

Milder phenotype in facioscapulohumeral dystrophy with 7–10 residual D4Z4 repeats

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ABSTRACT

Objective: To examine the relationship of clinical and genetic features of patients with facioscapulohumeral muscular dystrophy (FSHD) with 7–10 residual D4Z4 repeats in a large genetically defined FSHD1 cohort.

Methods: We performed a prospective cross-sectional observational study of 74 clinically affected patients with FSHD1. Measures of clinical severity were compared between patients with 1–6 D4Z4 repeats and 7–10 repeats, and included D4Z4 CpG methylation, age at diagnosis, age-adjusted clinical severity score, a muscle pathology grade of quadriceps biopsies (0 = normal, 12 = severe dystrophic changes), quantitative myometry of biopsied muscles, global manual muscle testing scores, and frequency of wheelchair use.

Results: Twenty-eight (37.8%) participants had 7–10 D4Z4 repeats, and compared to participants with 1–6 repeats, were diagnosed 6.6 years older ($p = 0.17$); had lower CpG methylation than would be predicted by D4Z4 repeat size ($p = 0.04$); had age-adjusted clinical severity 39.8 points lower ($p = 0.004$); had muscle pathology grades that were 2.4 points less severe ($p < 0.0001$); had quantitative myometry 28.3% predicted of normal higher ($p = 0.002$); had global manual muscle testing scores 0.6 higher ($p = 0.005$); and did not require wheelchairs.

Conclusion: Patients with FSHD with 7–10 D4Z4 repeats have milder disease than other genetically defined patients with FSHD1. The lower than predicted methylation in the 7–10 residual repeat group may suggest that additional epigenetic factors play a role in the severity of disease expression. *Neurology*® 2015;85:2147–2150

GLOSSARY

FSHD = facioscapulohumeral muscular dystrophy.

Facioscapulohumeral muscular dystrophy (FSHD) is a common muscular dystrophy due in its most prevalent form (FSHD1) to deletions in the D4Z4 macrosatellite repeat region on chromosome 4q35.^{1,2} While normal individuals have >10 repeats, patients with FSHD1 have between 1 and 10 repeats. The current model for disease proposes FSHD is an epigenetic disease: deletions lead to decreased CpG methylation and opening of chromatin structure.³ The open chromatin structure in combination with a polyadenylation signal polymorphism allow the transcription of a normally repressed gene located within the D4Z4 repeat, *DUX4*, which causes disease by a toxic gain of function.⁴

Patients with the smallest number of repeats demonstrate a more severe phenotype, including earlier wheelchair use and increased frequency of extramuscular manifestations (retinal vasculopathy and hearing loss).^{5–7} A number of articles have looked at the relationship of D4Z4 methylation levels to penetrance of disease expression, with decreased methylation in patients expressing disease.^{8,9} A linear relationship exists between the number of residual D4Z4 repeats and estimates of methylation in the D4Z4 region.⁹ So for most patients with FSHD1, the repeat contraction is sufficient to decrease methylation and cause disease. Symptomatically affected patients with 7–10 repeats, unlike patients with 1–6 repeats, have divergent methylation values

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from those predicted by D4Z4 contractions alone, suggesting that the contraction per se is insufficient to cause disease but requires additional (epigenetic) factors.⁹ Here we sought to determine if symptomatic patients with 7–10 D4Z4 repeats have differences in disease severity compared to patients with 1–6 repeats.

METHODS We performed a prospective cross-sectional observational study of genetically confirmed and clinically affected patients with FSHD1 at the University of Rochester Medical Center conducted from 2002 to 2013.

Standard protocol approvals, registrations, and patient consents. The study was approved by the institutional review board, and written and informed consent was obtained from all participants.

FSHD participants were between 18 and 75 years of age. D4Z4 residual fragment size was determined by Southern Blot after double digestion with *EcoRI/BlnI* restriction enzymes.^{2,10} CpG methylation measurements were determined by Southern Blot after cleavage with the methylation-sensitive endonuclease *FseI*.¹⁰ The Δ methylation value is the observed methylation value corrected for the repeat array size: Δ methylation = (observed CpG methylation) – (predicted methylation based on repeat size).⁹ The predicted methylation model was derived from a large cohort of patients and healthy controls (e-Methods on the *Neurology*[®] Web site at Neurology.org).⁹

