Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2025;50(3):702-711
DOI: 10.17826/cumj.1736413

RESEARCH

Chromosome 4q D4Z4 contraction status in Turkish patients with facioscapulohumeral muscular dystrophy

Türk fasiyoskapulohumeral müsküler distrofi hastalarında kromozom 4q D4Z4 kontraksiyon durumu

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Abstract

Purpose: This study aimed to provide an updated overview of the genetic and clinical features of patients with facioscapulohumeral muscular dystrophy1 (FSHD1) followed between 2006 and 2025 in the Mediterranean region of Turkey.
Materials and Methods: A total of 46 patients diagnosed as having FSHD1 through Southern blot analysis were included. The cohort consisted of 26 males and 20 females, with a mean age of 32.93 ± 17.01 years. Clinical severity scores (CSS) and age-corrected CSS (ACSS) were assessed based on neurologic examinations.
Results: The most frequent D4Z4 repeat size was 4 units (30.4%). Although CSS and ACSS appeared lower in female patients, the difference was not statistically significant. Patients aged under 30 years exhibited significantly lower CSS and ACSS compared with those aged over 30 years. A strong correlation was observed between age and both CSS and ACSS; no significant correlation was found between D4Z4RU and clinical severity. Among the 46 patients, 20 families were represented, and one patient had a *de novo* mosaic mutation.
Conclusion: Our findings highlight the importance of longitudinal and population-specific data in understanding FSHD. Increased molecular diagnosis and regular follow-up of patients may facilitate future research and the development of targeted therapies.

Keywords: Facioscapulohumeral muscular dystrophy, D4Z4 repeat contraction; clinical severity; diagnosis; 4qter

Öz

Amaç: Bu çalışmada, 2006–2025 yılları arasında Türkiye'nin Akdeniz bölgesinde takip edilen Fasiyoskapulohumeral müsküler distrofi 1 (FSHD1) hastalarının genetik ve klinik özelliklerine dair güncel bir değerlendirme sunulması amaçlanmıştır.
Gereç ve Yöntem: Southern blot analizi ile FSHD1 tanısı alan toplam 46 hasta çalışmaya dahil edilmiştir. Kohort, yaş ortalaması 32,93 ± 17,01 yıl olan 26 erkek ve 20 kadından oluşmaktadır. Klinik Şiddet Skoru (CSS) ve yaşa göre düzeltilmiş CSS (ACSS), nörolojik muayeneler temelinde değerlendirilmiştir.
Bulgular: En sık gözlenen D4Z4 tekrar sayısı 4 olup, hastaların %30,4'ünde saptanmıştır. Kadın hastalarda CSS ve ACSS değerleri daha düşük görünmekle birlikte, bu fark istatistiksel olarak anlamlı bulunmamıştır. 30 yaş altındaki hastalarda CSS ve ACSS değerleri, 30 yaş üzerindekilere kıyasla anlamlı düzeyde daha düşük bulunmuştur. Yaş ile hem CSS hem de ACSS arasında güçlü bir pozitif korelasyon gözlenmiştir, buna karşın D4Z4RU ile klinik şiddet arasında anlamlı bir ilişki saptanmamıştır. Kohortta 20 farklı aile yer almakta olup, bir olguda mozaik yapıda *de novo* mutasyon tespit edilmiştir.
Sonuç: Elde edilen bulgular, FSHD'nin daha iyi anlaşılabilmesi için longitudinal ve popülasyona özgü verilere duyulan ihtiyacı vurgulamaktadır. Moleküler tanı oranlarının artırılması ve hastaların düzenli takibi, gelecekteki araştırmaların ve hedefe yönelik tedavi stratejilerinin geliştirilmesine katkı sağlayabilir.

Anahtar kelimeler: Fasyoskapulohumeral müsküler distrofi, D4Z4 tekrar kısalması, klinik şiddet, tanı, 4qter

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Received: 07.07.2025 Accepted: 23.08.2025

INTRODUCTION

Facioscapulohumeral muscular dystrophy (FSHD) is a condition characterized by muscle degeneration, typically manifesting initially with muscles in the face and upper extremities, followed by scapular and humeral muscles, and finally the lower extremities[1] Different muscle involvement patterns are also possible, and this variable involvement pattern remains one of the unknown aspects of genotype-phenotype correlation in FSHD. Estimates suggest that FSHD affects approximately 1 in 8000 to 1 in 20.000 individuals on a global scale, with significant variations in prevalence observed across different studies. The estimated prevalence in China is 1:13.000,[2] whereas in Italy it is 1:20.000[3] In the United States, the estimated prevalence is 1:100[4]. This variation may be attributed to differences in the underlying genotype among populations.

