10.1097/WCO.0000000000000289
13. Dyck PJ. Polyneuropathy dysfunction scores. *Journal of Neurology, Neurosurgery & Psychiatry*. 2006;77(8):899-900. doi:10.1136/jnnp.2006.093781

14. Brannagan TH, Wang AK, Coelho T, et al. Early Data on Long-Term Efficacy and Safety of Inotersen in Patients With Hereditary Transthyretin Amyloidosis: A 2-Year Update From the Open-Label Extension of the NEURO-TTR Trial. *Eur J Neurol*. Published online April 28, 2020:ene.14285. doi:10.1111/ene.14285
15. Kollmer J, Weiler M, Purrucker J, et al. MR neurography biomarkers to characterize peripheral neuropathy in AL amyloidosis. *Neurology*. 2018;91(7):e625-e634. doi:10.1212/WNL.0000000000006002
16. Kollmer J, Hegenbart U, Kimmich C, et al. Magnetization transfer ratio quantifies polyneuropathy in hereditary transthyretin amyloidosis. *Ann Clin Transl Neurol*. 2020;7(5):799-807. doi:10.1002/acn3.51049
17. Kollmer J, Hund E, Hornung B, et al. In vivo detection of nerve injury in familial amyloid polyneuropathy by magnetic resonance neurography. *Brain*. 2015;138(3):549-562. doi:10.1093/brain/awu344
18. Kollmer J, Sahn F, Hegenbart U, et al. Sural nerve injury in familial amyloid polyneuropathy: MR neurography vs clinicopathologic tools. *Neurology*. 2017;89(5):475-484. doi:10.1212/WNL.0000000000004178
19. Moore CW, Allen MD, Kimpinski K, Doherty TJ, Rice CL. Reduced skeletal muscle quantity and quality in patients with diabetic polyneuropathy assessed by magnetic resonance imaging: Muscle Quality in Diabetes. *Muscle Nerve*. 2016;53(5):726-732. doi:10.1002/mus.24779
20. Leporq B, Le Troter A, Le Fur Y, et al. Combined quantification of fatty infiltration, T1-relaxation times and T2\*-relaxation times in normal-appearing skeletal muscle of controls and dystrophic patients. *Magn Reson Mater Phy*. 2017;30(4):407-415. doi:10.1007/s10334-017-0616-1
21. Lareau-Trudel E, Le Troter A, Ghattas B, et al. Muscle Quantitative MR Imaging and Clustering Analysis in Patients with Facioscapulohumeral Muscular Dystrophy Type 1. Fan Y, ed. *PLoS ONE*. 2015;10(7):e0132717. doi:10.1371/journal.pone.0132717
22. Ansari B, Salort-Campana E, Ogier A, et al. Quantitative muscle MRI study of patients with sporadic inclusion body myositis. *Muscle Nerve*. 2020;61(4):496-503. doi:10.1002/mus.26813
23. Morrow JM, Evans MRB, Grider T, et al. Validation of MRC Centre MRI calf muscle fat fraction protocol as an outcome measure in CMT1A. *Neurology*. 2018;91(12):e1125-e1129. doi:10.1212/WNL.0000000000006214
24. Bas J, Ogier AC, Le Troter A, et al. Fat fraction distribution in lower limb muscles of patients with CMT1A: A quantitative MRI study. *Neurology*. 2020;94(14):e1480-e1487. doi:10.1212/WNL.0000000000009013
25. Sinclair CDJ, Morrow JM, Hanna MG, et al. Correcting radiofrequency inhomogeneity effects in skeletal muscle magnetisation transfer maps: CORRECTION OF MUSCLE MTR MAPS. *NMR Biomed*. 2012;25(2):262-270. doi:10.1002/nbm.1744

