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ORIGINAL ARTICLE

Quantification of muscle involvement in familial amyloid polyneuropathy using MRI

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Abstract

Background and purpose: Transthyretin familial amyloid polyneuropathy (TTR-FAP) is a rare genetic disease with autosomal-dominant inheritance. In this study, we aimed to quantify fatty infiltration (fat fraction [FF]) and magnetization transfer ratio (MTR) in individual muscles of patients with symptomatic and asymptomatic TTR-FAP using magnetic resonance imaging. Secondarily, we aimed to assess correlations with clinical and electrophysiological variables.

Methods: A total of 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 in the nondominant lower limb from T1-weighted anatomical images. The corresponding masks were propagated on the MTR and FF maps. Detailed neurological and electrophysiological examinations were conducted in each group.

Results: The MTR was decreased (42.6 AU; $p=0.001$) and FF was elevated (14%; $p=0.003$) in the lower limbs of the symptomatic group, with preferential posterior and lateral involvement. In the asymptomatic group, elevated FF was quantified in the gastrocnemius lateralis muscle (11%; $p=0.021$). FF was significantly correlated with disease duration ($r=0.49$, $p=0.015$), neuropathy impairment score for the lower limb ($r=0.42$, $p=0.041$), Overall Neuropathy Limitations Scale score ($r=0.49$, $p=0.013$), polyneuropathy disability score ($r=0.57$, $p=0.03$) and the sum of compound muscle action potential ($r=0.52$, $p=0.009$). MTR was strongly correlated to FF ($r=0.78$, $p<0.0001$), and a few muscles with an FF within the normal range had a reduced MTR.

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

KEYWORDS
biomarker, familial amyloid polyneuropathy, quantitative MRI

INTRODUCTION

Transthyretin familial amyloid polyneuropathy (TTR-FAP) is a rare genetic disease with autosomal-dominant inheritance. TTR-FAP progresses rapidly, and is characterized by axonal, length-dependent polyneuropathy, primarily affecting the small fibers of the autonomic nervous system [1, 2]. Onset age, manifestation, and disease progression are highly variable, depending on the type of TTR gene variant and the patient's ethnicity [3, 4]. In the absence of therapy, progression is rapid and leads to death within approximately 10 years

[5]. Early diagnosis is crucial, as various effective therapies are available [6, 7] some of which are under trial with promising preliminary results [8, 9].

The diagnosis of hereditary transthyretin amyloidosis is based on genetic study, the aim of which is to detect amyloidogenic variants in the TTR gene. Supplementary tests, such as nerve conduction studies using electroneuromyography and clinical scoring (peripheral neuropathy disability [PND] score, neuropathy impairment score, Medical Research Council testing, Overall Neuropathy Limitations Scale [ONLS] score, Rasch-Built Overall Disability Scale [RODS]) can be used to evaluate the disease stage of TTR-FAP, to initiate treatment in asymptomatic patients [10] and to monitor therapeutic efficiency [11, 12]. Despite their utility, however, these tools have been noted to have limited sensitivity [13, 14].

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

The main objective of this study was to quantify and characterize potential MRI abnormalities in individual muscles of TTR-FAP patients. We aimed to integrate an MRI-based quantitative approach and a dedicated segmentation method to quantify both fat fraction (FF) and MTR values in symptomatic and asymptomatic TTR-FAP patients and to investigate the potential correlations of these values with clinical scores.

PATIENTS AND METHODS

Clinical assessment

Thirty-nine patients with a confirmed mutation in 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 (defined as time between the first symptoms and date of the MRI scan), and details of previous and ongoing therapies. A neurologist specializing in neuromuscular diseases (E.D.) carried out the neurological examinations, which included testing of motor and sensory functions in each limb. Peripheral neuropathy was scored using the ONLS, the RODS, and neuropathy impairment score for the lower limb (NIS-LL). The severity of disease was assessed based on the PND score.

