

Distinct inter- and intra-muscular features observed by MR imaging and spectroscopy in patients with FSHD uncover pathobiological processes in disease development

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Introduction: Facioscapulohumeral dystrophy (FSHD) is the third most common muscular dystrophy with a prevalence of 1:20000. FSHD is associated with contraction of D4Z4 microsatellite repeats on chromosome 4q35, leading to a stabilized distal DUX4 transcript [1]. The disease is typically characterized by initial facial and scapular muscle weakness, followed by pelvic and lower limb muscles dysfunction. The affected muscles in a FSHD patient are distributed unevenly. Until recently, non-invasive biomarkers to follow disease progression and treatment effects were lacking, but from MR studies of the lower leg we deduced 2 such biomarkers. One from ¹H MRI assessment of T2 values identifying that affected muscles have high fractions of fat infiltration [2] and another from ³¹P MRSI which showed an abnormal PCr/ATP ratio as a reflection of abnormal energy metabolism in FSHD [3]. The aim of this study was to exploit these non-invasive biomarkers for a more detailed assessment of muscular involvement and progression towards a diseased state in FSHD. The examinations were performed on the upper leg to prepare for the evaluation of a therapeutic training regime targeting upper leg muscles.

Materials and methods: Recruitment: 30 genetically proven FSHD patients, 19 males and 11 females, average age 53 +/- 15 years (range 21-77) were included. They were randomly divided in 3 groups: i) control group: usual care, ii) four months of aerobic exercise training or iii) four months of cognitive behavioral therapy [4].

MR protocol: All patients underwent a baseline MR measurement, the therapy groups have a 2nd MR exam after 7 months and the control group after 4 months. MR was performed on a Siemens Trio 3T system using a ¹H volume coil for imaging and a surface coil for ³¹P MR spectroscopy. The coils were placed to acquire images of the upper leg of the patient. A marker was positioned at 1/3 of the distance between spina iliaca anterior superior and patella. For qualitative assessment T1-weighted images were acquired with a spin-echo sequence (TR/TE: 530 ms/16 ms, 23 slices, slice thickness/gap: 4 mm/0.4 mm, FOV 175 mm x 175 mm). Multi spin echo MR images were recorded of the same location (TR: 3 sec, TE: 16 echo times 7.7 ms - 123.2 ms, 4-8 slices; limited by SAR, slice thickness/gap 6 mm/9 mm, FOV 175 mm x 175 mm). Finally 3D ³¹P MRSI data is acquired (TR/TE: 1 sec/0.1 ms, matrix size: 16 x 16 x 8, FOV: 150 x 150 x 200 mm, 12 averages)

Analysis: Fat content was derived from multi spin echo images by fitting the signal intensity to a biexponential function with fixed T2 relaxation times for muscle (40 ms) and fat (143 ms) [3], this was done with a custom-made IDL program calculating muscle and fat fractions. Muscles of the upper leg investigated are the: rectus femoris (RF), vastus lateralis (VL), vastus intermedius (VI), vastus medialis (VM), Sartorius (S), adductor longus (AL), adductor magnus (AM), gracilis (G), semimembranosus (SM), semitendinosus (ST), biceps femoris long head (BFL) and biceps femoris short head (BFS). Linear correlation analysis was performed on the fat infiltration over the slices of each muscle to estimate if intramuscular fat was linearly distributed along the length of the muscle.

The ³¹P MR spectra of the quadriceps muscles: RF, VM, VI and VL, were fitted in the time domain with AMARES.

Myometry: Muscle strength of quadriceps and hamstring (BFS, BFL, ST, and SM) muscles, was determined with a quantitative fixed myometry testing system [5].

Results: Of 389 thigh muscles the fraction of infiltrated fat was determined. The analysis of this data revealed that muscles of FSHD patients are divided nearly binary over a high and a low fat fraction (see Fig 1). With 37% of the muscles having a fatty infiltration of less than 33 and 52% of the muscles having a fatty infiltration of more than 66%, leaving only 11% with an intermediate fat infiltration of between 34 and 66%. Interestingly, in this intermediate group with an average fatty infiltration between 30 and 70% muscular fat content increased linearly from proximal to distal with on average 7±6 percent per slice (correlation coefficient of >0.55, see Fig. 1). Moreover, results of 59 muscles showed that in these intermediately affected muscles the muscular fraction significantly decreased over time within 4 - 7 months (P <0.001, paired t-test), while the muscles with fat infiltration > 70% or < 30% did not change in muscle fraction over the same period (Fig.2).