FSHD predominantly exhibits an autosomal dominant inheritance pattern, although a small number of patients exhibit autosomal recessive or digenic inheritance patterns. The disease originates from a specific genetic mutation carried by patients with FSHD. In the majority of cases (95%), the mutation is characterized by a contraction of the D4Z4 repeat on chromosome 4q35[5]. This deletion results in an autosomal dominant inheritance pattern and is classified as FSHD1. In a minority of cases (<5%), mutations have been identified in the SMCHD1 (structural maintenance chromosome hinge domain 1)[6] or DNMT3B (DNA methyltransferase 3B)[7] or LRIF1 (Ligand Dependent Nuclear Receptor Interacting Factor 1)[8] These mutations are inherited in an autosomal recessive or digenic manner and are grouped as FSHD2.

From a clinical perspective, there are no discernible differences between FSHD1 and FSHD2. Therefore, a clinical pre-diagnosis alone is insufficient for definitive diagnosis, necessitating the identification of the underlying genetic defect through molecular testing. The gold standard for diagnosing FSHD is Southern blot analysis, which detects the contracted D4Z4 allele that segregates with the permissive 4qA haplotype[9]. The contracted region is defined by a reduced number of D4Z4 repeat units (RU), typically ranging from 1 to 10 units in affected individuals, whereas the general population carry arrays consisting of 11 to 100 RUs. Each repeat unit is 3.3 kilobases (3300 base pairs) in length[10]. The genetic diagnosis of FSHD is achieved by demonstrating the presence of the short allele, which is defined as a length of less than 38 kilobases and fewer than 11 repeats, on chromosome 4.

The number of patients with molecularly confirmed FSHD1 in Turkey is quite limited. Our previous research has contributed to establishing the foundational context of the pathogenesis of FSHD[11,12].

Previous studies have mostly been limited to the D4Z4 repeat number and clinical severity, whereas this study comprehensively analyzed demographic parameters such as clinical severity scores (CSS), age-corrected CSS (ACSS), age, and sex, and discussed their effects on clinical course using statistical modeling (regression, correlation, group comparisons). In this regard, the present study is one of the rare studies that attempt to bridge the gap between clinical scoring systems and epigenetic effects. Data from the Mediterranean region represent one of the most comprehensive Turkish FSHD1 cohort analyses that jointly address genetic and clinical characteristics. The current study highlights the limitations of classic genetic markers in predicting clinical course, raising new questions that encourage further investigation into the effects of non-genetic factors.

MATERIALS AND METHODS

Patients

The data of 53 patients were retrieved through a retrospective review of patient records documented in the institutional archives. In the retrospective archive search, patient files were systematically reviewed using a predefined data extraction form to minimize variability.

Patients with comprehensive and up-to-date documentation, including both clinical and molecular diagnostic records, were included in the study. The subjects were under regular clinical follow-up, with their medical records systematically maintained, ensuring the reliability and continuity of the data.

Patients who exhibited characteristic clinical signs and symptoms of FSHD, specifically scapulohumeral and facial muscle weakness, and whose clinical involvement severity had been assessed through standardized evaluation methods were included in the study. Additionally, individuals with a molecularly

confirmed diagnosis of FSHD1, defined by a contraction of the D4Z4 macrosatellite repeat array on chromosome 4q35 to 1–10 units and the presence of a permissive 4qA allele, were enrolled. Patients who had secondary causes of muscle weakness, such as endocrine disorders (e.g., hypothyroidism, hypercortisolism), inflammatory myopathies (e.g., polymyositis, dermatomyositis), or motor neuron diseases, were excluded from the study. Furthermore, individuals without molecular confirmation of FSHD1, including those lacking D4Z4 repeat contraction analysis or with inconclusive genetic test results, were not considered eligible for participation. Seven patients were excluded from the study because they fulfilled the clinical diagnostic criteria but lacked molecular confirmation (Figure 1).

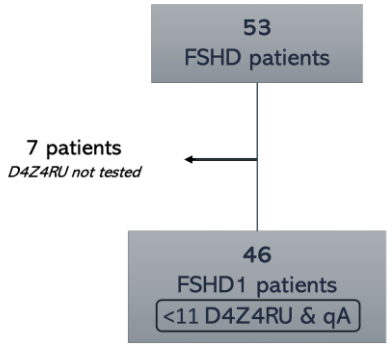

Figure 1. Number of patients with FSHD included in the study.

Neurological examination

In this study, clinical severity score (CSS) and age-corrected clinical severity score (ACSS) were used with the data measured in the neurologic examinations of the patients with FSHD1. A CSS is a semi-quantitative scale that yields a total score ranging from 0 to 10. Higher scores indicate greater disease severity. This scale is specifically designed to assess the extent of muscle weakness and is based on an evaluation of the facial, scapular, humeral, abdominal, and pelvic girdle muscles. The ACSS adjusts the CSS for the patient's age, providing a more standardized measure of disease progression relative to age-related expectations. The ACSS is calculated using the following formula: ((CSS x 2) / age at examination) x 1000. This correction helps to mitigate the variability in clinical presentation caused by age differences among patients. Both the CSS and the ACSS have been widely used in observational studies to evaluate disease burden, track progression over time, and correlate with molecular findings, such as D4Z4 repeat size[13,14].