Additionally, patients underwent electrophysiological assessment for each limb, which included measurement 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 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 the CMAP amplitudes in both lower limbs and considered this to represent nervous function. Autonomic dysfunction was assessed by testing sympathetic skin response, electrochemical skin conductance (sudoscan values), and 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 clinical examination was normal, ONLS, RODS and NIS-LL scores were within the normal range, and they had normal electrophysiological assessment. Symptomatic was defined as the presence of functional complaints related to the disease and at least one abnormal test result in a complementary examination. The control group was made up of individuals with no 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 was obtained, two-dimensional anatomical T1-weighted, three-dimensional (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 A1.

As previously described [22, 24], the 3D gradient echo multi-echo dataset was used to generate FF maps, while the MTR map was computed from the normalized ratio of an image acquired with and without saturation and after correction for B1 field inhomogeneities [25].

Segmentation protocol

Using FSLeyes (FSL, FMRIB Software Library) 16 muscles (10 at the thigh level and six at the leg level) were manually delineated in a limited number of slices (3–4 on average) from T1-weighted images. The rectus femoris, vastus lateralis, vastus medialis, vastus intermedius,

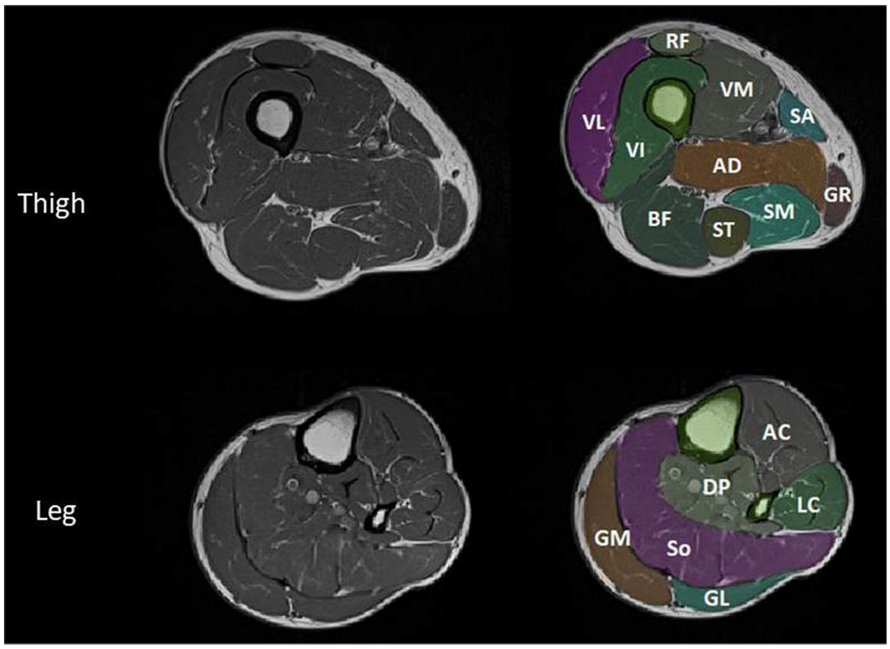

FIGURE 1 T1-weighted axial images and regions of interest in a control subject. Thigh: AD, adductor; BF, biceps femoris; GR, gracilis; RF, rectus femoris; SA, sartorius; SM, semimembranous; ST, semitendinous; VI, vastus intermedius; VL, vastus lateralis; VM, vastus medialis. Leg: AC, anterior compartment; DP, deep posterior compartment; GL, gastrocnemius lateralis; GM, gastrocnemius medialis; LC, lateral compartment; So, soleus.

sartorius, gracilis, semitendinous, semimembranous, biceps femoris and long adductor were delineated at the thigh level, while the anterior muscle compartment (tibialis anterior, extensor hallucis longus, extensor digitorum longus, and fibularis tertius), deep posterior compartment (tibialis posterior, flexor hallucis longus, flexor digitorium longus, and popliteus]), lateral compartment (fibularis longus and brevis]), soleus, gastrocnemius lateralis and medialis were delineated at the lower-leg level (Figure 1). These masks were then automatically propagated to the remaining unsegmented slices as previously described [26, 27]. Based on resampling and registration processes, the resulting masks were propagated to the quantitative maps, namely, the FF and MTR maps.

