Natural History of Facioscapulohumeral Dystrophy in Children: A 2-Year Follow-up

Author(s):
Jildou N. Dijkstra, MD1,2; Rianne J.M. Goselink, MD, PhD3; Nens van Alfen, MD, PhD4; Imelda J.M. de Groot, MD, PhD4; Maaike Pelsma4; Nienke van der Stoep, PhD5; Thomas Theelen, MD PhD6; Baziel G.M. van Engelen, MD PhD1; Nicol C. Voermans, MD PhD1; Corrie E Erasmus, MD PhD2

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Equal Author Contributions:
Nicol Voermans and Corrie Erasmus contributed equally to this work

Corresponding Author:
Corrie E Erasmus
corrie.erasmus@radboudumc.nl

Affiliation Information for All Authors: 1. Department of Neurology, Donders Centre of Neurosciences, Radboud University Medical Centre, Nijmegen, The Netherlands; 2. Department of Pediatric Neurology, Amalia Childrens Hospital, Radboud University Medical Centre, Nijmegen, The Netherlands; 3. Department of Neurology, Jönköping, Region Jönköping County, and Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden; 4. Department of Rehabilitation, Donders Centre for Neuroscience, Radboud University Medical Centre, Nijmegen, The Netherlands; 5. Department of Clinical Genetics, Leiden University Medical Centre, Leiden, The Netherlands; 6. Department of Ophthalmology, Radboud University Medical Centre, Nijmegen, The Netherlands

Contributions:
Jildou N. Dijkstra: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data
Rianne J.M. Goselink: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data
Nens van Alfen: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data
Imelda J.M. de Groot: Study concept or design
Maaike Pelsma: Major role in the acquisition of data
Nienke van der Stoep: Major role in the acquisition of data; Analysis or interpretation of data
Thomas Theelen: Major role in the acquisition of data
Baziel G.M. van Engelen: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data
Nicol C. Voermans: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data
Corrie E Erasmus: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data

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Abstract

Background and objectives

Data on the natural history of facioscapulohumeral dystrophy (FSHD) in childhood is limited and critical for improved patient care and clinical-trial readiness. Our objective was to describe the disease course of FSHD in children.

Methods

A nationwide, single-centre, prospective cohort study of FSHD in childhood assessing muscle functioning, imaging and quality of life over two years of follow-up.

Results

We included 20 children with genetically confirmed FSHD aged 2 to 17 years. Overall, symptoms were slowly progressive, and the mean FSHD clinical score increased from 2.1 to 2.8 (p=0.003). The rate of progression was highly variable. At baseline, 16/20 symptomatic children had facial weakness, after two years this was observed in 19/20 children. Muscle strength did not change between baseline and follow-up. The most frequently and most severely affected muscles were the trapezius and deltoid. The functional exercise capacity, measured using the six-minute walk test improved. Systemic features were infrequent and non-progressive. Weakness-associated complications like lumbar hyperlordosis and dysarthria were common and their prevalence increased during follow-up. Pain and fatigue were frequent complaints in children and their prevalence increased too during follow-up. Muscle ultrasonography revealed a progressive increase in echogenicity.

Discussion

FSHD in childhood has a slowly progressive, but variable, course over two years of follow-up. The most promising outcome measures to detect progression were the FSHD clinical score and muscle ultrasonography. Despite this disease progression, an improvement on functional capacity may still occur as the child grows up. Pain, fatigue and a decreased quality of life were common symptoms,
and need to be addressed in the management of childhood FSHD. Our data can be used to counsel patients and as baseline measures for treatment trials in childhood FSHD.