Neurologic examinations were performed by expert neuromuscular specialists at the departments of neurology, Akdeniz University and Çukurova University, Faculty of Medicine where files of patients with FSHD are regularly archived and updated. To ensure inter-rater reliability, the retrospective clinical severity data were reviewed by a second independent specialist, and clinical scores were recalculated.

The study protocol received approval from the Akdeniz University Medical Scientific Research Ethics Committee (08.09.2010/008356, 31.07.2025/TBAEK-631). Procedures performed in the study involving human participants were conducted in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all participants.

Genetic analysis

Genetic diagnosis of all included FSHD1 cases was performed using Southern blot analysis, targeting the D4Z4 repeat array on chromosome 4q.

Southern blot analysis

A total of 10 mL of peripheral blood was collected from each patient in K3EDTA tubes. DNA isolation was performed using the salting-out method, and the quantity and purity of the isolated DNA were measured. For each patient, tubes were prepared using EcoRI, EcoRI/BlnI, and XapI. Spermidine was added to each tube, followed by overnight enzyme digestion at 37°C. A 0.88% agarose gel was prepared. The BioRad Chef II system was used for pulse field gel electrophoresis (PFGE). After electrophoresis, the DNA was transferred onto a nylon membrane via Southern blotting and hybridized overnight at 65°C with a labelled probe. Images were captured using phosphor imaging (Bio-Rad), and the results were interpreted accordingly (Supplementary Figure 1).

Statistical analysis

Assuming a large effect size (Cohen's d = 0.8) and a significance level of p = 0.05, a minimum of 31 participants was required to achieve a statistical power of 80%. Given that our study included 46 patients, the statistical power exceeded 90%,

indicating that the sample size was sufficient to detect clinically meaningful differences with a high degree of confidence.

The analyses were performed using the SAS version 9.4 software. For the analysis of normality Shapiro-Wilk test was performed. The Wilcoxon test was used to identify significant disparities in the comparison between male and female patients, as well as between patients aged under and over 30 years. The Spearman correlation analysis was performed to investigate the relation of age and D4Z4 with CSS or ACSS. P-values below 0.05 were considered statistically significant. In addition, regression analysis was performed with CSS and ACSS designated as dependent variables. Variables included in the regression model were selected based on clinical relevance, prior literature, and statistical assumptions. Age, sex, and D4Z4RUs were considered due to their potential impact on clinical severity. Prior to inclusion, variables were assessed for distribution characteristics and inter-variable correlations. Only non-collinear predictors with theoretical justification and significant univariate association with the outcome (CSS) were retained in the final model.

RESULTS

A total of 46 patients with FSHD1 were monitored over the period from 2010 to 2025 to contribute to the study. The distribution of subjects by sex was found to be homogeneous, with 26 males and 20 females (56.6% and 43.4% respectively). The mean age of the subjects was found as 32.93 years, with a standard deviation of ±17.01 years (Table 1).

The median value was used as a reference to determine the cut-off point because the age variable did not follow a normal distribution. Accordingly, 30 years of age was selected to stratify the cohort into younger and older subgroups for comparative analyses. Similarly, D4Z4RU counts and ACSS also deviated from normality, whereas CSS was found to be normally distributed. Therefore, non-parametric statistical methods were applied for both group comparisons and correlation analyses.

Table 1. Demographic characteristics of 46 patients with FSHD1 and family information.

Patient	Sex	Age	D4Z4RU	CSS	Family	ACSS	Patient	Sex	Age	D4Z4RU	CSS	ACSS	Family
P1	M	12	3	1.0	Family 1	166.67	P24	M	46	5	3.5	152.17	Family 11
P2	F	17	3	1.5	Family 1	176.47	P25	M	16	5	3.0	375.00	Family 11
P3	M	12	3	1.0	Family 1	166.67	P26	M	17	5	2.0	235.29	Family 11
P4	M	26	4	2.5	Family 2	192.31	P27	F	64	8	3.5	109.38	Family 12
P5	M	45	8	3.5	Family 3	155.56	P28	M	45	8	2.5	111.11	Family 12
P6	F	61	8	3.0	Family 4	98.36	P29	M	41	8	2.5	121.95	Family 12
P7	M	13	7	1.0	Family 4	153.85	P30	F	56	4	4.5	160.71	Family 13
P8	M	32	8	1.5	Family 4	93.75	P31	F	41	4	1.0	48.78	Family 13
P9	M	37	8	2.5	Family 4	135.14	P32	M	63	9	4.0	126.98	Family 14
P10	M	18	6	3.0	Family 5	333.33	P33	M	55	4	5.0	181.82	Family 13
P11	M	18	10	2.5	Family 6	277.78	P34	M	53	4	2.5	94.34	Family 13
P12	F	8	10	1.5	Family 6	375.00	P35	F	64	4	4.5	140.63	Family 13
P13	F	15	10	1.5	Family 6	200.00	P36	F	28	3	1.5	107.14	Family 15
P14	F	21	10	3.0	Family 6	285.71	P37	F	47	8	2.5	106.38	Family 16
P15	M	19	4	3.0	Family 7	315.79	P38	M	36	8	1.5	83.33	Family 16
P16	M	23	4	2.5	Family 7	217.39	P39	M	51	3	2.0	78.43	Family 17
P17	F	38	4	3.5	Family 7	184.21	P40	F	20	3	1.5	150.00	Family 17
P18	M	20	4	3.0	Family 8	300.00	P41	F	36	4	2.5	138.89	Family 18
P19	M	51	2	1.5	Family 9	58.82	P42	F	11	4	0.5	90.91	Family 18
P20	M	21	2	3.0	Family 9	285.71	P43	F	9	4	0.0	0.00	Family 18
P21	F	57	6	1.0	Family 10	35.09	P44	M	47	6	3.0	12.,66	Family 19
P22	F	29	6	1.5	Family 10	103.45	P45	F	25	6	0.5	40.00	Family 19
P23	M	22	6	3.0	Family 10	272.73	P46	F	29	4	2.0	137.93	Family 20