Participants filled out a standardized self-reported medical history (<https://www.urmc.rochester.edu/fields-center/protocols/>). For each participant, clinical severity was determined based on pattern of muscle involvement (0–10, increasing from face and shoulders, to lower extremity) and adjusted for age: $1,000 \times (2XCS)/age$

(table e-1).^{e1} Manual muscle testing was performed utilizing a modified 13-point Medical Research Council Scale (bilateral shoulder abductors, forward flexors, elbow flexors/extensors, wrist extensors, hip flexors, knee flexors/extensors, and ankle dorsiflexors).^{e2} Quadriceps muscle biopsy pathology was determined by one neuropathologist rater using an ordinal severity scale (0 = normal, 12 = severely dystrophic). Quantitative myometry of the quadriceps was performed prior to biopsy and normalized to percent predicted based on age, sex, and height (e-Methods).^{e2,e3}

Descriptive statistics (mean, median, and frequency) were used to evaluate each outcome. Participants were divided into 2 groups based on residual D4Z4 repeat size for comparisons (1–6 vs 7–10 repeats). Differences between groups were determined by 2-sample *t* test for continuous measures, Wilcoxon rank-sum test for ordinal values, and χ^2 for frequencies. Ninety-five percent confidence limits were calculated by standard procedures. The relationship between age-adjusted clinical severity and D4Z4 repeat was investigated visually first and mathematically by fitting trendlines (higher R^2 values determining optimal fit). For box and whisker plots: boxes represent 50% of the distribution; thick line, median values; whiskers to nearest outlier, or 1.5 times box size; and dots, outliers. Missing data were assumed to be missing at random. All statistical testing was 2-sided with $p < 0.05$ used as a cutoff. Statistical testing was performed using SAS version 9.4 (SAS Institute Inc, Cary, NC) and STATA version 11.2 (Stata-Corp, College Station, TX).

RESULTS Seventy-four participants with FSHD1 were included (table 1). Just over 1/3 of participants had 7–10 D4Z4 repeats. There were no differences based on sex or patient-reported age at diagnosis, although participants with 7–10 repeats were older at evaluation. No participants had documented extramuscular manifestations of FSHD1. After adjusting for age, participants with 7–10 repeats

Table 1 Clinical characteristics of patients with FSHD with 1–6 D4Z4 repeats compared to 7–10 repeats

Measure	1–3 D4Z4 repeats	4–6 D4Z4 repeats	1–6 D4Z4 repeats	7–10 D4Z4 repeats	Diff (7–10) – (1–6) (95% CI)	p Value ^a
No. (%)	9 (12.2)	37 (50.0)	46 (62.2)	28 (37.8)	—	—
Male sex, n (%)	4 (44.4)	26 (70.3)	30 (65.2)	19 (67.9)	2.7 (–19.4 to 24.8)	0.81
Mean (SD) age at evaluation, y	42.3 (15.3)	46.8 (12.2)	45.9 (12.8)	51.9 (9.6)	6.0 (0.4 to 11.6)	0.04
Mean (SD) D4Z4 residual fragment kb	13.9 (2.0)	21.8 (2.6)	20.2 (4.0)	30.1 (3.0)	9.9 (8.1 to 11.6)	<0.0001
Mean (SD) % methylation	34.6 (13.3)	35.8 (13.0)	35.6 (12.9)	33.1 (7.3)	–2.5 (–7.8 to 2.9)	0.36
Mean (SD) Δ methylation	0.5 (11.1)	–0.7 (12.1)	–0.5 (11.8)	–5.4 (6.0)	–5.0 (–9.8 to –0.2)	0.04
Mean (SD) diagnosis age, y ^b	22.4 (12.5)	38.4 (14.9)	34.5 (15.8)	41.1 (34.2, 48.0)	6.6 (–2.9 to 16.1)	0.17
Mean (SD) age-adjusted clinical severity	187.0 (83.5)	119.3 (52.5)	132.5 (64.7)	92.7 (38.6)	–39.8 (–66.7 to –12.9)	0.004
Mean (SD) combined MMT ^c	3.5 (0.8)	4.3 (0.6)	4.2 (0.7)	4.7 (0.4)	0.6 (0.2 to 0.9)	0.005
Mean (SD) quantitative myometry PP ^d	45.9 (27.9)	61.0 (22.9)	57.8 (24.3)	86.1 (27.9)	28.3 (11.4 to 45.2)	0.002
Mean (SD) pathology grade ^e	4.9 (2.1)	4.4 (2.4)	4.6 (2.3)	2.1 (1.2)	–2.4 (–3.5 to –1.3)	<0.0001
No. (%) wheelchair use ^f	3 (37.5)	3 (12.0)	6 (18.2)	0 (0)	–18.2 (–5.0 to –31.3)	0.07

CI = confidence interval; Diff = difference; FSHD = facioscapulohumeral muscular dystrophy; MMT = manual muscle testing; PP = percent predicted of normal.