For anatomical standardization purposes among individuals, 18 slices were eventually selected at the thigh level for the FF and MTR maps. The proximal limit selected for the thigh was the appearance of the short head of the biceps femoris. At the leg level, 20 slices were selected so that the distal limit of the 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.

Statistical analyses

Quantitative values were compared between groups using one-way analysis of variance and the T3 Dunnett test for post hoc comparisons. Spearman's rank correlation was used to analyze the correlations between metrics (Spearman's r). Values are presented as mean ± SD, and differences were considered significantly different when p values were lower than 0.05. Analyses were performed with IBM SPSS Statistics (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 patients (20 males) were classified as having symptomatic and 14 (seven males) as having asymptomatic TTR-FAP. The control group was composed of 14 individuals (eight males).

Body mass indices were similar in the symptomatic patients ($23.9 \pm 4.6\,kg/m^2$), asymptomatic patients ($24.6 \pm 2.8\,kg/m^2$) and their respective matched controls ($22.1 \pm 2.9\,kg/m^2$). Symptomatic patients and controls were of a similar age (60 and 54 years, respectively), whereas asymptomatic patients were younger (41 years).

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

Fatty infiltration

Visual analysis of FF maps showed 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 (11.3% and 16.8%, respectively; $p < 0.05$ [Figure 3]). For each individual muscle, except for some of the thigh muscles, namely, the sartorius, gracilis, rectus femoris, and vastus medialis, FF was significantly greater in patients than controls (Figure 4). The muscles with the greatest fatty infiltration 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.

TABLE 1 Nerve conduction studies and clinical characteristics of patients.

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

Note: Values are presented as mean ± SD, unless otherwise stated.
Abbreviations: CMAP, compound muscle action potential; NIS-LL, neuropathy impairment score for the lower limb; ONLS, Overall Neuropathy Limitations Scale; PND, peripheral neuropathy disability; RODS, Rasch-Built Overall Disability Scale.

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

Magnetization transfer ratio

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 (44.5 and 40.6 AU, respectively; $p < 0.05$ [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, and the 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.