P:patient, F:female, M:male; D4Z4 repeat units (D4Z4RU), Clinical Severity Score (CSS), and Age-corrected CSS (ACSS).

Distribution of D4Z4 repeat units

A thorough examination of the distribution of RUs among the patients revealed that the most prevalent number of units was 4, with a frequency of 30.4% (14/46) (see Figure 2).

D4Z4RU				
D4Z4RU	Frequency	Percent	Cumulative Frequency	Cumulative Percent
2	2	4.35	2	4.35
3	6	13.04	8	17.39
4	14	30.43	22	47.83
5	3	6.52	25	54.35
6	6	13.04	31	67.39
7	1	2.17	32	69.57
8	9	19.57	41	89.13
9	1	2.17	42	91.30
10	4	8.70	46	100.00

Figure 2. Frequency of D4Z4 repeat units (D4Z4RU) in the study sample.

Comparison of Clinical and Molecular Parameters in sub-groups of patients with FSHD

The clinical and genetic data of 46 patients were analyzed to assess potential differences based on sex (female vs. male) and age (≤30 years vs. >30 years). Both age distribution and D4Z4RU counts were found to be homogeneous across the groups. Although the CSS and ACSS appeared lower in females compared with males, statistical analysis revealed that neither CSS nor ACSS showed a significant association with sex (Table 2).

In contrast, age-based comparisons demonstrated a significant difference. Although the D4Z4RU distribution remained homogeneous between the age groups, individuals aged under 30 years exhibited significantly lower CSS (p=0.01) and ACSS (p<0.001) values compared with those aged over 30 years. Detailed results are presented in Table 2.

Table 2. Results of difference analyses between females and males, aged <30 and >30 years.

		Age	D4Z4RU	CSS	ACSS
Female	n=20	33.8±18.9	5.65±2.47	2.05±1.28	134.45±86.09
Male	n=26	32.3±15.8	5.53±2.28	2.53±0.94	185.13±88.28
age<30	n=24	18.7±6.1[a]	5.25±2.48	1.89±0.94[b]	206.63±101.23[c]
age>30	n=22	48.5±9.8[a]	5.95±2.17	2.79±1.13[b]	115.61±40.17[c]

n: number; [a] statistical differences (p<0.001) between age <30 and >30 years; [b] statistical differences (p=0.01) between age <30 and >30 years; [c] statistical differences (p<0.001) between age <30 and >30 years

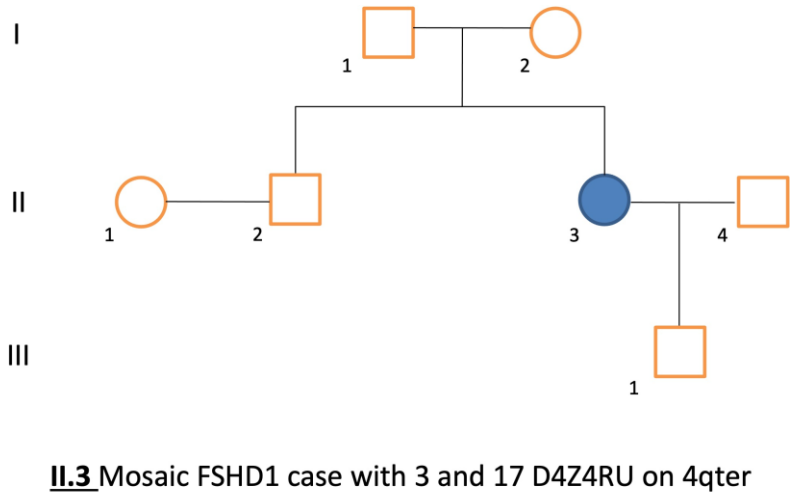

Figure 3. Pedigree of patient with mosaic FSHD1.

