Original Article

Quantitative Three-dimensional Scanning of Facial Movements in Facioscapulohumeral Dystrophy

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Abstract

Background: Facioscapulohumeral dystrophy (FSHD) is the third prevalent neuromuscular disorder, particularly with facial and upper limb involvement. Due to its characteristics, quantitative assessment of facial function is challenging but essential for determining severity. We aimed to evaluate facial participation of patients with FSHD using three-dimensional (3D) scanning to determine whether 3D evaluations differed in patients with FSHD compared to healthy controls and if facial involvement was correlated with genetic mutation and clinical severity. **Methods:** Twenty-one healthy controls (57.1% of females) and 12 patients with FSHD (58.3% of females) were investigated using 3D facial scanning in a three-axis coordinate system to evaluate eyebrow elevation, maximal eye closing, maximal teeth showing, whistling, and happy facial expressions. These expressions were evaluated in terms of intensity and asymmetry. Genetic mutations were investigated to reveal the D4Z4 repeat (D4Z4-RU) on 4q35. Clinical severity scores (CSS) were determined through neurologic examinations. For Q1, 3D data were compared between patients with FSHD and controls. For Q2, the association of 3D variables with D4Z4-RU and CSS was examined. **Results:** Intensity of whistling, maximal eye closing, and happy expressions were significantly different in patients with FSHD compared with controls ($P < 0.001$, $P < 0.002$). Asymmetry-related measurements were not different between groups. Facial movements did not correlate with CSS and D4Z4-RU in patients with FSHD. **Conclusions:** 3D estimations can provide quantitative data on the function of facial muscles. By converting 3D scans into colored face scales, additional information on facial parts affected by specific movements could be obtained. The use of 3D in FSHD has the potential to provide reliable quantitative data for routine, research, and follow-up studies.

Keywords: *Clinical severity, D4Z4 repeat, facial asymmetry, facioscapulohumeral dystrophy, facioscapulohumeral muscular dystrophy, three-dimensional morphology scan*

Submitted: 30-Dec-2024
Revised: 12-Apr-2025
Accepted: 06-May-2025
Published: 27-Jun-2025

Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is a muscle disease with a frequency of 1:8000.[1] Despite ongoing therapeutic studies on both natural and pharmaceutical agents, there is no applicable treatment.[2] FSHD tends to select facial, scapular, and humeral muscles, and foot dorsiflexors in its initial stages, and progresses to the other muscle groups.[3] The initial active muscle groups, the rate of disease progression may be variable, resulting in different clinical severity and divergent outcomes among FSHD patients.[3]

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Supplementary material available online: www.nsnjournal.org

Access this article online

Quick Response Code:

Website: www.nsnjournal.org

DOI: 10.4103/nsn.nsn_174_24

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How to cite this article: Hangul C, Ozsoy U, Hizay A, Karauzum SB, Sauer E, Firat MZ, *et al.* Quantitative three-dimensional scanning of facial movements in facioscapulohumeral dystrophy. Neurol Sci Neurophysiol 2025;42:48-55.

More than 95% of FSHD cases are related to the deletion of D4Z4 repeat units located on chromosome 4q35.[4] Together with a specific qA allele, D4Z4 deletion leads to toxic and stable DUX4 protein expression, impairing skeletal muscles.[5] For a certain diagnosis of FSHD, revealing the D4Z4 deletion is essential as the root cause of the disease. Clinical variability cannot be explained only by D4Z4 deletion. Even in family members carrying the same deletion,[6] different clinical signatures can be observed. This clinical diversity leads to difficulties in the standardization of severity and follow-up. Accordingly, reliable evaluation methods providing quantitative data are needed to detect disease progression and measure the effect of treatment applications.

Quantitative manual testing, the manual muscle test, clinical severity score (CSS), the two-minute-walk test (2MWT), magnetic resonance imaging (MRI), the instrumented timed up and go test (iTUG), spatio-temporal gait, and reachable workspace (three-dimensional (3D) evaluation)[7,8] are examples of quantitative evaluation methods of disease severity.[9-16] However, these methods do not provide specific information for facial involvement, which is important because it also causes reduced psychosocial function.[17] Loonen *et al.* suggested two-dimensional (2D) imaging but declared that it was not appropriate for longitudinal studies.[18] Konstantonis *et al.* used a scale system for the facial evaluation of patients with FSHD and stated that orofacial muscle weakness might not be a prognostic biomarker.[19] However, the evaluations in these studies used discontinuous data in a scale form. Besides these studies, no comprehensive and quantitative evaluation methodology has been provided. In the present study, we aimed to provide 3D quantitative data for the facial involvement of patients with FSHD for the first time.

The purpose of this study was to examine the severity of facial involvement and the amount of asymmetry in FSHD. It has been hypothesized that quantitative data from 3D evaluations could discriminate patients with FSHD from healthy individuals. Therefore, 3D change estimations were performed on patients with FSHD and healthy controls. In addition, the correlation between facial 3D findings with genetic mutation and total clinical severity was investigated.