^aTwo-sample *t* test for continuous variables, χ^2 for frequencies, Wilcoxon for ordinal data.

^bAge at diagnosis is patient-reported.

^cCombined MMT available for n = 51: 1–6 D4Z4 repeats (n = 35) and 7–10 D4Z4 repeats (n = 16).

^dQuantitative myometry available for n = 42: 1–6 D4Z4 repeats (n = 28) and 7–10 D4Z4 repeats (n = 14).

^ePathology grade available for n = 56: 1–6 D4Z4 repeats (n = 34) and 7–10 D4Z4 repeats (n = 22).

^fWheelchair use reported by n = 49: 1–6 D4Z4 repeats (n = 33) and 7–10 D4Z4 repeats (n = 16).

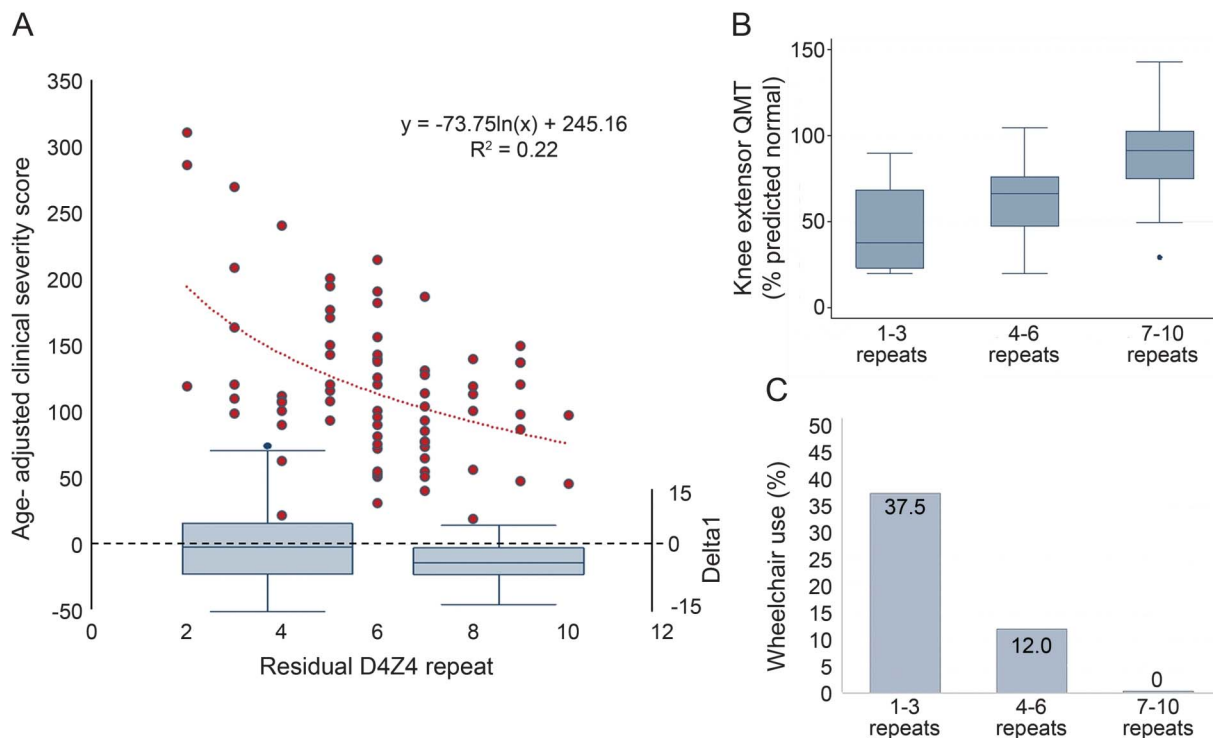
were less severely affected. The relationship of the age-adjusted clinical severity to residual D4Z4 repeat number was not linear (figure, A, left y-axis). For participants with 1–6 repeats, the residual repeat size appears to have a linear effect on clinical severity. But the relationship flattens out for participants with 7–10 repeats. Looking at the distribution of Δ methylation values for the 2 groups shows (1) a Δ methylation with a median value that approaches zero for participants with 1–6 repeats; and (2) a shift to negative values for participants with 7–10 repeats, which corresponds to the flatter portion of the age-adjusted clinical severity vs repeat size curve (figure, A, right y-axis). Overall, looking at a broad group of clinical severity measures, participants with 7–10 residual D4Z4 repeats were more mildly affected, including increased combined average manual muscle testing scores, increased quantitative myometry of quadriceps muscle (figure, B), and lower quadriceps muscle pathology scores. No participant with 7–10 residual repeats was using a wheelchair for mobility, despite the older age of the population (figure, C).