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 score,

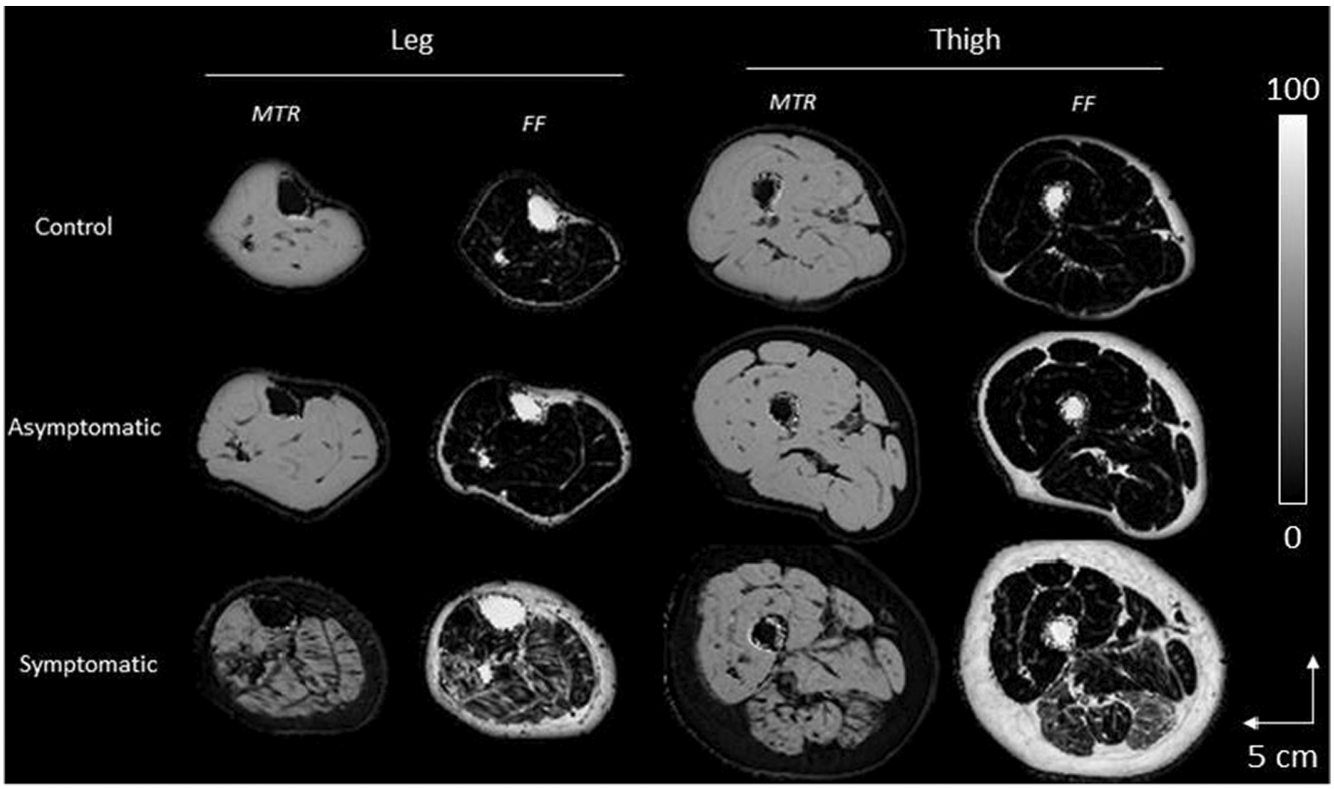

FIGURE 2 Fat fraction (FF) and magnetization transfer ratio (MTR) maps in controls, asymptomatic patients and symptomatic patients (peripheral neuropathy disability [PND] score 3, neuropathy impairment score for the lower limb [NIS-LL] 40 and 1 year of disease progress). Preferential involvement of the posterior and lateral compartments in the symptomatic patient can be observed.