Methods

Study design and sample

This study was performed as a prospective case–control study. Age and sex were the covariates. Patients with FSHD and controls who presented within a 6-month period were included in the study [Table 1]. Controls were selected from healthy individuals. The control group comprised individuals with no psychiatric, neurologic, cognitive, or chronic illnesses that could affect facial measurements and who were not using medications. The inclusion criterion for patients with FSHD was deletion of the D4Z4 repeat unit number (D4Z4-RU) on the terminal region of the q arm on chromosome 4. Exclusion criteria for both patients with FSHD and the control group were the presence of additional diseases such as palsy, dystrophy, or any other diseases with facial involvement. Experimental protocols were approved by the Institutional Review Board in accordance with ethical guidelines.

Table 1: Demographic data of the patients with facioscapulohumeral dystrophy and healthy controls

	Control, *n* (%)	Patient, *n* (%)	*P*
Female	12 (57.1)	7 (58.3)	0.9
Male	9 (42.8)	5 (41.6)	
Age	49.3±12	32.3±13	0.02

Outcome variables

Three types of variables were present in this study. First was 3D estimation variables. 3D variables reflect facial expressions specified as neutral, eyebrow elevation, maximal eye closing, maximal teeth showing, whistling, and happy. Each variable was evaluated in positions as they were named (e.g., maximal eye closing: most tightly positioned eye closure). All of these motions were measured using a handheld 3D light scanner.

The second variable was the genetic mutation corresponding to the D4Z4-RU. This variable was specific to patients with FSHD and was necessary for the molecular diagnosis of FSHD. The genetic analysis of D4Z4-RU was performed using Southern blot analysis. Patients with FSHD carried repeats of <11 units (scaled from 2 to 10 repeat units).

The last variable was the CSS. This variable was determined through neurologic examinations. The CSS is a 10-point scale that evaluates whole-body muscle involvement, scored from 0 to 5; 0 corresponds to normal muscle strength with no muscle involvement. Healthy individuals scored 0. A score of 0.5 corresponds to facial weakness, 1 is mild scapular involvement, 1.5 is moderate scapular involvement, 2 is severe scapular involvement, and 2.5 is lower-extremity involvement corresponding to pelvic and proximal leg muscles. Respectively, 3 = mild, 3.5 = moderate, and 4 = severe involvement of pelvic and proximal leg muscles. A score of 4.5 corresponds to the inability to stand up from a chair. The progression of FSHD causes increased muscle involvement with an increased CSS score. The highest score[5] corresponds to the most severe form, wheelchair-bound.[10,11]

Facial scan and three-dimensional assessment of facial morphology

Facial scans were performed using a handheld Artec EVA 3D light scanner (2013; Artec Group, Luxembourg). Participants sat on a stool with a natural head position and were asked to remain still during scanning to avoid motion artifacts. Each participant was scanned during neutral, eyebrow elevation, maximal eye closing, maximal teeth showing, whistling, and happy facial expressions. Our previous publications and supplementary material can be viewed for detailed information about image processing [Supplementary Material 1].[20-22]

Images for each facial expression were used to discriminate both evaluations of the (i) intensity of motion and (ii) asymmetry changes in healthy individuals and patients with FSHD. Intensity measurements correspond to 3D displacement changes on the face as a result of certain movements. Asymmetry measurements are based on determining differences between displacement changes by dividing the face into two vertical halves [Supplementary Material 1].

Intensity and asymmetry estimations were first calculated for the whole face and named as total intensity and total asymmetry. Furthermore, a calculation of sub-regions was performed for the upper-middle-lower parts of the face and named partial intensity and partial asymmetry [Supplementary Material 1].

Colored mapping with red-green-blue was used in facial maps Figures 1 and 2]. Here, red and yellow represent the positive distance from the reference point of the neutral position, and blue represents the negative distance from the reference point of the neutral position. The darkening in color indicates an increase in the distance. The green color indicates more stationary load points where the drift from the reference point is minimal [color scales with distance numbers are represented in the middle of Figures 1 and 2].

Statistical analyses

Data were obtained as root mean square (RMS). Raw RMS data have been shared as a supplementary table [Supplementary Material 2]. Analyses were performed using the SAS Institute Inc. SAS® 9.4 software. The Wilcoxon test was used to reveal significant differences regarding facial asymmetry and intensity of movement between patients and healthy individuals [Tables 2 and 3]. In addition, correlation analysis was applied to investigate the relationship of 3D values with D4Z4 and CSS [Table 4]. $P < 0.05$ were considered statistically significant.