Background and purpose

Facioscapulohumeral muscular dystrophy (FSHD) is a slowly progressive muscular dystrophy and one of the most common hereditary muscle diseases, with an estimated prevalence of 12 per 100,000 in the Netherlands. FSHD typically causes progressive, asymmetric weakness of the facial, scapulohumeral, tibial and axial muscles, with a highly heterogeneous disease severity. Type 1 FSHD, responsible for over 95% of the FSHD cases, is associated with a contraction of the D4Z4-repeat on chromosome 4q35. In the normal population this array contains 11-100 D4Z4 repeats, whereas in FSHD there is a contraction of the D4Z4 region to 1-10 repeat units, leading to D4Z4 hypomethylation. In type 2 FSHD, hypomethylation of the D4Z4 repeats is caused by mutations in SMCHD1 or DNMT3B gene. Both types lead to disease in the setting of a permissive allele, with aberrant DUX4 expression in skeletal muscle. The age of symptom onset in FSHD is variable; in approximately one fifth of all the patients FSHD starts in childhood. Approximately half of the children with FSHD fulfill the criteria of early-onset FSHD, a subgroup defined by facial weakness before the age of 5 and scapular weakness before the age of 10. This subgroup is associated with a fewer number of D4Z4 repeats, more extensive muscle weakness, and more systemic features (including hearing loss, retinal abnormalities, epilepsy, intellectual disability and cardiac arrhythmias) compared to those of similar age and disease duration with a "classic onset FSHD". Childhood FSHD is characterized by a significant variability in symptoms and clinical disease course. However, so far only cross-sectional observations are available, and little is known about the natural course in this age group. The rate of disease progression and the presence of systemic features within this population are also unknown. There is a need for the identification of prognostic factors that can be used in patient care and counseling. Knowledge of the natural history is also required for the design of clinical trials.
In this study we present the two-year follow-up of a nationwide prospective cohort study of 28 children diagnosed with FSHD in The Netherlands. Baseline data were reported as the iFocus FSHD study. The primary aim of the current work was to assess the clinical features and natural course of FSHD in childhood in a two-year follow up period, to identify prognostic factors of disease severity and progression rate, and to find promising outcome measures that can serve as a base for further clinical trials.

Patients and methods

Patients and Design
This is a prospective cohort study of FSHD in childhood. The iFocus FSHD study cohort includes 28 children with genetically confirmed type 1 FSHD, aged 0-17 years and living in the Netherlands, of whom one was asymptomatic at baseline. A detailed protocol and description of the baseline characteristics can be found elsewhere.

Clinical Assessments
Clinical assessment of the patients was performed by the same examiners at study initiation and after two years of follow-up. The follow-up measurements took place from March 2018 until March 2020, all assessments were performed as a part of this study. The study visit took approximately four hours, including a lunch break. When patients were unable to visit the study site, information was obtained by a phone interview with the patient and/or a parent, supplemented with information from the electronic health record including a recent evaluation by the participant’s pediatric neurologist and/or rehabilitation physician. A full description of the study procedures can be found in the published protocol.

In brief, the clinical phenotype of each participant was assessed by the Motor Function Measure (MFM), the shoulder dimension of the Performance of the Upper Limb (PUL) module, by manual muscle force testing using the Medical Research Council (MRC)-sum score from the trapezius, deltoid, biceps brachii, finger flexors, quadriceps femoris, tibialis anterior and gastrocnemius muscle on both sides, and by testing motor performance using the six-minute walk test. The MFM assesses proximal, distal and axial motor functions and has been developed for the...
broad spectrum of neuromuscular diseases. A short form is validated for the use in children aged 2 to 7 years. The PUL scale was developed to measure upper limb function in patients with Duchenne muscular dystrophy. Weakness is tested through three domains: shoulder, mid and distal, of which the shoulder domain is relevant for the FSHD population (minimal score 0, maximum score 16). Results on the six-minute walk test were expressed as a z-score (the number of standard deviations from the mean for sex and age in healthy individuals). In addition, the FSHD clinical score (an evaluation scale that assesses the strength and functionality of six different groups of muscles, typically involved in FSHD. The total score ranges from 0 when no symptoms are present to a maximum of 15) and (age-corrected) FSHD clinical severity scale (CSS) (a score ranging from 0-10 that evaluates the extent of weakness in FSHD, considering the descending spread of symptoms) were scored. The burden of disease on the quality of life was assessed by asking about the presence and location of pain, evaluating fatigability (NeuroQol fatigue domain), and scoring the Kidscreen questionnaire. These questionnaires were completed by children aged 8 years and older, if participants were younger their caregivers were asked to complete these forms. Information about (para)medical treatments was obtained from an interview with the patient and/or parent, and concerned the doctors and therapists involved in the treatment and the frequency of these visits. Muscle imaging was performed using muscle ultrasonography, with visual grading according to the Heckmatt scale (a four-point visual grading scale to classify muscle intensity, ranging from 1 for normal ultrasound appearance to 4 for very strongly increased echogenicity) and quantitative grayscale analysis (QMUS) to determine echogenicity of the bilateral trapezius, biceps brachii, rectus abdominis, rectus femoris and tibialis anterior muscles. Grayscale results were expressed as a z-score, i.e. the number of standard deviations from the mean, after comparing the echogenicity to a muscle specific reference value. Patients were screened for systemic disease features using ophthalmologic screening including a fundoscopy and visual acuity testing by an ophthalmologist, tone- and speech audiometry by an audiometrist, an electrocardiogram (ECG), and observation of possible spinal deformities.
Statistical Analyses