Inheritance characteristics

An analysis of the inheritance patterns within the pedigrees of patients with FSHD1 revealed that a total of 20 families were represented in the study cohort. Among these, 39 patients were identified as members of distinct family lineages. Despite the presence of additional FSHD1-affected individuals within their respective families, patients P4 (Family 2), P5 (Family 3), P10 (Family 5), P18 (Family 8), P32 (Family 14), and P46 (Family 20) were the only participants from their families included in the study (Table 1).

Furthermore, a single patient (P36) exhibited a *de novo* mosaic mutation, representing a sporadic occurrence (Figure 3). The cohort also comprised large families with multiple affected members. For instance, within one extended pedigree, individuals IV.2, IV.3, IV.8, and V.5 were all enrolled in the study, reflecting familial aggregation of the disease (Figure 4).

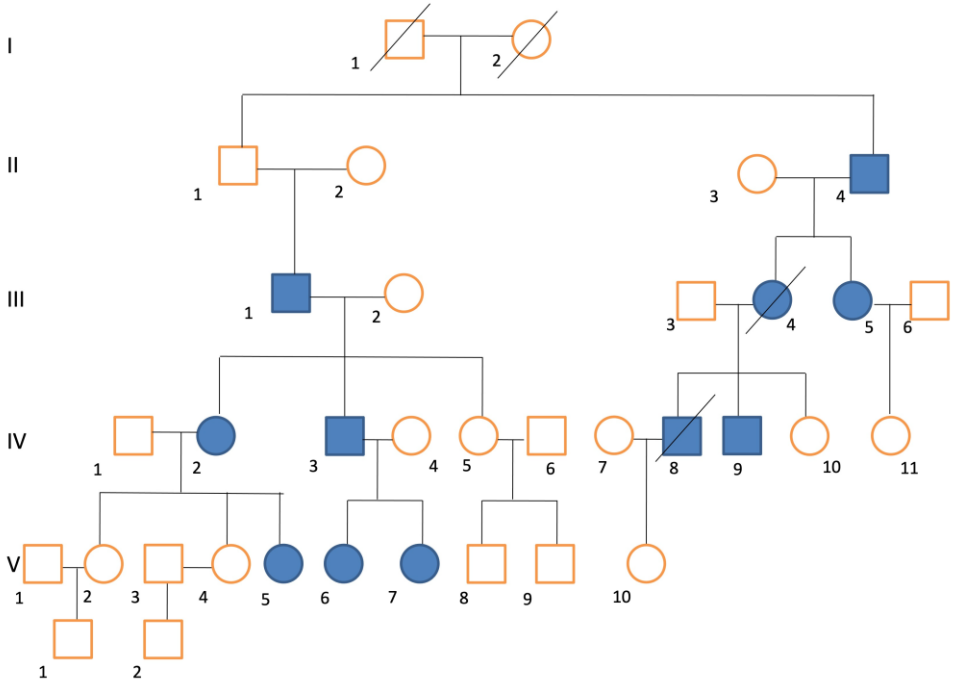

Figure 4. Pedigree of largest family included in the study.

Pearson Correlation Coefficients, N = 46

Prob > |r| under H0: Rho=0

	Age	D4Z4RU	CSS	ACSS
Age				
D4Z4RU	0.08			
CSS	0.55[c]	0.09		
ACSS	-0.48[d]	0.12	0.37	

-1
0
1

[c,d] p<0.001

Table 3. Results of correlation analysis.

n: number, D4Z4RU: D4Z4 repeat unit, CSS: Clinical Severity Score, ACSS: Age-corrected Clinical Severity Score

Association of clinical and molecular parameters

An evaluation of the association between clinical and molecular parameters revealed a significant correlation between age and disease severity, as reflected by both the CSS and ACSS ($p<0.001$). Conversely, no statistically significant relationship was identified between D4Z4RU size and CSS (Table 3).

Consistent with the correlation analysis, regression analysis also revealed a significant association between CSS and age. When CSS was modelled as the dependent variable, the resulting regression equation was formulated as CSS = 1.013 + (0.0357 × Age). D4Z4 repeat number was excluded from the final model because it did not exhibit a significant relationship with CSS.

DISCUSSION

The genetic and clinical characterization of patients with FSHD in Turkey remains largely underrepresented in the current literature, with only a few studies focusing on this population. In this context, the present study aimed to provide a comprehensive assessment of molecular findings and their clinical correlations in a well-defined cohort of patients with FSHD followed between 2006 and 2025 in the Mediterranean region of Turkey. Building upon our earlier findings,[11,12] this study offers a larger cohort evaluation of genetic mutations and their association with phenotypic variability, addressing a critical gap in the literature and contributing to a deeper understanding of genotype-phenotype correlations in the pathogenesis of FSHD within the Turkish population.