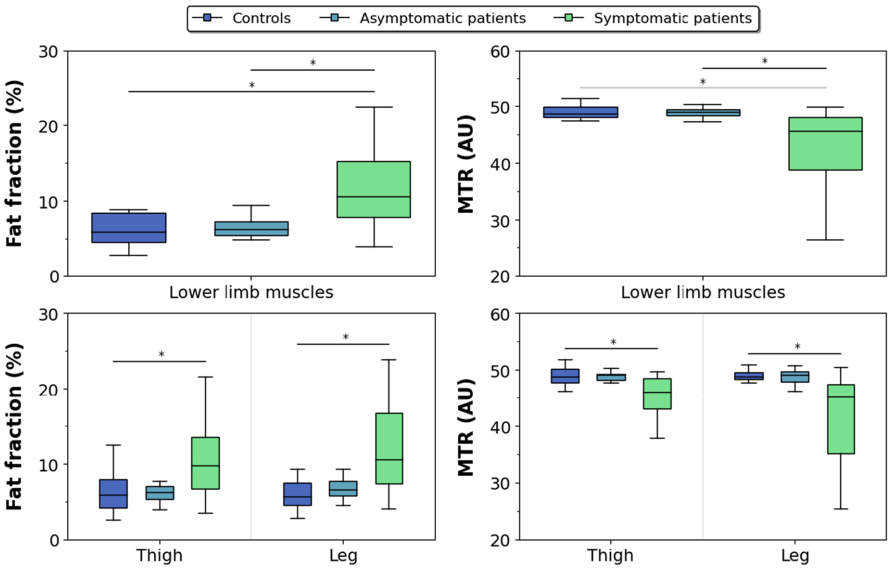

FIGURE 3 Fat fraction (FF) and magnetization transfer ratio (MTR) in control subjects, asymptomatic patients, and symptomatic patients. *Indicates *p* value <0.05. FF was significantly elevated and MTR was significantly decreased in symptomatic patients compared with controls. There was no difference between asymptomatic patients and control subjects.

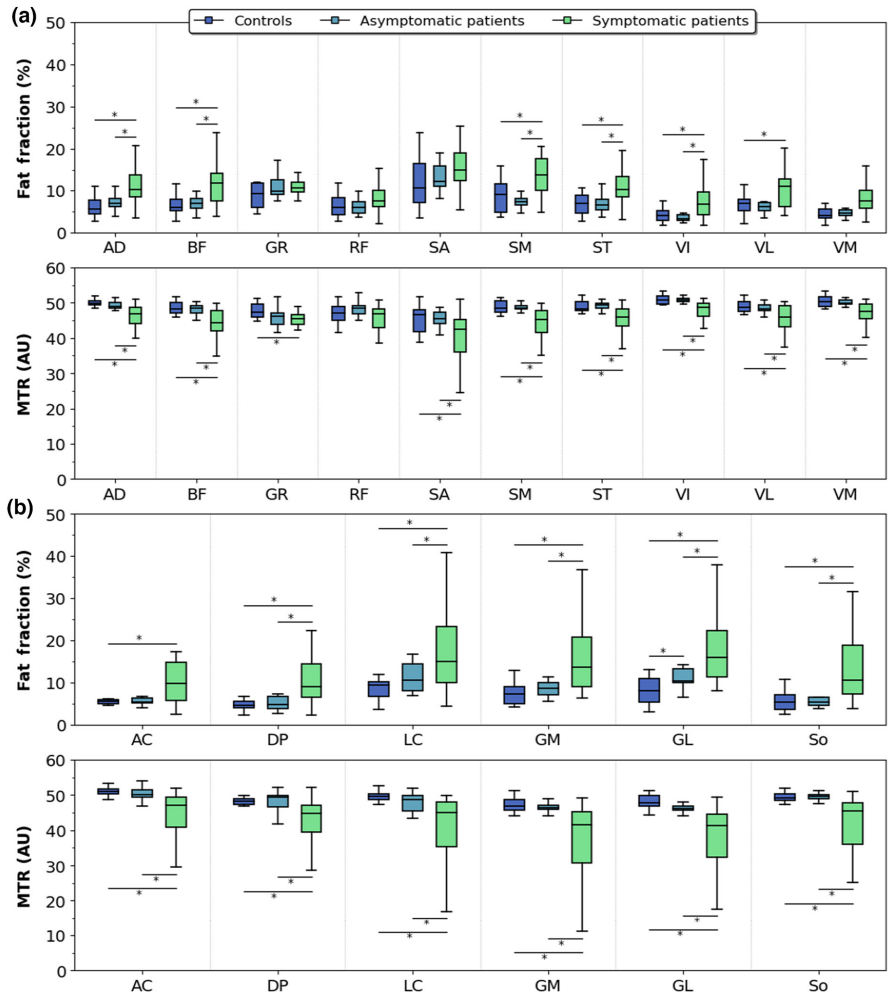

FIGURE 4 Fat fraction (FF) and magnetization transfer ratio (MTR) for each muscle in the thigh (a) and leg (b) in control subjects, asymptomatic patients, and symptomatic patients. *Indicates *p* value <0.05. Thigh: AD, adductor; BF, biceps femoris; GR, gracilis; RF, rectus femoris; SA, sartorius; SM, semimembranous; ST, semitendinous; VI, vastus intermedius; VL, vastus lateralis; VM, vastus medialis. Leg: AC, anterior compartment; DP, deep posterior compartment; GL, gastrocnemius lateralis; M, gastrocnemius medialis; LC, lateral compartment; So, soleus. In the symptomatic group MTR was decreased and FF was elevated in multiple muscles with a preferential posterior and lateral involvement. For SA, GR and VM, MTR was decreased with an FF within the normal range. In the asymptomatic group, an increased FF was quantified only in the GL.