Statistical analyses were performed using SPSS version 25 (IBM SPSS inc., Chicago IL, USA).

Continuous parametric variables were expressed as the mean and standard deviations, whereas continuous nonparametric variables were expressed as the median and interquartile ranges.

Categorical data were given as a percentage. The Kolmogorov-Smirnov test was used for testing normality assumptions in the distribution of the data. For continuous variables with a normal distribution a paired t-test was performed to test a mean difference between the baseline and the two-year follow-up. The Wilcoxon signed rank test was performed to compare nonparametric continuous variables, and the McNemar test was used for comparing paired categorical data. For comparison between the group "early onset" and "classic onset" at two-year follow-up, the Mann-Whitney U test was used for nonparametric continuous variables, the independent t-test for continuous variables with a normal distribution, and the Fisher’s exact test for categorical variables. Linear mixed models were applied to analyze differences in disease progression. The continuous variable of the FSHD clinical score was used as the primary outcome, whereas the repeat length (divided in 1-3, 4-6 and 7-10 D4Z4 repeat unit’s categories), the age at the baseline, and sex were used as the fixed effect predictors. The level of statistical significance was set at ≤0.05.

Standard Protocol Approvals, Registrations, and Patient Consents

The protocol has been approved by the Medical Review Ethics Committee region Arnhem-Nijmegen (NL53213.091.15). Written consent for study participation was provided by parents/legal guardians of all participants and by participants aged 12 to 18 years.18, 19

Data availability

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

Demographics and genetic characteristics

Of the 28 participants in the baseline study, a total of 20 children from 17 families, including three sibling pairs, could be included for this follow-up study. Thirteen of them were clinically examined at the study location. An electronic health record review combined with a telephone interview was performed in seven participants; two of them could not be examined at the study location because of the COVID-19 pandemic restrictions; the other five were unable to visit for other reasons, mainly because of the added burden of a hospital visit. (Figure 1) Participants were very cooperative, all of them gave permission to approach them again for future study visits.

The demographic and genetic characteristics of the cohort of 20 children who completed the two-year follow-up are shown in Table 1. We observed a significant correlation between mean number of units in the pathogenic D4Z4 repeat and the age at onset, with a lower number of D4Z4 units associated with a younger age of onset ($r = 0.498, p=0.015$). No significant correlation between the number of D4Z4 repeats and disease severity as measured by the FSHD clinical score and clinical severity scale was found at either baseline or follow up. Additionally, no correlation between the age of onset and disease severity (FSHD clinical score and clinical severity scale) was found.