FSHD has a much milder course and a lower rate of progression in women compared with men.[12,15,16] Within the context of the study sample, the mean CSS values were found to be lower in the female group; however, no statistically significant difference was identified (Table 2). ACSS was borderline, with a p-value of 0.06. The underlying rationale for this phenomenon may, once again, be attributable to the limited number of cases. On the other hand, CSS is a severity indicator and may not cover variable muscle involvements. There are multiple measurement methods available for obtaining further information on clinical severity such as the Lamperti scoring scale,[17] the 2-minute walk test, or three-dimensional estimations of body[18] and face involvement[19] Inclusion of these measurement methods may provide more detailed quantitative and functional data, which could help capturing sex-related differences.

Given that FSHD progresses with age, it is known to manifest with greater severity in older age groups. Following the separation of the study group by age as above and below 30 years, in line with the literature, it was found that the CSS increased significantly with age (Table 2).[20] Supporting this, the only parameter found to be associated with disease severity in the correlation analysis was age (Table 3). These findings suggest that, within this cohort, age serves as a more robust predictor of clinical disease burden than repeat sequence length.

The sporadic mosaic case represented a piece of evidence demonstrating the potential for D4Z4 deletion to occur *de novo* in the postzygotic period (Figure 3). Although the transmission of RUs appears to be uniform within our families, there may be variations among family members. About one-fifth of relatives of the proband carrying small fragments were asymptomatic or minimally affected.[15] This may be due to technical limitations, such as measurement sensitivity, and biologic variability, such as germline or somatic mosaicism. However, the formation of the phenotype is dependent on the number of repeats, the haplotype, and methylation status.[6]

Several studies have investigated the association between D4Z4 repeat number and clinical severity in FSHD. Some reports have described an inverse correlation, indicating that shorter D4Z4 repeat arrays (1–3 RU) are often associated with earlier onset and more severe disease, whereas longer arrays (8–10 RU) tend to be linked with milder or even asymptomatic presentations.[21,22] However, there are individuals with very short repeat arrays who exhibit mild or even absent clinical symptoms, as well as patients with longer repeat lengths who display significant disease manifestations[23–25] In 2024, an another data set from Turkey, clinical severity was also measured and found to be compatible with extant literature.[26] It is noteworthy that in this study, the high-severity case group included an individual with a D4Z4 repeat size of 8 units highlighting that severe phenotypes can also occur in patients with longer contracted D4Z4 repeat arrays. Consistent with these heterogeneous findings, our study revealed no statistically significant correlation between D4Z4 repeat number and CSS. This supports a growing

consensus that D4Z4 repeat number alone is insufficient to fully explain clinical heterogeneity in FSHD.

Research has highlighted the contribution of additional genetic and epigenetic changes, such as the methylation status of the D4Z4 locus,[14,27] which can influence DUX4 expression and disease activity. Moreover, variability in clinical penetrance—such as asymptomatic carriers with short arrays—may reflect the influence of additional genetic modifiers (e.g., SMCHD1 mutations[6]) and individual-specific factors, including sex hormones,[12,28,29] which may modulate disease expression. Therefore, the pathogenesis of FSHD appears to be a multifactorial process, where D4Z4 repeat number acts as a necessary but not sufficient determinant of clinical outcome.

Our study used data from Southern blot analysis, which was initially employed in FSHD and is considered the gold standard to determine the number of D4Z4RUs and identify the presence of a permissive 4qA allele.[10] With the development of new technologies, more advanced methods such as molecular combing,[30] optical genome mapping (OGM),[31] and nanopore sequencing[32] allowed for high-resolution visualization of the D4Z4 repeat array and are increasingly used due to their speed and accuracy. In patients suspected of having FSHD2, next-generation sequencing (NGS) or Sanger sequencing is used to identify pathogenic variants in genes such as *SMCHD1*, *DNMT3B*, or *LRIF1*. Additionally, methylation-sensitive techniques such as bisulfite sequencing or methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) are employed to assess the epigenetic status of the D4Z4 region, which is crucial in distinguishing FSHD2 cases.

As this study was conducted within a hospital-based cohort, there exists a potential for selection bias. Patients referred to tertiary care centers may represent more severe or atypical phenotypes, which might not be representative of the broader FSHD patient population. To enhance the correlation between molecular findings and clinical severity, the inclusion of functional outcome measures—such as muscle performance tests (e.g., 2-minute walk test) and advanced quantitative assessments such as 3D imaging techniques—alongside standardized scales such as CSS, could provide a more comprehensive evaluation of disease burden.