DISCUSSION FSHD1 is an epigenetic disease with considerable variability across the size of the D4Z4

repeat array.^{e1,e4} While attempts to split out groups for examination may be arbitrary, a number of points related to deletion size and methylation are becoming clear:

1. Patients with the smallest number of residual fragments^{1–3} have more severe disease. These patients are typically diagnosed younger, have higher penetrance by age,^{e4} are more likely to use a wheelchair,^{5,e5} and are more likely to experience extramuscular manifestations of FSHD, including symptomatic retinal vasculopathy, hearing loss requiring hearing aids, or seizures or cognitive changes.^{6,7,e6} These patients have decreased methylation determined by the number of residual repeats with Δ methylation values approximating zero.⁹ However, low methylation in itself does not completely explain severity, as patients with FSHD2, who have low methylation values, have not been described with severe extramuscular manifestations of disease or with early onset.¹⁰
2. Patients with FSHD with the largest number of residual D4Z4 fragments have a milder disease or remain nonpenetrant,^{5,e5,e7} with an older age at onset,^{e5} lower penetrance by age, lower frequency of wheelchair use, and typically do not have

Figure The relationship of facioscapulohumeral muscular dystrophy 1 residual D4Z4 repeats and Δ methylation to disease severity



(A) The relationship of age-adjusted clinical severity to D4Z4 repeat size is not linear (left y-axis, $n = 74$). While the relationship of severity to repeat size is linear from 1 to 6 repeats, some other factor besides repeat size appears to determine severity for 7–10 repeats. Below box plots of the Δ methylation (right y-axis) for 1–6 repeats (left) and 7–10 repeats (right). Note the negative Δ methylation values for 7–10 repeats. (B) Participants with 7–10 repeats have increased quadriceps strength by quantitative myometry (1–3 repeats [$n = 6$], 4–6 repeats [$n = 23$], and 7–10 repeats [$n = 14$]). (C) No participants with 7–10 repeats reported using wheelchairs (1–3 repeats [$n = 8$], 4–6 repeats [$n = 26$], and 7–10 repeats [$n = 17$]).

extramuscular manifestations. In this group, the methylation values do not appear to be completely driven by the number of residual repeats—the Δ methylation values are negative, suggesting some other factor, whether genetic, epigenetic, or environmental, is required for them to express disease.⁹ A genotype–phenotype cohort in Japan did not identify disease in this group.⁶⁸ Additionally, mutations in *SMCHD1* are not only associated with FSHD2 but also modify FSHD1: an article described families in the 7–10 repeat range, with a mild phenotype, where certain individuals with more severe disease were found to have unexpectedly low methylation values, then subsequently found to also carry mutations in *SMCHD1*.⁶⁹

Ultimately, disease expression in FSHD1 appears to be a unique combination of factors, which includes deletion of repetitive elements that affects not only methylation, but may have additional physical properties related to size in the 1–3 repeat group, and a second group with 7–10 repeats where the size of the repeat is not sufficient to cause disease, but some other (epigenetic) factor is also required.

AUTHOR CONTRIBUTIONS

Jeffrey Statland and Rabi Tawil contributed to drafting/revising the manuscript for content, study concept or design, analysis or interpretation of data, acquisition of data, and statistical analysis. Stephen Tapscott, Richard Lemmers, and Silvère van der Maarel contributed to study concept or design, drafting/revising the manuscript for content, and acquisition of data. Colleen Donlin-Smith contributed to drafting/revising the manuscript for content and acquisition of data.

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DISCLOSURE

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