Clinical Characteristics and changes over two year

Muscle function

At baseline, 15/20 patients (75%) had facial weakness and two years later this had increased to 19/20 (95%). One child developed new scapular weakness during follow-up; a total of six out of 15 patients had functional impairment of shoulder and/or arm after two years. The mean MRC sum score of the group did not change between baseline and follow-up, and varied between 60 to 70 (median of 69, maximum score 70). The most frequently and most severely affected muscles were the trapezius and deltoid, but we also observed weakness of the biceps brachii, quadriceps, tibialis anterior and gastrocnemius muscles. The mean clinical severity scale was stable over the two year period, despite variability between individuals. The mean FSHD clinical score increased from 2.1 to
The mean result of the six-minute walk test and motor function measure showed a tendency towards increase, but this did not reach statistical significance. Despite better test scores during follow-up, the six-minute walk distance was on average still below the mean for sex and age (mean: -1.3 SDs; range -2.7 to 0.2; SD 1.0), and a large variability between patients was observed. During follow-up, 11/20 patients (55%) were given physical therapy, compared to 8/20 (40%) at baseline.

All muscle measurements are summarized in Table 2. Figure 2 displays the change in FSHD clinical score, motor function measure, the six-minute walk test and the quantitative muscle ultrasonography results of individual patients over the course of two years.

Muscle imaging

Visual muscle ultrasound grading from 14 children at baseline and 9 children at follow-up showed a mean Heckmatt score of 1.4 and 1.6, respectively. Quantitative muscle ultrasound showed an increase in the mean z-scores from 1.0 to 2.1 after two years (Table 2).

The visual grading correlated moderately to strongly with the quantitative score (baseline r=0.55 p=0.02; 2-year follow-up r=0.88, p=0.001). Figure 3A shows mean z-score per muscle at two-year follow-up. All clinically affected muscles had an increased echogenicity, except for the rectus abdominis. Three patients with an increased lumbar lordosis, considered to be a sign of weakness of the abdominal muscles in FSHD, did not have an increased echo intensity of the bilateral rectus abdominis. In addition, spinal and hip girdle muscle weakness contribute to postural instability in FSHD. These muscles were not evaluated by muscle ultrasound. Not all muscles with an increased echogenicity were clinically affected. We found a negative correlation between the mean QMUS z-score and the MRC sum score (r = -0.74, p=0.01). Disease severity as measured by the FSHD clinical score demonstrated a trend towards a positive correlation with the mean z-scores, but this did not reach statistical significance (r = 0.51, p=0.07). One child showed a deterioration on muscle ultrasound with a z-score increase of 1.06, but no clinical changes as measured by the FSHD clinical score. Figure 2D shows the changes in mean echogenicity in individual patients over the course of 2 years, and demonstrates the variability in progression. Figure 3B and 3C show the distribution of the
measured muscles at baseline and at follow-up by visual (Heckmatt score) and quantitative (z-score) analysis.

Systemic features

The presence of systemic features is shown in Table 3.

Vision. None of the children or parents reported vision loss. Despite normal visual acuity, retinal abnormalities were frequently observed on fundoscopy, in six out of eight patients tested. Retinal changes consisted of mild tortuosity of the retinal arteries. The degree of retinal abnormalities did not change over 2 years. Coats' disease with retinal detachment was not observed.

Hearing. One child reported hearing problems, and in two children (12.5%) a hearing loss was detected by audiometry. One child had a conductive hearing deficit at baseline and a normal audiometry during follow up. There were no patients with a newly diagnosed hearing deficit on follow up.

Cardiac function. No changes were observed in the frequency of abnormalities seen on the electrocardiogram. We found a right-axis deviation without clinical consequences in one child.

Skeletal features. The percentage of children with lumbar hyperlordosis showed an increase from 35% to 53% over 2 years, but this change was not found to be statistically significant (Table 3). At baseline, a mild scoliotic posture was seen in two participants, however this was no longer observed during the neurological examination at the two year follow-up visit.

Speaking and swallowing. Bulbar dysarthria was found in 25% of children on baseline and its occurrence increased to 50% after two-year follow-up. All children with severe facial weakness had a dysarthric speech. No changes were observed in the number of children with swallowing difficulties over time.