FSHD remains an incurable neuromuscular disorder, necessitating a deeper understanding of its pathomechanisms to advance therapeutic development.[33] Comprehensive data collection from larger patient cohorts is essential for elucidating the molecular and clinical complexity of the disease. Engaging more patients in diagnostic workflows and performing thorough molecular analyses will be pivotal in unraveling disease mechanisms. Investigating coincident cases[34,35] and expanding research beyond skeletal muscle tissue to include other affected systems, such as bone marrow[36] and immune system responses[37] could yield critical insights into disease progression and variability. In Turkey, diagnostic capabilities remain limited, largely due to the lack of reimbursement for the standard diagnostic protocol. Broadening access to molecular diagnostics and increasing patient enrollment in registries could pave the way for more detailed, population-specific studies. Prospective longitudinal studies with extended follow-up will be invaluable in characterizing the natural history of FSHD and refining genotype-phenotype correlations. Furthermore, integrating advanced imaging modalities, such as magnetic resonance imaging-based muscle quantification and 3D facial mapping, alongside patient-reported outcome measures, may enhance the sensitivity of clinical assessments.

Author Contributions: Concept/Design : CH, OBS, SBK, ES, HU, FK; Data acquisition: CH, OBS, SBK, ES, HU, FK; Data analysis and interpretation: CH, OBS, SBK, ES, HU, FK; Drafting manuscript: CH, OBS, SBK, ES, HU, FK; Critical revision of manuscript: CH, OBS, SBK, ES, HU, FK; Final approval and accountability: CH, OBS, SBK, ES, HU, FK; Technical or material support: CH, OBS, SBK, ES, HU, FK; Supervision: CH, OBS, SBK, ES, HU, FK; Securing funding (if available): n/a.
Ethical Approval: The study protocol received approval from the Akdeniz University Medical Scientific Research Ethics Committee (08.09.2010/008356, 31.07.2025/TBAEK-631).
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: This study was funded by the Akdeniz University Research Foundation and Cukurova University Research Foundation.
Acknowledgement: Southern Blot experiments and haplotype analyses were performed in Germany Institute for Human Genetics, University of Wuerzburg and Netherlands-Leiden University Medical Center.

REFERENCES

1. Lunt PW, Harper PS. Genetic counselling in facioscapulohumeral muscular dystrophy. J Med Genet. 1991;28:655-64.
2. Wang Z, Qiu L, Lin M, Chen L, Zheng F, Lin L et al. Prevalence and disease progression of genetically-confirmed facioscapulohumeral muscular dystrophy type 1 (FSHD1) in China between 2001 and 2020: a