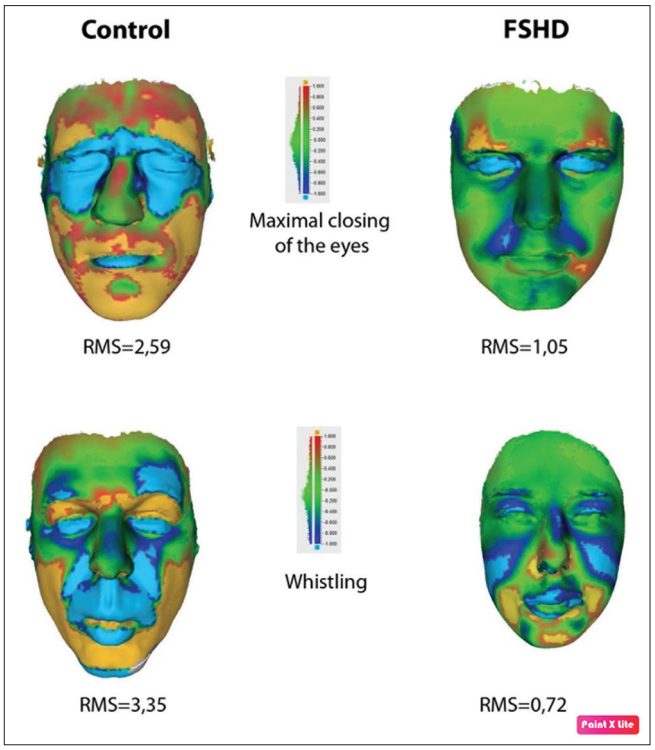


Figure 1: The images of maximal closing of the eye and whistling were represented in control and facioscapulohumeral dystrophy. The evaluations in this figure were performed as a total face analysis. Color maps after superimposition of the neutral face and whistling face position or maximal eye closing of the same subject. The colored deviation map legends between the figures show the millimeter scale. The map changes in color from blue, which corresponds to negative distance, to red, which corresponds to positive distance; green means that the distance between surfaces at that particular point is close to zero

Table 2: Results of three-dimensional intensity analysis (partial and total) performed between the facioscapulohumeral dystrophy and healthy control groups

Variable	Control		FSHD		*P*
	n	Mean±SD	*n*	Mean±SD	
Partial intensity of motion					
Eye(brow) elevation	21	1.722±0.873	12	1.104±0.639	0.059
Eye closing	21	2.093±1.081	12	1.168±0.277	0.001
Teeth showing	21	5.597±1.636	12	5.261±0.594	0.330
Whistling	21	3.320±0.965	12	2.101±0.677	0.001
Happy expression	21	4.878±1.001	12	3.308±1.106	0.001
Total intensity of motion					
Eye(brow) elevation	21	1.192±0.505	12	0.789±0.398	0.016
Eye closing	21	1.663±0.911	12	0.980±0.186	0.002
Teeth showing	21	3.709±1.073	12	3.441±0.529	0.321
Whistling	21	2.266±0.613	12	1.399±0.380	<0.001
Happy expression	21	3.261±0.833	12	2.208±0.788	0.002

SD: Standard deviation, Mean: Estimated value

Results

The study sample comprised 21 healthy individuals (57.1% females, mean 49.3 ± 12 (range, 31–79) years) and 12 patients with FSHD (58.3% females, mean

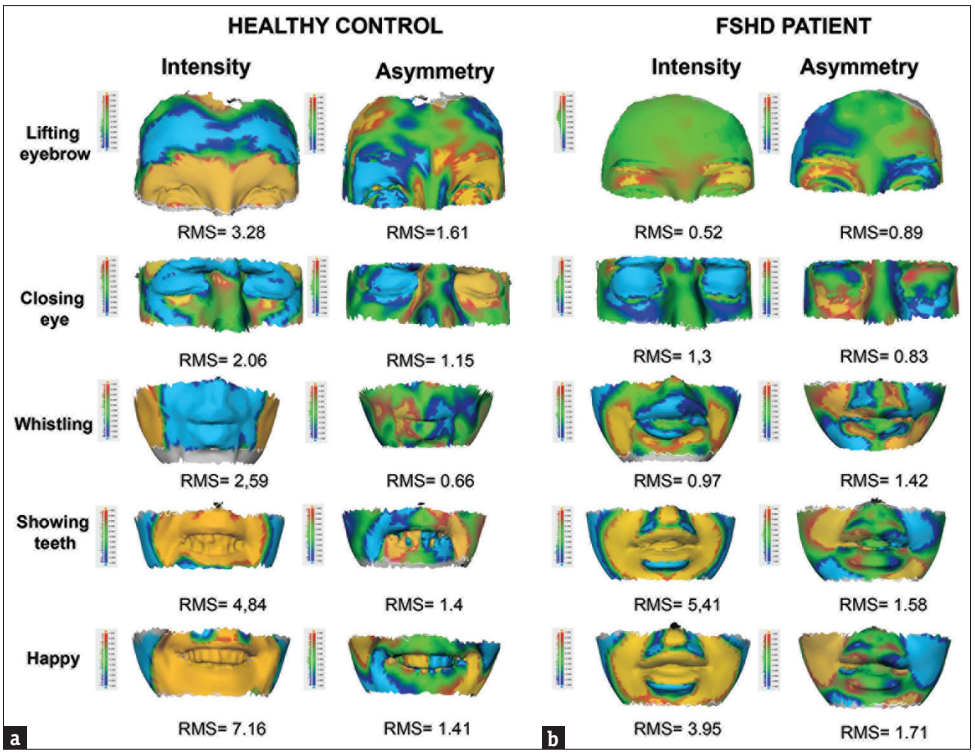


Figure 2: The images of eyebrow elevation (eyelift), maximal eye closing, maximal teeth showing, whistling, and happy expressions were evaluated as a partial face analysis. Intensity maps (left) and asymmetry maps (right) for the control and facioscapulohumeral dystrophy individuals. The colored deviation map legends between the figures show the millimeter scale. The map changes in color from blue, which corresponds to negative distance, to red, which corresponds to positive distance; green means that the distance between surfaces at that particular point is close to zero