Central nervous system. None of the participants had an intellectual disability or developmental impairment. One child was diagnosed by dyslexia and one child had psychological support to deal with anxiety. Seventeen out of 20 children (85%) attended regular education. None of the participants had or developed epilepsy.
Pain, fatigue and quality of life

Pain and fatigue were frequently reported, and their occurrence increased during the two-year follow-up (75% and 70%, respectively). The location of pain was mainly in the lower legs, in 53%, but also in the scapular region (27%) and lower back (20%). The pain was typically induced by muscle exercise and exertion. The NeuroQoL fatigue questionnaire showed that the children experienced more fatigue compared to healthy children, both at baseline and after two-year follow-up. The mean z-score did not increase during follow-up (Table 3).

Quality of life was lower in children with FSHD compared to their healthy peers. Low scores were found especially in the domains "physical well-being" and "social acceptance". Overall, there was no difference in quality of life at baseline and after two years. On follow up there was a significant improvement of the scores in the domains "parent relation and home life" and "school environment". Details are shown in Table 3.

*Differences early-onset and classic onset type*

The differences between children with an early-onset and classic onset type are listed in Table 4. Two of the 20 children were too young to determine their specific phenotype and were excluded from this analysis. Early-onset FSHD patients had a shorter number of units within the pathogenic D4Z4 repeat compared to classic onset patients, as expected. Patients with an early-onset type were significantly younger than patients in the classic onset group. Motor functioning after two years as measured by MRC-sum score, FSHD score, clinical severity scale and six-minute walking test did not differ significantly between these subgroups. The age-adjusted clinical severity scale score was higher in the early-onset subgroup ($p=0.02$). No significant difference in disease progression was found between the two onset types, with a mean change in FSHD clinical score of 0.63 points in the early-onset subgroup and 0.80 in the classic-onset subgroup ($p=0.65$).

The visual and quantified muscle echogenicity tended to be higher in early-onset patients, but these changes did not reach statistical significance ($p=0.11$ and $p=0.07$). During follow up, early-onset patients showed a bigger mean echogenicity change compared to classic onset patients: in early-onset patients an increase in mean z-score of $2.0\pm1.4$ was seen, compared to an increase of $0.1\pm0.5$.
in the classic-onset subgroup \(p=0.02\). The presence of pain, fatigue and systemic features did not vary significantly between the two groups, although hearing abnormalities were only observed in the children with an early onset FSHD who had a D4Z4 repeat size of two units.

**Determinants of disease progression**

The rate of disease progression in two years as measured by the change of FSHD clinical score was not influenced by sex, current age, the number of D4Z4 repeats or the mean echogenicity z-score at baseline.

**Discussion**

This 2-year follow-up study in children with FSHD clinically showed mild progression of facial weakness, pain and fatigue, and lumbar hyperlordosis. This was reflected by an increase of disease severity as measured by the FSHD clinical score. As in adults, the disease course was variable. The muscle ultrasound abnormalities increased over two years and correlated with disease severity. The early-onset subtype had shorter D4Z4 repeats and a more severe phenotype as indicated by a higher age-corrected clinical severity score (CSS), more ultrasound abnormalities, and a higher prevalence of hearing- and retinal abnormalities.

While the clinical scores indicated disease progression, the actual changes were limited. The FSHD clinical score, a sum score of six independent sections that describes strength and functionality of several muscle regions, was the only motor function outcome measurement that showed a significant deterioration. The children’s performance on functional exercise tests improved over two years, although it was still below the average performance for healthy peers. This most likely indicates the delayed, but still ongoing motor development in children with FSHD compared to healthy children, which is in line with observations in Duchenne muscular dystrophy patients, where a delay in gross motor development is seen in one third of children\(^{34}\). Physical therapy and functional training might also improve functional capacities\(^{35}\), but we did not find a relation between attending physical therapy and improvement. This result might also point to limitations of the six-minute walk...
test: its result is affected by the effort of the child. It gives us an estimate of the child’s functional status, but does not assess the mechanism of exercise intolerance. The improvement found emphasizes the importance of natural history studies to ensure correct implementation of these outcome measurements in future clinical trials in children.