ONLS score and the sum of CMAP. Regarding the muscle compartments identified as pathologic, a significant relationship was found between most of muscle FF 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) mostly showed significant correlations between MTR values, clinical and electrophysiological data.

TABLE 2 Spearman correlation values (*r*) for mean fat fraction and magnetization transfer ratio in the lower limb muscles, clinical scores and electrophysiological data.

		Age		Disease duration		PND score		RODS score		ONLS score		NIS-LL		Sum of CMAP	
		r	*p* value	*r*	*p* value	*r*	*p* value	*r*	*p* value	*r*	*p* value	*r*	*p* value	*r*	*p* value
Mean Lower Limb	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**	**0.00001**	**0.7**	**0.0001**	**−0.76**	**0.00001**	**−0.70**	**0.0001**	**0.78**	**0.000001**
Thigh															
AD	FF	**0.63**	**0.001**	0.37	0.07	**0.70**	**0.00009**	**−0.68**	**0.0003**	**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	0.22	**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**	**0.00009**	0.30	0.15	**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	0.16	**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	0.14	**−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	0.11	**−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**	**0.00006**	**−0.66**	**0.003**	**0.78**	**0.000008**
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**
Leg															
LC	FF	**0.52**	**0.007**	**0.44**	**0.030**	**0.51**	**0.008**	−0.28	0.195	**0.47**	**0.018**	**0.41**	**0.043**	**−0.56**	**0.004**
	MTR	**−0.63**	**0.001**	**−0.46**	**0.02**	**−0.66**	**0.0003**	**0.58**	**0.03**	**−0.69**	**0.0001**	**−0.58**	**0.003**	**0.74**	**0.00003**
AC	FF	**0.70**	**0.00008**	**0.42**	**0.037**	**0.47**	**0.016**	−0.30	0.167	0.36	0.072	0.31	0.130	**−0.44**	**0.031**
	MTR	**−0.73**	**0.00003**	**−0.42**	**0.04**	**−0.70**	**0.0002**	**0.64**	**0.001**	**−0.69**	**0.0002**	**−0.61**	**0.001**	**0.86**	**0.00001**
DP	FF	**0.60**	**0.002**	**0.61**	**0.001**	**0.65**	**0.0004**	**−0.44**	**0 0.033**	**0.53**	**0.006**	**0.47**	**0.018**	**−0.62**	**0.001**
	MTR	**−0.67**	**0.00002**	**−0.5**	**0.01**	**−0.90**	**0.0001**	**0.75**	**0.0001**	**−0.82**	**0.0001**	**−0.65**	**0.001**	**0.86**	**0.000005**
So	FF	**0.57**	**0.003**	**0.50**	**0.012**	**0.65**	**0.0004**	**−0.42**	**0.047**	**0.53**	**0.006**	**0.51**	**0.010**	**−0.53**	**0.002**
	MTR	**−0.67**	**0.00001**	**−0.49**	**0.01**	**−0.86**	**0.0001**	**0.70**	**0.0002**	**−0.80**	**0.0001**	**−0.78**	**0.0001**	**0.84**	**0.000002**
GM	FF	**0.62**	**0.001**	**0.59**	**0.002**	**0.59**	**0.002**	**−0.50**	**0.015**	**0.60**	**0.001**	**0.47**	**0.020**	**−0.61**	**0.001**
	MTR	**−0.61**	**0.001**	**−0.55**	**0.005**	**−0.80**	**0.0001**	**0.67**	**0.001**	**−0.82**	**0.0001**	**−0.74**	**0.0001**	**0.86**	**0.000009**