Table 3: Results of three-dimensional analysis of asymmetry (partial and total) performed between the facioscapulohumeral dystrophy and healthy control groups

Variable	Control		FSHD		*P*
	n	Mean±SD	*n*	Mean±SD	
Partial asymmetry					
Eye(brow) elevation	21	1.115±0.431	12	1.073±0.310	0.6804
Eye closing	21	1.187±0.469	12	0.945±0.429	0.1659
Teeth showing	21	2.174±0.808	12	1.704±0.624	0.1204
Whistling	21	0.995±0.347	12	1.275±0.448	0.0920
Happy expression	21	1.906±0.638	12	1.465±0.348	0.0695
Total asymmetry					
Eye(brow) elevation	21	1.206±0.409	12	1.023±0.244	0.2857
Eye closing	21	1.278±0.439	12	1.024±0.237	0.0587
Teeth showing	21	1.686±0.436	12	1.413±0.367	0.1249
Whistling	21	1.211±0.332	12	1.194±0.317	0.8957
Happy expression	21	1.499±0.422	12	1.213±0.246	0.0562

SD: Standard deviation, Mean: Estimated value

32.3 ± 13 (range, 16–53) years). Sex distribution between the groups was homogeneous (P = 0.947); the mean age of the control group was higher than the patient group (P = 0.021) [Table 1].

Colored face maps allow a precise detection of movement differences

The total intensity of motion: Whole face [Figure 1]

In the maximal eye closing, the control individual (RMS = 2.59) had large blue around the eyes; while, patients with FSHD (RMS = 1.05), had tiny blue around the eyes with a major green area.

In the whistling position, control individuals had a large blue area around the lips (RMS = 3.35), whereas a narrower blue and wider green area was observed in patients with FSHD (RMS = 0.72).

The partial intensity of motion: Upper and middle and lower face [Figure 2a]

In eye elevation (lifting eyebrow), broad yellow and blue regions were observed in controls (RMS = 3.28), whereas tiny yellow and broad green regions with no blue were present in patients with FSHD (RMS = 0.52). In closing the eyes, yellow-red regions were minimal in patients with FSHD compared with the controls. In whistling, we observed a distinct blue region due to protrusion in controls (RMS = 2.59), while the blue area was considerably reduced in patients with FSHD (RMS = 0.97). Showing teeth exhibited the most minimal disparity between the patients with FSHD (RMS = 5.41) and controls (RMS = 4.84). In the happy expression, we observed that almost the entire area around the mouth was yellow in individuals with FSHD (RMS = 3.95), and green in controls (RMS = 7.16).

Table 4: Results of correlation analysis between D4Z4 repeat unit, clinical severity score, and significant facial parameters

	CSS	Partial intensity			Total intensity			
		Eye closing	Whistling	Happy expression	Eye elevation	Eye closing	Whistling	Happy expression
D4Z4 RU								
r	0.166	0.015	0.435	−0.008	0.047	−0.103	0.320	−0.025
P	0.60	0.96	0.15	0.97	0.88	0.74	0.30	0.93
CSS								
r	1	−0.330	−0.255	−0.139	−0.089	0.027	−0.219	−0.128
P	1	0.29	0.42	0.66	0.78	0.93	0.49	0.69

CSS: Clinical severity score, D4Z4 RU: D4Z4 repeat unit

The partial asymmetry: Upper and middle and lower face [Figure 2b]

Left–right asymmetry appeared similar in the patient and control groups. Only the size of the colored areas was observed variable between the two groups.

Specific intensity estimations were able to distinguish patients with facioscapulohumeral dystrophy from healthy individuals

The neutral position had lower RMS scores in FSHD; however, the results were insignificant ($P = 0.092$).

In total measurements, eye elevation ($P = 0.016$), eyes closing ($P = 0.002$), whistling ($P < 0.001$), and happy expressions ($P = 0.002$) were significantly lower in the FSHD group; of these, whistling was the most prominent variable [Table 2].

In partial measurements, the most prominent result was the happy expression ($P = 0.001$). Whistling and eye closing ($P = 0.001$ and $P = 0.001$, respectively) also exhibited high significance. Eye elevation ($P = 0.059$) and showing teeth ($P = 0.330$) were not significant; however, the median in the FSHD group was lower compared with the controls [Table 2].

Asymmetry measurements did not differ between the patient and control groups

In neither partial nor total estimations of asymmetry, no difference could be found between the FSHD and control groups. Total asymmetry estimations of FSHD patients were lower compared to controls but not significant [Table 3].