The prevalence and severity of facial weakness, dysarthria and swallowing difficulties increased during follow-up. All children with severe facial weakness also had a dysarthric speech. The swallowing difficulties were mild, with a score of 7 on the neuromuscular disease swallowing status scale, that indicates that the person is totally orally fed without difficulties, but avoids more difficult to eat foods. The prevalence of these symptoms was higher than in the adult population and no difference between children with a classic onset or early-onset type was found, suggesting that orofacial function improves during the normal development in childhood. The low prevalence of systemic features found in this cohort, that did not increase after two-year follow-up, is similar to what is seen in the adult population with classic FSHD.

Our studied cohort confirmed the previous finding that muscle ultrasound is correlated with disease severity and a responsive biomarker for FSHD disease progression. Ultrasound was previously shown to have a similar sensitivity as quantitative MR imaging. Visual grading correlated with quantified muscle ultrasound, both could be used in evaluating muscle ultrasound abnormalities in children.

When comparing the subgroups defined by onset type, after two-year follow-up patients with an early-onset subtype are deteriorating faster in mean echogenicity than patients with a classic onset type. On the other hand, the progression on the FSHD clinical score did not differ, suggesting that muscle ultrasound abnormalities precede clinical deterioration and thus seem a reliable biomarker. This resembles the finding in Duchenne muscular dystrophy patients, where muscle echogenicity of patients already deviates from healthy controls in an early stage and is of great importance in the early detection of muscle functioning impairments.
Pain, fatigue and a decreased quality of life appear to be major problems in childhood, with a prevalence similar to that of the adult population. The current study reports an even higher proportion of children experiencing pain as compared to another recent study on FSHD in childhood (83% vs 61%), despite the fewer patients with systemic features or loss of ambulation in our cohort. This difference could be explained by the different approach to exploring the presence of pain in the current study, where we consciously asked for the presence of pain without the use of a pain scale, thus including all levels and patient perceptions of pain. Another possible explanation is that the children in our cohort experience more pain secondary to exercise, because of their maintained ambulation. The high prevalence of pain and fatigue in children with FSHD is a reason for concern, especially when taking into account the correlation between age and fatigue severity and increasing age leading to a further deterioration of the quality of life. For children this could have a substantial effect on participation in school and sports, and their professional development. We would suggest to create more focus on the management of pain and fatigue to ameliorate physical impairment and optimize the child’s abilities.

A strength of this nationwide study is that it provided information about the full spectrum of FSHD in childhood. Yet, our study also has some limitations. First, the small number of participants makes it hard to determine correlations. This could be addressed by an even more encompassing international study or prospective data collection initiative for patients with FHSD. Second, FSHD in childhood appears to be a slowly progressive disease, so two-year of follow-up is a short period to evaluate clinically relevant changes. A longer follow-up period is expected to provide further information about the natural history and support longer running treatment trials. Lastly, missing data were caused by COVID-19 and by the substantial proportion of patients and/or their parents who preferred not to visit the study center and participated by a phone interview and chart review only.
The slowly progressive course of childhood FSHD offers the opportunity for a future drug trial to delay or thwart this progression rate. Based on our results, the most promising outcome measures for analyzing the effects of future therapeutics are the FSHD clinical score and muscle ultrasound. We expect to further define the natural history and course in a subsequent five-year follow-up study of this cohort.

References:


### Table 1. Demographic and genetic characteristics in current two-year follow-up study

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<td>Male sex (%)</td>
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<td>D4Z4(^a) repeat units</td>
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\(^a\) Mean number of units within the pathogenic D4Z4 repeat.

\(^b\) The observed methylation minus the predicted methylation based on the D4Z4 repeat size.

Y = year; AD: autosomal dominant

* One child was from a family with FSHD and had been tested and diagnosed asymptotically.