TABLE 2 (Continued)

		Age		Disease duration		PND score		RODS score		ONLS score		NIS-LL		Sum of CMAP	
		r	*p* value	*r*	*p* value	*r*	*p* value	*r*	*p* value	*r*	*p* value	*r*	*p* value	*r*	*p* value
GL	FF	**0.55**	**0.004**	**0.41**	**0.044**	**0.52**	**0.007**	−0.40	0.06	**0.40**	**0.046**	0.33	0.109	**−0.41**	**0.044**
	MTR	**−0.62**	**0.001**	**−0.44**	**0.03**	**−0.80**	**0.0003**	**0.75**	**0.00003**	**−0.68**	**0.0001**	**−0.65**	**0.001**	**0.71**	**0.000009**

Note: Significant correlations are indicated in bold. -, - Correlation not realized for muscles without pathological fatty infiltration.

Abbreviations: AC, anterior compartment; AD, adductor; BF, biceps femoris; DP, deep posterior compartment; FF, fat fraction; GL, gastrocnemius lateralis; GM, gastrocnemius medialis; GR, gracilis; LC, lateral compartment; MTR, magnetization transfer ratio; NIS-LL, Neuropathy Impairment Score for the Lower Limb; ONLS, Overall Neuropathy Limitations Scale; PND, peripheral neuropathy disability; RODS, Rasch-Built Overall Disability Scale; SA, sartorius; SM, semimembranous; So, soleus; ST, semitendinous; VI, vastus intermedius; VL, vastus lateralis; VM, vastus medialis.

Interestingly, a very strong correlation was identified between MTR and fatty infiltration in patients and controls ($r = 0.78$, $p < 0.0001$; Figure 5).

DISCUSSION

The use of specific imaging sequences and a semi-automatic image segmentation technique allowed us to study, in depth, 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. FF abnormalities were also found in asymptomatic patients.

Fatty infiltration in TTR-FAP patients was previously reported by Primiano et al. [28]. Their observations were based on a visual analysis of T1-weighted images and on a single slice in 10 patients without control subjects. This is a descriptive and semi-quantitative approach, as it does not allow exact quantification of the anomalies. In the present study, 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 previously been used to assess fatty infiltration in the muscle in CMT disease, a genetically inherited length-dependent neuropathy. Using a similar quantitative approach, Bas et al. [24] reported 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 [29]. This pattern is similar to that reported in the present study in TTR-FAP patients.

Two quantitative MRI parameters, 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 gastrocnemius medialis 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 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 amyloid neuropathy, it is plausible to hypothesize that the gastrocnemius medialis would be the first to be affected in the natural course of TTR-FAP. Therefore, the gastrocnemius medialis could be a good muscle candidate for assessing disease progression after the onset of clinical symptoms.

For asymptomatic patients, the gastrocnemius lateralis muscle in the leg, could be a more interesting candidate, as abnormal fatty infiltration was observed in this muscle. Interestingly, this muscle showed fewer correlations with clinical scores, particularly with the NIS-LL, which includes neurological deficits. This is not surprising as these muscle abnormalities were quantified in asymptomatic patients with normal clinical examination and electroneuromyography