26. Ogier A, Sdika M, Foure A, Le Troter A, Bendahan D. Individual muscle segmentation in MR images: A 3D propagation through 2D non-linear registration approaches. In: *2017 39th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*. IEEE; 2017:317-320. doi:10.1109/EMBC.2017.8036826
27. Ogier AC, Heskamp L, Michel CP, et al. A novel segmentation framework dedicated to the follow-up of fat infiltration in individual muscles of patients with neuromuscular disorders. *Magn Reson Med*. 2020;83(5):1825-1836. doi:10.1002/mrm.28030
28. Primiano G, Verdolotti T, D'Apolito G, et al. Muscle MRI as a Useful Biomarker in Hereditary Transthyretin Amyloidosis: A Pilot Study. *Genes*. 2021;12(11):1786. doi:10.3390/genes12111786
29. Chung KW, Suh BC, Shy ME, et al. Different clinical and magnetic resonance imaging features between Charcot–Marie–Tooth disease type 1A and 2A. *Neuromuscular Disorders*. 2008;18(8):610-618. doi:10.1016/j.nmd.2008.05.012
30. Henkelman RM, Stanisz GJ, Graham SJ. Magnetization transfer in MRI: a review. *NMR Biomed*. 2001;14(2):57-64. doi:10.1002/nbm.683
31. Morrow JM, Sinclair CDJ, Fischmann A, et al. MRI biomarker assessment of neuromuscular disease progression: a prospective observational cohort study. *The Lancet Neurology*. 2016;15(1):65-77. doi:10.1016/S1474-4422(15)00242-2
32. Sinclair CDJ, Morrow JM, Miranda MA, et al. Skeletal muscle MRI magnetisation transfer ratio reflects clinical severity in peripheral neuropathies. *J Neurol Neurosurg Psychiatry*. 2012;83(1):29-32. doi:10.1136/jnnp.2011.246116



**Figure 1 :** T1 axial images and regions of interest in a control subject  
 VL= vastus lateralis, RF= rectus femoris, VI= vastus intermedius, VM= vastus medialis, AD= adductor, BF= biceps femoris, ST= semi tendinious, SM= semi membranous, SA= Sartorius, GR= gracilis, AC= anterior compartment, LC= lateral compartment, DP= deep posterior compartment, So= soleus, GL= gastrocnemius lateralis, GM= gastrocnemius medialis