### Table 2. Clinical and imaging characteristics at baseline and two-year follow-up

<table>
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<tr>
<th>Number of participants (at baseline – at 2-year follow-up)</th>
<th>Baseline scoring</th>
<th>Two-year follow-up scoring</th>
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<td>Motor functioning</td>
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<table>
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<tr>
<th></th>
<th>Baseline scoring</th>
<th>Two-year follow-up scoring</th>
<th>p-value</th>
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</table>
| **Facial weakness**
  No weakness             | 15/20 (75%)      | 19/20 (95%)                  | 0.13     |
  Moderate weakness       | 5 (25%)          | 1 (5%)                       |          |
  Severe weakness         | 12 (60%)         | 15 (75%)                     |          |
| Scapular weakness       | 5/15 (33%)       | 6/15 (40%)                   | 1.00     |
| MRC sum score (0-70)    | 70 [2]           | 69 [4]                       | 0.30     |
| Clinical severity scale | 2.2±1.5 (0-6)   | 2.6±1.8 (0-6)                | 0.18     |
| FSHD clinical score     | 2.1±1.7 (0-6)   | 2.8±1.9 (0-8)                | 0.003    |
| Six-minute walk test    | -2.1±1.5         | -1.3±1.0                     | 0.07     |
| Motor Function Measure  | 99% [1.8]        | 100% [0.8]                   | 0.11     |
| Muscle imaging
  MUS (z-score)        | 1.0±1.4          | 2.1±2.0                      | 0.02     |
| Heckmatt score         | 1.4±0.4          | 1.6±0.4                      | 0.07     |

*Based on one or more points on the facial weakness domain of the FSHD clinical score
Based on a score of 15 or lower on the Performance of the upper limb (PUL) shoulder module
Mean z-score of the quantified echogenicity per muscle measured by muscle ultrasound.
Mc Nemar test; Wilcoxon signed rank test; Paired t-test
FSHD = facioscapulohumeral dystrophy; MRC = Medical Research Council; MUS = muscle ultrasound; SD = standard deviation.