Facial severity was not correlated with genetic mutation or total severity

We performed correlation analysis with genetic deletion and clinical severity. Significant 3D-intensity variables (whistling, happy expression, eye closing, and eye elevation) had no association with D4Z4 repeat size and CSS [Table 4].

Male patients with facioscapulohumeral dystrophy exhibited significantly higher scores in part of the facial 3D estimations

In the neutral position, we could not detect sex differences. Teeth showing provided higher RMS in males compared with females with significant asymmetry in males [Supplementary Material 3].

Discussion

This study investigated whether quantitative 3D scanning data of facial movements differed in patients with FSHD compared with healthy control individuals. Compatible with our hypothesis, we found a significant decline in intensity estimations of patients with FSHD: whistling, happy expressions, and eye closing reflected facial involvement more accurately. In the FSHD literature, the orbicularis oris, orbicularis oculi, and zygomaticus major muscles were reported as the most frequently involved muscle groups.[18] This may explain why there were significantly lower whistling, happy facial expression, and eye-closing scores in patients with FSHD compared with the control group [Table 2]. Among the significant parameters, whistling and happy expressions exhibited the highest significance ($P < 0.001$ and $P < 0.002$). This finding indicates a more pronounced difference in the oral movements of patients. Therefore, whistling and happy expressions can be more discriminative for clinical practice to evaluate the degree of facial involvement and follow progression in a time-dependent manner.

The effect of the genetic mutation on the severity of the disease is controversial.[23-25] Based on our data, no relation was present between genetic mutation and facial 3D scores [Table 4]. A recent study revealed a mild-to-moderate correlation between orofacial weakness and FSHD clinical scores, facial weakness scores, and the facial function scale.[26] In our study, conflictingly, total CSS showed no significant correlation with 3D measurements. To reach a definitive conclusion, repeated studies with more patients are needed.

FSHD is an asymmetrical neuromuscular disorder.[27-29] An MRI study with 12 patients with FSHD and 20 controls revealed asymmetrical atrophy and fatty infiltration in the masseter-medial and lateral pterygoid muscles.[30] However, the study did not provide detailed

information on the functional capacity of these muscles. Even though they observed a visible change in muscle volumes, we could not detect functional asymmetry in patients with FSHD compared with controls in our study sample [Table 3]. The facial involvement could be less asymmetrical compared with the trunk or extremities, or a slight asymmetry difference in patients may be overlooked because healthy individuals also have facial asymmetry.[20]

Recent studies have related FSHD to sex steroids such as progesterone,[31,32] estrogen,[33,34] and testosterone.[35] Accordingly, we also analyzed the 3D variables of patients with FSHD by sex. We showed that males had significantly higher asymmetry and intensity values in teeth showing [Supplementary Material 2]. This result supports the severe phenotype observed more in males.[36]

There are a few atypical FSHD cases without apparent facial involvement.[37] Colored 3D facial maps additionally allowed the detection of movement differences in the three axes [Figures 1 and 2]. The application of colored maps to atypical cases in future studies can help investigate these cases in detail. Until now, photographic methods or clinical observer approaches have been insufficient in providing the desired level of data for facial involvement in FSHD.[18] Unlike other striated muscles, mimetic muscles insert into the skin and cause complex facial deficits, which are challenging to characterize. 3D maps allowed motion loss to be observed in a visible manner, as well as provide quantitative data. Compared with raw 2D photographs, the data clarity provided by colorized 3D scans can be noticed [Figure 3a and b]. We can suggest that 3D data are reliable because our previous studies proved the intraobserver and interobserver reliability with the 3D approach.[21] We have also shown the intra-subject repeatability of facial movements by analyzing facial expressions at 3-month intervals.[21] However, specific studies need to be designed to draw a definitive conclusion on the discriminatory efficacy of 3D in mildly affected or subclinical cases.



Figure 3: 2D photographic image of (a) eye closure and (b) neutral position of the patient. Facial involvement of the patients was evaluated as Grade II in the House Brackmann grading scale and 3.5 in the Clinical Severity Score scale. Written permission has been obtained for the publication of his photographic image

3D facial scanning can potentially diagnose FSHD and conditions with facial involvement, such as peripheral facial palsy. 3D facial scanning provides detailed, quantitative assessments of facial soft-tissue morphology and function and has demonstrated effectiveness in evaluating facial palsy by analyzing facial asymmetry and movement dynamics.[38,39] In addition, studies have shown that integrating electromyography (EMG) with motion capture systems enables simultaneous recording of muscle activity and facial movement, offering a comprehensive view of neuromuscular function.[40,41] Given that EMG has been used to predict 3D facial shapes and recognize various facial movements with high accuracy,[41,42] future research should explore the correlation between EMG and 3D scanning in FSHD to assess its diagnostic sensitivity and specificity. Furthermore, the potential role of 3D scanning in longitudinal studies of FSHD progression warrants investigation because it has been successfully applied in grading and monitoring facial palsy.[41,43] Incorporating these aspects into future studies could enhance the clinical use of 3D facial scanning as a complementary tool for FSHD assessment alongside existing diagnostic methods.[26]

Conclusion

Sophisticated analysis methods are needed to analyze the complex movements of the face. 3D scanning is easy to apply in clinical routine and can provide quantitative data on the function of facial muscles. The results of our study support that 3D scanning is suitable for quantitatively analyzing patients with FSHD. Future detailed 3D analysis studies, including different sexes, may provide valuable information for routine follow-up, research studies, including clinical trials.

