

MRI validation of a transcriptional cascade propagation model in FSHD muscular dystrophy

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Introduction:

Facioscapulohumeral muscular dystrophy (FSHD) is the third most common hereditary muscular disorder. FSHD is linked to contractions of the D4Z4 repeat array on chromosome 4q35, leading to lost repression of DUX4, a protein that exerts toxic effects on muscle cells[1]. However in recent biopsy studies it was found that DUX4 is only expressed in 1/1000 FSHD myoblasts [2]. Furthermore it was found that also non-affected first degree siblings showed DUX4 expression, even though at significantly lower levels [3]. These findings demonstrate that apart from DUX4 expression other mechanisms must be involved for unfolding of FSHD muscle pathology.

In the search for an explanation on how such a rare occurring protein could cause a myopathy Tassin et al. [4], presented cellular evidence for a model of "dynamic propagation and initiation of a transcriptional cascade". In this model the DUX4 gene is activated in one or few myonuclei yielding the DUX4 proteins. These diffuse into the cytoplasm towards neighboring nuclei where they activate target genes, causing expansion into a transcriptional cascade of deregulation (oxidative stress, inflammation, fatty infiltration, atrophy).

The aim of this study was to uncover patterns in the data of our quantitative MRI exams of muscles of patients that could validate the value of this propagation model in human FSHD.

Materials and methods:

Recruitment: Thirty-six genetically proven FSHD patients were included (24 male, age range: 21-81 years). Eleven patients received a follow-up exam after four months (8 male, age range 34-76 years).

MR protocol: MR was performed on a 3T Siemens Trio using a ¹H volume coil to measure 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). Turbo Inversion Recovery Magnitude (TIRM) images were collected with nulled fat signals to visualize inflammation associated edema. (TR/TE/IT 4000 ms/41 ms/ 22 ms, 23 slices slice thickness/gap: 4mm/0.4 mm, FOV 175 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 bi-exponential function with fixed T2 relaxation times for muscle (40 ms) and fat (143 ms) [5], this was done with a custom-made IDL program calculating muscle and fat fractions. Muscle fraction = 1 - fat fraction. By visual assessment and marker position the overlapping slices between the first and second measurement were selected, an average over the slices was taken so an average muscle fraction was derived for every investigated muscle for every patient at two time points. The ³¹P MR spectra of the quadriceps muscles: RF, VM, VI and VL, were fitted in the time domain with AMARES. The muscles were divided in three groups having either a normal (>0.75), intermediate (<0.75 and >0.25) or low muscle fraction (<0.25).

Results:

In total 427 individual FSHD muscles were investigated, of which 262 (61%) had a normal, 54 (13%) an intermediate and 111 (26%) a low muscle fraction. A prominent feature of the intermediate fraction was that the fat content was higher in the distal than proximal part of muscles (Fig. 2). These muscles showed a significantly steeper fatty infiltration gradient over the length of the muscle compared to muscles with a normal or low muscle fraction: $7 \pm 1\% \text{cm}^{-1}$ (mean \pm SEM) compared to $1.3 \pm 0.3\% \text{cm}^{-1}$ and $1.1 \pm 0.1\% \text{cm}^{-1}$ respectively. Furthermore PCr/ATP ratio was significantly decreased in muscles with an intermediate muscle fraction compared to normal muscles (3.48 ± 0.88). Follow-up measurements were successfully performed in 85 muscles of eleven patients. Progression of fatty infiltration was fastest in muscles with an intermediate fat fraction (Fig 2): $18 \pm 15\% \text{year}^{-1}$ (mean \pm sd). This was significantly faster compared to muscles with a normal muscle fraction ($4 \pm 10\% \text{year}^{-1}$) or low muscle fraction ($0 \pm 10\% \text{year}^{-1}$). Inflammation as reflected in TIRM images was more prominent in (intermediate phase) muscles prone to progress.

Conclusion and discussion:

We identified a quasi-binary distribution of fatty infiltration in FSHD muscles, which suggests an abrupt transition from normal to heavy fat infiltrated muscles. Moreover, the minor intermediate muscle fraction showed rapid disease progression, which agrees with the clinical observation that FSHD has long periods of stable disease and short periods of rapid progression in one muscle or muscle group. In addition we showed that this progressive phase has a gradient of fatty infiltration over the length of the muscle and decreased PCr/ATP. Our findings match very well with the transcriptional cascade model [4], which predicts that a stochastic event of DUX4 gene activation, leads to rapid propagation of muscle deregulation towards the fully diseased phase. Because of the multinucleated nature of muscle fibers the model also predicts a gradient of deregulation over the length of the muscles, exactly as reflected in our study by a fatty infiltration gradient. In addition to the propagation model we can add that fatty infiltration starts in the distal part of muscles by an as yet unknown trigger causing initiation of DUX4 expression. We conclude that propagation model is an important addition, next to the genetic abnormalities, in our understanding of FSHD and should be of value in the search for treatments.

References:

[1] Lemmers R, Science 329, 2010 [2] Snider L, PLoS Genet 6, 2010 [3] Jones T, Hum Mol Genet 21, 2012 [4] Tassin A, J Cell Mol Med, 17, 2013, [5] Kan H, Neuromuscul Disord 19, 2009

Acknowledgments: Prinses Beatrix Fonds (Grant WAR08-15), ZonMW (Grant 89000003), and FSHD Global Research Foundation.

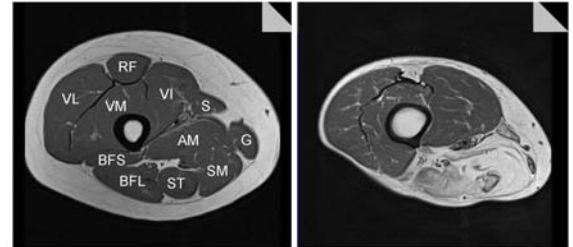


Figure 1: Muscles in FSHD present as either normal or highly fat infiltrated. Transversal T2 weighted images of the upper legs of two FSHD patients. Left: 24 year old female FSHD patient with normal appearing thigh muscles. Right: 62 year old male FSHD patient with clear fatty infiltration in the RF, AM, BFL, BFS, SM and ST. AM = adductor magnus; BFL = biceps femoris long head; BFS = biceps femoris short head; G = gracilis; RF = rectus femoris; S = Sartorius; SM = semimembranosus; ST = semitendinosus; VI = vastus intermedius; VL = vastus lateralis; VM = vastus medialis.

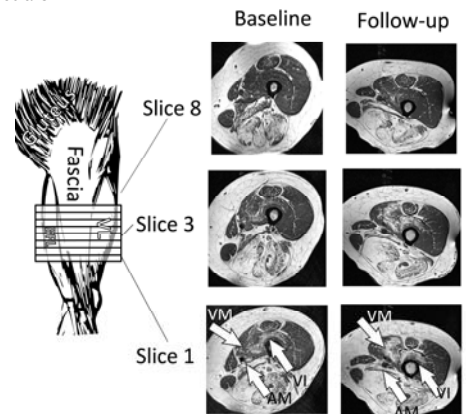


Figure 2: Intermediate muscle fraction muscles show an fatty infiltration gradient over the length of the muscles, suggesting that the the fatty infiltration progresses over the length of the muscles. In this figure it is clearly seen that for the VI, VM and AM, the fat progresses from the distal part to the more proximal part of the muscle.