Average fat infiltration was not specific for any muscle, and did not correlate with disease onset for all investigated muscles (P=0.10, R² = 0.12), with the exception of the quadriceps muscles, in which average fat infiltration did correlate with disease onset (P = 0.034, R² = 0.27).

The average remaining muscle fraction of the quadriceps and hamstring muscle groups, corrected for muscular area, inversely correlated with the corresponding muscle strengths (quadriceps: P= 0.0017, R² = 0.57, hamstrings: P= 0.019, R² 0.38), see Fig. 3).

PCr/ATP, Pi/ATP and pH were determined from the ³¹P spectra of the quadriceps in 23 patients. Only PCr/ATP showed a significant correlation with the corresponding muscle fraction (P = 0.036, R² = 0.22).

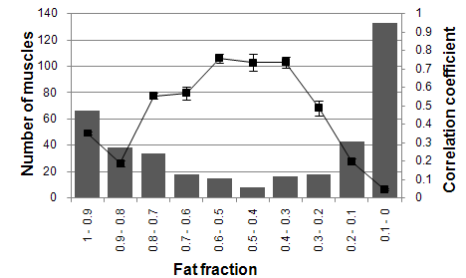


Figure 1: Histogram showing distribution of fatty infiltration of 398 thigh muscles of FSHD patients. The line gives the correlation coefficient for linear distribution of fat over the length of the muscle.

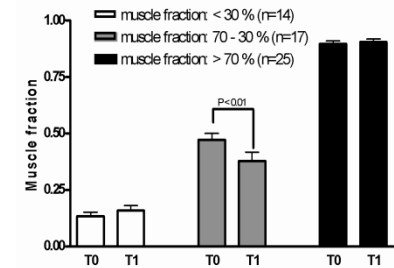


Figure 2: Muscle fraction measured at 2 time points (baseline, T0; after 4 months, T1). Only the intermediately affected group showed a significant decrease.

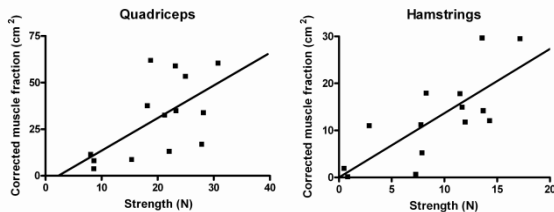


Figure 3: Correlation between muscle strength and corresponding remaining muscular

Discussion and conclusions: In this project we had the unique opportunity to study a relatively large group of individuals with FSHD. In this way a number of new biological features was identified in the skeletal muscles of these patients. *Firstly*, muscles in the legs of these patients are nearly binary divided over unaffected and completely affected categories as recorded from their fat content. *Secondly*, only for the intermediately affected muscles a heterogeneity in fatty infiltration over the length of the muscle was observed and also a change in fat content occurred over time. Combined, this suggests that once disease onset in a muscle is triggered by an event this inevitably rapidly leads towards nearly complete muscular dysfunction. Why this seems to hit different skeletal muscles randomly and at an unpredictable moment is unknown. An exception is the observation that fat infiltration in quadriceps correlates with disease onset, which may indicate that this muscle carries some specific properties related to FSHD etiology. *Thirdly*, we confirmed that muscular performance (strength) is inversely correlated with fat infiltration. And *finally* the abnormal PCr/ATP ratio indicates that in remaining functional muscles energy metabolism has changed, possibly in an attempt to compensate for overload in the development towards a FSHD condition, for instance adapting to a more oxidative phenotype.

References: 1. Lemmers *et al.*, Science (329), 2010; 2 Kan *et al.*, Neuromuscular Disorders (19), 2009 ; 3 Kan *et al.*, NMR Biomed (23), 2010; 4. Voet *et al.*, BMC Neurology (10), 2010; 5. Van der Kooi *et al.*, Neurology (63) 2004

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