Limitations of the study

This study is the first investigation reflecting muscle function using 3D methodology with its quantitative outputs in FSHD. Therefore, there are no literature data for a direct comparison. The age difference between the healthy controls and patients could be defined as a limitation. However, because the mean age of the control group was older, it had no negative effect on the analyses performed in the study.

The limited but statistically sufficient sample size of >10 patients and 20 controls in this pilot study provides

significant *P* values to establish a basis for future studies targeting functional and quantitative 3D estimations. The 3D method seems to be effective in differentiating patients with FSHD; however, the technique needs to be tested in a larger group of affected patients before it can be used clinically.

Author contributions

Concept and design, CH, UO, SBK, HU; methodology, UO, AH, CH, ES, MZF; software, UO, AH, CH, MZF; validation, UO, AH, CH; formal analysis, CH, UO, AH, MZF; investigation, CH, UO, AH, HU, SBK, ES, MZF; resources, HU, SBK, ES; data curation, UO, AH, CH, MZF; writing – original draft preparation, CH, UO, SBK, AH, HU, ES, MZF; writing – review and editing, CH, UO, SBK, HU, AH, ES, MZF; visualization, CH, UO, AH, SBK, HU, ES, MZF; supervision, UO, SBK, HU; project administration, UO, HU; funding acquisition, HU, UO. All authors have read and agreed to the published version of the manuscript.

Ethical approval

All procedures performed in the study involving human participants were under the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent from all subjects and/or their legal guardian(s) was obtained for both study participation AND publication of identifying information/images in an online open-access publication.

Availability of data and materials

All data supporting the findings of this study are available within the paper and its Supplementary Information.

Acknowledgment

Akdeniz University Research Foundation financially supported this work. Thanks, Nevruz ILHANLI, for her additional support on statistics. Thanks, Serdar CEYLANER, for his support in finding a collaborative institute.

Financial support and sponsorship

This study was funded by the Akdeniz University Research Foundation.

Conflicts of interest

There are no conflicts of interest.

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Supplementary Materials

Supplementary Material 1: Detailed information on image processing

Facial Scan and Three-Dimensional Assessment of Facial Morphology

Each subject's scanned masks were imported into Artec Studio 11 Software (version 11.2.2.16; Artec Group, Luxembourg, licensed) and processed. Each scan was oriented manually according to the established coordinate system (X-axis, left/right; Y-axis, superior/inferior; Z-axis, anterior/posterior) using translation and rotation in all three directions. The three axes' common origin was set at a mid-endocanthion point (halfway between the inner corners of the eyes); as previous research has shown, its position changes the least with time. The ears, the hairline, and the neck were excluded from the complete analysis of facial movements.

Calculations of Intensity and Asymmetry Estimations

(i) The intensity of movement was determined by comparing facial morphology during facial expression and resting. The 3D facial mask obtained while resting was superimposed on the facial mask obtained during the movement. Each mask consists of approximately 10,000 points joined in triangles. Each junction point is called a vertex. The root means square (RMS) of the distance between the vertexes of superimposed masks gives the quantitative value of facial motion. The Artec Studio 11 software automatically computed the mean point-to-point distance between the superimposed images and colored distance maps created.

(ii) To determine the asymmetry of motion, Mimicry's mirror image was generated using Autodesk Netfabb software (Netfabb, Parsberg, Germany, Free trial version) and superimposed on the original image. The RMS values were calculated for the functional changes between the two halves of the face. A high RMS value indicates high asymmetry and vice versa. A zero value indicates that the two surfaces are identical for a perfect overlapping.

Sub-division of the face in three Partial Regions in Intensity Estimations: Upper-Middle-Lower Face

To detect partial changes in eyebrow elevation, the line passing through the right and left exocanthion and the horizontal line passing through the trichion (the intersection point of the hairline and midline of the head) was analysed. To detect partial changes eye closing, the line passing through the left and right orbitale and the horizontal line passing through the nasale was evaluated. Finally, to detect partial changes in teeth showing, whistling, and happiness expressions, the horizontal line passing through the subnasale and the horizontal line passing through the labiomental fold was considered.