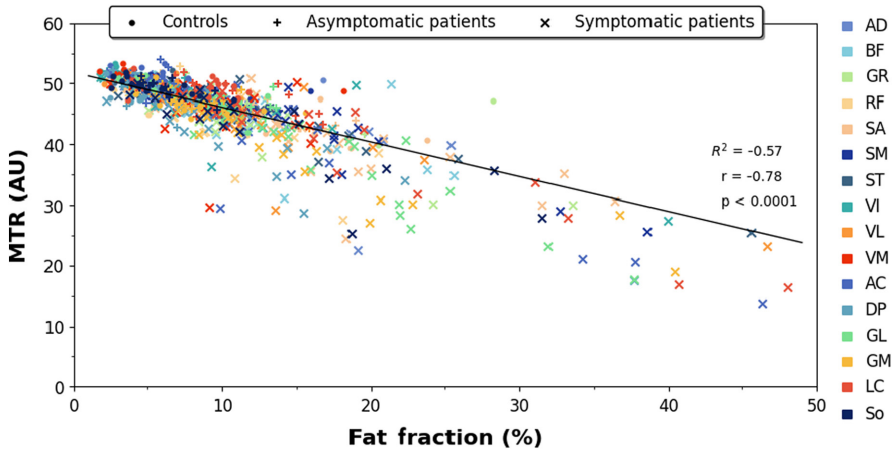

FIGURE 5 Correlation between fat fraction (FF) and magnetization transfer ratio (MTR) for each muscle in controls, asymptomatic patients, and symptomatic patients. Thigh: AD, adductor; BF, biceps femoris; GR, gracilis; RF, rectus femoris; SA, sartorius; SM, semimembranous; ST, semitendinous; VI, vastus intermedius; VL, vastus lateralis; VM, vastus medialis. Leg: AC, anterior compartment; DP, deep posterior compartment; GL, gastrocnemius lateralis; GM, gastrocnemius medialis; LC, lateral compartment; So, soleus.

results. These results suggest that fatty infiltration of the gastrocnemius lateralis 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 proton exchange between water and macromolecules [30]. Similar decreases in MTR have been reported in several neuromuscular diseases [31]. Sinclair et al. [32] reported a similar reduction in CMT and chronic demyelinating polyradiculoneuropathy. This reduction has been suggested to be 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 fatty infiltration might be related to the MTR decrease; therefore, these two variables might reflect the same pathological process. However, considering individual muscles, our results demonstrate some peculiarities for a few muscles (i.e., the 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 an early marker of the neuropathic process. However, in the asymptomatic group, the gastrocnemius lateralis muscle was abnormaly 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 in 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 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 gastrocnemius lateralis 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. We acknowledge the number of subjects included in this study was a limitation. This low number is attributable to the rarity of the disease, a factor that has similarly limited patient numbers in previous studies utilizing magnetic resonance neurography [17, 18]. 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 interesting potential biomarkers in familial amyloid polyneuropathy. Interestingly, MTR was abnormally decreased for muscles with an FF within the normal range, suggesting that MTR could be an early biomarker of muscle alterations. Moreover, in asymptomatic patients, fatty infiltration in the gastrocnemius lateralis

muscle could be considered 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 to determine their sensitivity to disease progression would be highly interesting.

AUTHOR CONTRIBUTIONS

Clémence Durelle: Investigation; writing—original draft; writing—review and editing; formal analysis. Emilien Delmont: Investigation; writing—review and editing; methodology; validation; supervision. Constance Michel: Software; methodology. Amira Trabelsi: Software; methodology. Marc-Adrien Hostin: Software. Augustin Ogier: Writing—review and editing; Software. David Bendahan: Investigation; writing—review and editing; validation; methodology; supervision; software; conceptualization. Shahram Attarian: Conceptualization; investigation; methodology; writing—review and editing; validation; supervision.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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APPENDIX A

TABLE A1 MRI sequences.

	TR, ms	TE, ms	Voxel size, mm * mm
2D T1	549	11	0,7 * 0,7
3D GR	22	2.38–19,6	1,7 * 1,7
3D GRE_{MTR}	36	3.5	1,7 * 1,7

Abbreviations: 2D, two-dimensional; 3D, three-dimensional; GRE, gradient echo; MTR, magnetization transfer ratio; TE, echo time; TR, repetition time.