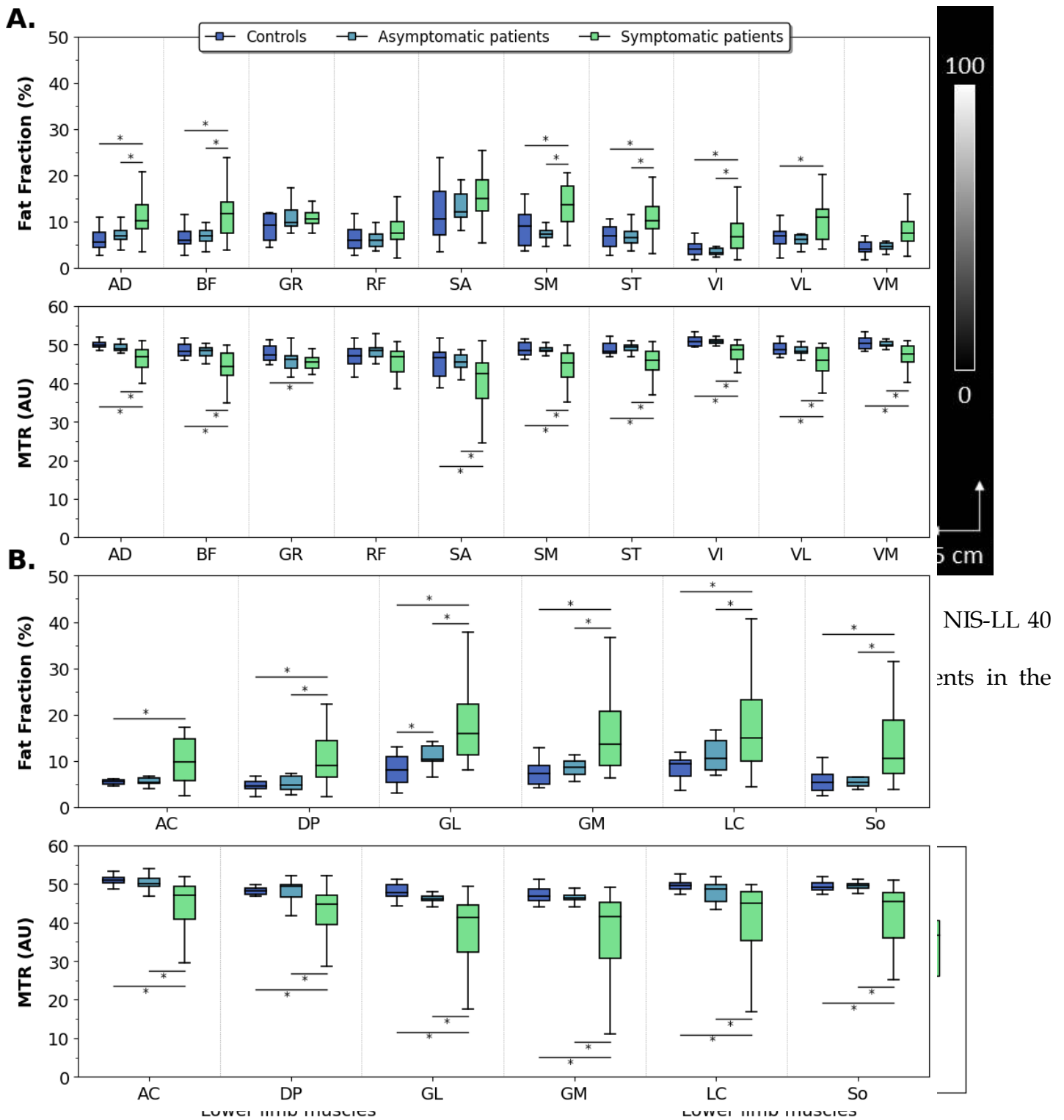


Figure 4: FF (%) and MTR (AU) for each muscle in the thigh (A) and leg (B) in control subjects, asymptomatic and symptomatic patients

\* indicates a p value <0.05.  
\* indicates a p value <0.05.  
VL= vastus lateralis, RF= rectus femoris, VI= vastus intermedius, VM= vastus medialis, AD= adductor, BF= biceps femoris, SI= semi tendinosus, SM= semi membranous, SA= Sartorius, GR= gracilis, AC= anterior compartment, LC= lateral compartment, DP= deep posterior compartment, So= Soleus. There was no difference between asymptomatic patients and control subjects.

In the symptomatic group MTR was lower and FF was higher in multiple muscles with a preferential involvement of posterior and lateral compartments. For SA, GR and VM, MTR was lower while FF was within the normal range.  
In the asymptomatic group, FF was only higher in the GL

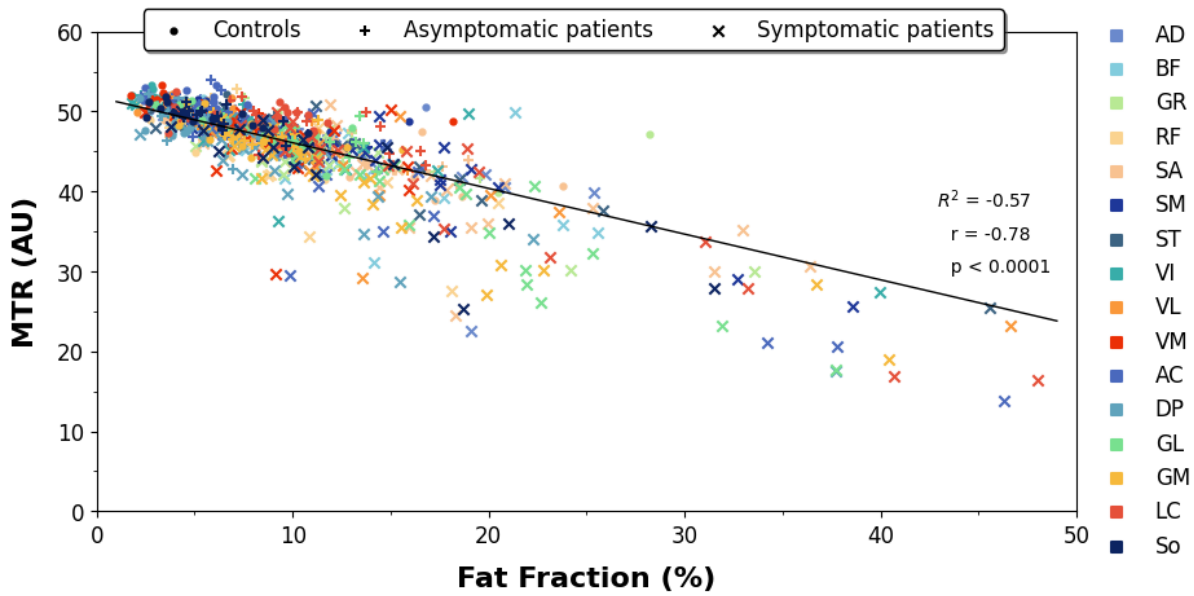


Figure 5: Correlation between MTR and FF for each muscle in controls, asymptomatic and symptomatic patients.

VL= vastus lateralis, RF= rectus femoris, VI= vastus intermedius, VM= vastus medialis, AD= adductor, BF= biceps femoris, ST= semi tendinosus, SM= semi membranous, SA= Sartorius, GR= gracilis, AC= anterior compartment, LC= lateral compartment, DP= deep posterior compartment, So= soleus, GL= gastrocnemius lateralis, GM= gastrocnemius medialis