Supplementary Material 2: Raw data of root mean square values on heatmap tables

Supplementary Table 2a: Heatmap of root mean square values corresponding partial movement estimations

	Partıal 3D scanning Movement					
	Neutral	Neutral - eyebrow elevation	Neutral - eye closing	Neutral - teeth showing	Neutral - whistling	Neutral - happy expression
Control 1	0.99	1.7	1.5	5.11	3.76	5.04
Control 2	0.94	0.5	1.71	6.06	2.27	5.06
Control 3	1.11	2.99	2.73	7.45	4.12	5.33
Control 4	0.9	0.89	1.49	4.96	1.39	3.84
Control 5	1.61	1.26	1.29	5.7	3.12	3.86
Control 6	1.04	2.51	2.76	6.76	3.66	5.84
Control 7	1.16	3.26	2.08	4.46	2.71	6.55
Control 8	2.66	2.55	3.57	7.29	4.96	4.43
Control 9	1.02	1.96	1.77	5.47	3.63	4.63
Control 10	0.68	1.81	0.74	3.68	4.89	3.39
Control 11	1.33	0.73	1.42	1.23	3.29	3.41
Control 12	1.07	1.007	3.77	7.8	4.1	6.97
Control 13	1.09	1.33	1.8	5.57	2.74	4.27
Control 14	1.84	2.04	1.83	4.91	2.22	5.13
Control 15	1.68	1.74	5.31	8.85	2.79	6.19
Control 16	1.67	3.39	2.91	5.45	2.84	4.48
Control 17	1.18	2.34	1.45	6.29	4.42	4.86
Control 18	0.68	1.76	1.91	5.29	3.02	4.26
Control 19	0.88	0.78	1.62	5.25	2.9	4.68
Control 20	1.13	0.86	1.2	6.31	2.21	6.13
Control 21	0.85	0.75	1.09	3.64	4.67	4.08
Patient 1	0.79	0.91	1.56	5.07	2.75	5.03
Patient 2	0.92	0.83	0.87	4.85	1.24	4.15
Patient 3	0.681	0.95	1.1	6.49	1.72	3.68
Patient 4	1.37	0.51	0.89	5.1	2.21	2.39
Patient 5	2.55	1.19	1.22	5.66	1.64	2.35
Patient 6	0.79	0.85	1.24	4.91	2.26	3.85
Patient 7	1.01	0.84	0.68	4.24	2.41	3.61
Patient 8	1.39	1.1	1.53	5.64	2.09	4.61
Patient 9	0.77	0.84	1.39	5.56	3.32	2.22
Patient 10	0.9	2.77	1.3	5.67	1.73	1.51
Patient 11	0.82	1.93	0.93	4.62	2.84	2.33
Patient 12	0.81	0.53	1.31	5.32	0.998	3.96

3D: Three-dimensional

Supplementary Table 2b: Heatmap of root mean square values corresponding to total movement estimations

	Total 3D scanning Movement				
	Neutral - eyebrow elevation	Neutral - eye closing	Neutral - teeth showing	Neutral - whistling	Neutral - happy expression
Control 1	1.04	1.17	3.44	2.45	3.54
Control 2	0.49	1.44	3.81	1.53	3.64
Control 3	1.73	1.8	5.04	3.35	3.69
Control 4	0.91	1.22	2.99	1.28	2.37
Control 5	0.89	0.99	4.25	2.32	2.76
Control 6	1.6	2.58	4.56	2.26	3.92
Control 7	2.21	1.58	3.25	1.92	5.05
Control 8	1.63	2.59	4.91	3.27	3.25
Control 9	1.15	1.34	3.87	2.61	1.75
Control 10	1.27	0.79	2.19	3.02	2.01
Control 11	0.75	0.95	1.04	2.79	2.46
Control 12	0.71	2.91	4.92	2.32	4.81
Control 13	0.86	1.23	3.68	2.06	2.79
Control 14	1.71	1.69	3.21	1.45	3.26
Control 15	1.14	4.72	5.83	1.74	3.63
Control 16	2.12	1.79	3.5	2.29	2.86
Control 17	1.53	1.08	3.92	2.73	3.55
Control 18	1.19	1.49	3.8	1.9	2.93
Control 19	0.47	1.13	3.31	1.64	3.1
Control 20	1.08	1.65	4.04	1.64	4.26
Control 21	0.56	0.78	2.32	3.02	2.86
Patient 1	0.66	1.15	3.32	1.57	3.38
Patient 2	0.63	1.04	3.03	0.78	2.69
Patient 3	0.64	0.87	4.51	1.34	2.685
Patient 4	0.55	1.05	3.096	1.39	1.29
Patient 5	0.88	1.11	3.52	1.39	1.93
Patient 6	0.6	0.88	3.51	1.34	2.52
Patient 7	0.69	0.6	2.67	1.47	2.07
Patient 8	0.69	1.23	3.85	1.366	3.28
Patient 9	0.54	1.099	3.83	2.16	1.51
Patient 10	1.796	0.869	3.887	1.578	1.1
Patient 11	1.375	0.75	2.73	1.69	1.305
Patient 12	0.42	1.11	3.34	0.717	2.73