<table>
<thead>
<tr>
<th><strong>Systemic features</strong></th>
<th>Number of participants (at baseline – at two-year follow-up)</th>
<th>Baseline scoring</th>
<th>Two-year follow-up scoring</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hearing loss</td>
<td>18-16</td>
<td>3/18 (17%)</td>
<td>2/16 (12.5%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Vision loss</td>
<td>20-12</td>
<td>0/18 (0%)</td>
<td>0/12 (0%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Retinal abnormalities</td>
<td>11-8</td>
<td>8/11 (73%)</td>
<td>6/8 (75%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Cardiac abnormalities</td>
<td>15-13</td>
<td>3/15 (20%)</td>
<td>1/13 (8%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Assisted ventilation</td>
<td>20-20</td>
<td>0/20 (0%)</td>
<td>0/20 (0%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Lumbar hyperlordosis</td>
<td>20-19</td>
<td>7/20 (35%)</td>
<td>10/19 (53%)</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Early onset (N=8)</td>
<td>Classic onset (N=10)</td>
<td>P-value</td>
<td></td>
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<td>----------------</td>
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<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at baseline examination (y)</td>
<td>8.3±2.5</td>
<td>14.2±2.9</td>
<td>&lt;0.001&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Age at two year follow-up (y)</td>
<td>10.3±2.5</td>
<td>16.4±3.0</td>
<td>&lt;0.001&lt;sup&gt;3&lt;/sup&gt;</td>
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<tr>
<td>Age onset symptoms (y)</td>
<td>2.8±2.2</td>
<td>10.5±4.2</td>
<td>0.01&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>D4Z4&lt;sup&gt;a&lt;/sup&gt; (mean±SD)</td>
<td>3.8±2.2</td>
<td>6.4±1.7</td>
<td>0.02&lt;sup&gt;2&lt;/sup&gt;</td>
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<tr>
<td>Motor functioning</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>MRC sum score (0-70), (mean±SD)</td>
<td>66.7±3.9</td>
<td>69.0±1.4</td>
<td>0.17&lt;sup&gt;2&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Clinical severity scale (0-10), (mean±SD)</td>
<td>3.1±2.0</td>
<td>2.7±1.8</td>
<td>0.70&lt;sup&gt;2&lt;/sup&gt;</td>
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<tr>
<td>Age-corrected CSS (0-2000), (mean±SD)</td>
<td>288±131</td>
<td>168±112</td>
<td>0.02&lt;sup&gt;2&lt;/sup&gt;</td>
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<tr>
<td>FSHD clinical score (0-15), (mean±SD)</td>
<td>3.0±1.9</td>
<td>2.5±1.1</td>
<td>0.27&lt;sup&gt;2&lt;/sup&gt;</td>
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<tr>
<td>Six-minute walk test (number of SDs, mean±SD)</td>
<td>-1.5±1.1</td>
<td>-1.0±0.9</td>
<td>0.40&lt;sup&gt;3&lt;/sup&gt;</td>
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<tr>
<td>Motor Function Measure (mean±SD)</td>
<td>97.2±3.9</td>
<td>100±0</td>
<td>0.06&lt;sup&gt;2&lt;/sup&gt;</td>
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<tr>
<td>Muscle imaging</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>MUS (z-score, mean±SD)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.4±2.5 (n=4)</td>
<td>1.1±0.4 (n=5)</td>
<td>0.07&lt;sup&gt;7&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Heckmatt score (1-4) (mean±SD)</td>
<td>1.9±0.6 (n=4)</td>
<td>1.4±0.1 (n=5)</td>
<td>0.11&lt;sup&gt;7&lt;/sup&gt;</td>
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</tr>
<tr>
<td>ΔMUS&lt;sup&gt;c&lt;/sup&gt; (z-score, mean±SD)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0±1.4 (n=4)</td>
<td>0.1±0.5 (n=5)</td>
<td>0.02&lt;sup&gt;7&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Feature</td>
<td>N/Total (%)</td>
<td>p-value</td>
<td>Notes</td>
<td></td>
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<td>-------------------------------------</td>
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</tr>
<tr>
<td>Pain, N/Total (%)</td>
<td>7/8 (87.5%)</td>
<td>0.59</td>
<td>Fisher’s Exact Test, 1-sided p-value</td>
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</tr>
<tr>
<td>Fatigue, N/Total (%)</td>
<td>7/8 (87.5%)</td>
<td>0.38</td>
<td>Mann-Whitney U Test</td>
<td></td>
</tr>
<tr>
<td>Hearing abnormalities, N/Total (%)</td>
<td>2/7 (28.6%)</td>
<td>0.18</td>
<td>Independent t-test</td>
<td></td>
</tr>
<tr>
<td>Retinal abnormalities, N/Total (%)</td>
<td>3/3 (100%)</td>
<td>0.36</td>
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<tr>
<td>Lumbar hyperlordosis, N/Total (%)</td>
<td>4/8 (50%)</td>
<td>0.68</td>
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<tr>
<td>Swallowing difficulties, N/Total (%)</td>
<td>2/8 (25%)</td>
<td>0.43</td>
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<tr>
<td>Dysarthria, N/Total (%)</td>
<td>5/8 (62.5%)</td>
<td>0.32</td>
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</tr>
</tbody>
</table>

FSHD = facioscapulohumeral dystrophy; CSS = clinical severity scale; MRC = Medical Research Council; MUS = muscle ultrasound; SD = standard deviation.
Figure legends

Figure 1:
Flow diagram of patient inclusion in previously published baseline study (grey) and current two-year follow-up study (blue). The eight children of whom only an electronic health record review had been performed at baseline were not invited for follow-up. However, their baseline characteristics, including the mean age at time of baseline examination, age at onset of symptoms, age at time of diagnose, sex and mean number of D4Z4 repeats, were similar to the group of children who were included in this follow-up study.
Figure 2:

Muscle function and imaging in individual patients over two years.

[A] FSHD clinical score (0-15), [B] performance on Motor Function Measure (0-100%), [C] performance on Six-minute walk test (Z-score, the number of standard deviations of the mean for sex and age in healthy individuals) and [D] muscle ultrasound mean echogenicity (mean Z-score) at baseline and two year visit. MFM = motor function measure; 6MWT = six-minute walk test.
Figure 3:
Figure 3A displays the mean Z-score of quantitative muscle ultrasound measuring echogenicity. This shows the combined muscle ultrasound measurements of 9 participants at two-year follow-up. Figure 3B shows the distribution of the measured muscles at baseline and follow-up by qualitative analysis using Heckmatt scores and 3C by quantitative analysis using z-scores.