3D: Three-dimensional

Supplementary Table 2c: Heatmap of root mean square values corresponding partial asymmetry estimations					
	Partial 3D scanning Asymmetry				
	Eyebrow elevation	Eye closing	Teeth showing	Whistling	Happy expression
Control 1	0.97	0.85	2.78	0.85	2.42
Control 2	0.69	0.85	2.25	0.85	1.84
Control 3	1.1	1.62	2.38	0.88	1.73
Control 4	1.05	1.28	0.79	0.57	1.35
Control 5	1.22	0.94	2.55	1.45	2.08
Control 6	1.19	1.6	1.98	1.2	2.24
Control 7	1.65	1.26	1.47	0.67	1.6
Control 8	0.9	1.52	3.15	1.51	2.48
Control 9	1.1	1.01	2.06	1.35	1.61
Control 10	0.97	0.61	2.81	0.83	2.33
Control 11	1.49	1.4	1.32	1.16	2.13
Control 12	0.68	1.03	2.18	1.01	3.62
Control 13	0.97	1.19	1.72	0.67	1.04
Control 14	1.13	1.08	1.37	0.86	1.52
Control 15	1.41	1.16	4.56	1.89	2.62
Control 16	2.57	2.81	1.51	1.32	2.6
Control 17	1.19	1.19	2.66	1.001	0.998
Control 18	0.64	0.87	1.65	0.77	1.12
Control 19	0.88	0.7	2.4	0.63	1.47
Control 20	1.03	1.23	1.6	0.74	1.53
Control 21	0.58	0.73	2.46	0.69	1.7
Patient 1	0.81	0.74	0.63	0.94	1.18
Patient 2	1	0.7	2.33	1.11	1.94
Patient 3	0.94	0	0.74	0.66	1.8
Patient 4	1.44	1.69	2.39	1.4	1.67
Patient 5	1.34	1.08	1.41	1.89	1.59
Patient 6	0.87	1.04	1.43	1.88	0.84
Patient 7	0.88	0.9	1.71	0.85	1.16
Patient 8	1.28	1.59	1.8	1.6	1.55
Patient 9	0.74	1.03	1.41	1.62	0.99
Patient 10	0.93	0.88	2.52	1.28	1.41
Patient 11	1.76	0.85	2.35	0.58	1.71
Patient 12	0.89	0.84	1.73	1.49	1.74

3D: Three-dimensional

Supplementary Table 2d: Heatmap of root mean square values corresponding to total asymmetry estimations

	Total 3D scanning Asymmetry				
	Eyebrow elevation	Eye closing	Teeth showing	Whistling	Happy expression
Control 1	1.08	1.04	1.83	1.03	1.57
Control 2	0.95	0.87	1.44	1.06	1.39
Control 3	1.17	1.32	1.72	1.23	1.33
Control 4	0.92	1	1.06	1.13	1.22
Control 5	1.53	1.5	1.93	1.55	1.84
Control 6	1.25	1.66	1.47	1.46	1.61
Control 7	1.39	1.22	1.93	1.31	1.64
Control 8	2.5	2.66	2.48	2.03	2.33
Control 9	0.95	1.06	1.58	1.23	1.24
Control 10	0.84	0.79	1.69	0.88	1.36
Control 11	1.39	1.36	1.37	1.46	1.7
Control 12	0.99	1.46	1.87	1.11	2.26
Control 13	0.95	0.99	1.19	0.91	0.96
Control 14	1.17	1.47	1.38	1.02	1.45
Control 15	1.73	1.72	2.87	1.7	1.93
Control 16	1.79	1.81	2.16	1.66	2.19
Control 17	0.99	1.03	1.67	1.09	1.02
Control 18	0.85	0.78	1.16	0.73	0.9
Control 19	0.83	0.95	1.71	1.04	1.02
Control 20	1.15	1.16	1.31	1.09	1.32
Control 21	0.91	0.99	1.59	0.72	1.19
Patient 1	0.82	0.77	0.71	0.8	0.95
Patient 2	1.002	1.05	1.59	0.86	1.25
Patient 3	0.68	0.73	2.04	0.998	1.34
Patient 4	1.39	1.43	1.77	1.77	1.55
Patient 5	1.32	1.26	1.29	1.59	1.6
Patient 6	0.93	1.086	1.095	1.2	0.9
Patient 7	0.96	0.97	1.21	0.95	1.04
Patient 8	1.32	1.42	1.63	1.51	1.47
Patient 9	0.68	0.88	1.14	1.46	0.88
Patient 10	0.88	0.96	1.74	1.12	1.22
Patient 11	1.18	0.8	1.52	0.93	1.12
Patient 12	1.11	0.93	1.22	1.14	1.24

3D: Three-dimensional

Supplementary Material 3

Supplementary Table 3: Statistical analysis of sex effect on facial 3D estimations indicated with *P*-values in patients with facioscapulohumeral dystrophy

Patient Variable	*P*			
	P3DI	T3DI	P3DA	T3DA
Eye elevation	0.255	0.290	0.104	0.370
Eye closing	0.999	0.684	0.256	0.256
Teeth showing	0.015 (male)	0.051 (male)	0.569	0.015 (male)
Whistling	0.256	0.935	0.626	0.074
Happy expression	0.516	0.417	0.516	0.015 (male)

Neutral P=0.193