nationwide population-based study. Lancet Reg Health West Pac. 2022;18:100323.
3. Mostacciuolo ML, Pastorello E, Vazza G, Miorin M, Angelini C, Tomelleri G et al. Facioscapulohumeral muscular dystrophy: Epidemiological and molecular study in a north-east Italian population sample. Clin Genet. 2009;75:550-5.
4. Winnen A, Srivastsa S, Eldar-Lissai A, Kouchlev I, Jones G, Zuroske T. EPH75 applying machine learning (ml) to estimate prevalence of facioscapulohumeral muscular dystrophy (fshd) and related disease burden: findings from United States claims analysis. Value in Health; 2022;25(Suppl 12):S205–6.
5. Wijmenga C, Sandkuijl LA, Moerer P, Van Der Boorn N, Bodrug SE, Ray PN et al. Genetic linkage map of facioscapulohumeral muscular dystrophy and five polymorphic loci on chromosome 4q35-qter. Am J Hum Genet. 1992;51:411-5.
6. Lemmers RJLF, Tawil R, Petek LM, Balog J, Block GJ, Santen GWE et al. Digenic inheritance of an SMCHD1 mutation and an FSHD-permissive D4Z4 allele causes facioscapulohumeral muscular dystrophy type 2. Nat Genet. 2012;44:1370-4.
7. Van Den Boogaard ML, Lemmers RJLF, Balog J, Wohlgemuth M, Auranen M, Mitsuhashi S et al. Mutations in dnmt3b modify epigenetic repression of the d4z4 repeat and the penetrance of facioscapulohumeral dystrophy. Am J Hum Genet. 2016;98:1020-29.
8. Hamanaka K, Šikrová D, Mitsuhashi S, Masuda H, Sekiguchi Y, Sugiyama A et al. Homozygous nonsense variant in LRIF1 associated with facioscapulohumeral muscular dystrophy. Neurology. 2020;94:e2441-e2447.
9. Thomas NST, Wiseman K, Spurlock G, MacDonald M, Üstek D, Upadhyaya M. A large patient study confirming that facioscapulohumeral muscular dystrophy (FSHD) disease expression is almost exclusively associated with an FSHD locus located on a 4qA-defined 4qter subtelomere. J Med Genet. 2007;44:215-8.
10. Hewitt JE, Lyle R, Clark LN, Valleley EM, Wright TJ, Wijmenga C et al. Analysis of the tandem repeat locus D4Z4 associated with facioscapulohumeral muscular dystropothhy. Hum Mol Genet. 1994;3:1287-95.
11. Şahan ÖB. Fasioskapulohumeral musküler distrofili olgularda 4q`35`te lokalize D4Z4 tekrar dizilerindeki delesyonların gösterilmesi. Antalya, Akdeniz University Faculty of Medicine. 2012.
12. Hangül C, Bozkurt S, Bilge U, Özdem S, Altunbaş H, Uysal H et al. The ratios of estradiol and progesterone to testosterone influence the severity of facioscapulohumeral muscular dystrophy. Neurol Sci Neurophysiol. 2020;37:190-6.
13. Ricci E, Galluzzi G, Deidda G, Cacurri S, Colantoni L, Merico B et al. Progress in the molecular diagnosis of facioscapulohumeral muscular dystrophy and correlation between the number of KpnI repeats at the 4q35 locus and clinical phenotype. Ann Neurol. 1999;45:751-7.
14. Van Overveld PGM, Enthoven L, Ricci E, Rossi M, Felicetti L, Jeanpierre M et al. Variable hypomethylation of D4Z4 in facioscapulohumeral muscular dystrophy. Ann Neurol. 2005;58:569-76.
15. Tonini MMO, Passos-Bueno MR, Cerqueira A, Matioli SR, Pavanello R, Zatz M. Asymptomatic carriers and gender differences in facioscapulohumeral muscular dystrophy (FSHD). Neuromuscular Disorders. 2004;14:33-8.
16. Zatz M, Marie SK, Cerqueira A, Vainzof M, Pavanello RCM, Passos-Bueno MR. The facioscapulohumeral muscular dystrophy (FSHD1) gene affects males more severely and more frequently than females. Am J Med Genet. 1998;77:155-61.
17. Lamperti C, Fabbri G, Vercelli L, D'Amico R, Frusciante R, Bonifazi E et al. A standardized clinical evaluation of patients affected by facioscapulohumeral muscular dystrophy: The FSHD clinical score. Muscle Nerve. 2010;42:213-7.
18. Hatch MN, Kim K, Kurillo G, Nicorici A, McDonald CM, Han JJ. Longitudinal study of upper extremity reachable workspace in fascioscapulohumeral muscular dystrophy. Neuromuscul Disord. 2019;29:503-513.
19. 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.
20. Statland JM, Tawil R. Facioscapulohumeral muscular dystrophy: Molecular pathological advances and future directions. Curr Opin Neurol. 2011:24:423-8.
21. Tawil R, Forrester J, Griggs RC, Mendell J, Kissel J, McDermott M et al. Evidence for anticipation and association of deletion size with severity in facioscapulohumeral muscular systrophy. Ann Neurol. 1996;39:744-8.
22. Statland JM, Donlin-Smith CM, Tapscott SJ, Lemmers RJLF, Van Der Maarel SM, Tawil R. Milder phenotype in facioscapulohumeral dystrophy with 7-10 residual D4Z4 repeats. Neurol. 2015;85:2147-50.
23. Klinge L, Eagle M, Haggerty ID, Roberts CE, Straub V, Bushby KM. Severe phenotype in infantile facioscapulohumeral muscular dystrophy. Neuromuscular Disorders. 2006;16:553-8.
24. Butz M, Koch MC, Müller-Felber W, Lemmers RJLF, Van Der Maarel SM, Schreiber H. Facioscapulohumeral muscular dystrophy: Phenotype-genotype correlation in patients with borderline D4Z4 repeat numbers. J Neurol. 2003;250:932-7.
25. Van Overveld PGM, Lemmers RJFL, Sandkuijl LA, Enthoven L, Winokur ST, Bakels F et al. Hypomethylation of D4Z4 in 4q-linked and non-4q-linked facioscapulohumeral muscular dystrophy. Nat Genet. 2003;35:315-7.

26. Yunisova G, Eraslan S, Avci S, Eren İ, Demirhan M, Kayserili H et al. Clinical and molecular characteristics of patients with fascio-scapulo-humeral dystrophy 1 (fshd 1): the first cohort investigating the genotype-phenotype characteristics of fshd 1 patients in turkey (P3-11.019). Neurol. 2024:P3-11.019.
27. Erdmann H, Scharf F, Gehling S, Benet-Pagès A, Jakubiczka S, Becker K et al. Methylation of the 4q35 D4Z4 repeat defines disease status in facioscapulohumeral muscular dystrophy. Brain. 2023;146:1388-1402.
28. Hangul C, Celik EG, Kaya H, Eroglu O, Uysal H, Karauzum SB. Estradiol differentially regulates DUX4, β-catenin and PAX3/PAX7 in primary myoblasts of facioscapulohumeral muscular dystrophy patients. Turk Biyokim Derg. 2021;46:435-44.
29. Hangul Ceren, Ozcan Filiz, Darbas Sule, Uysal Hilmi, Koc Filiz Ayse, Karauzum Berker Sibel. Progesterone may be a regulator and B12 could be an indicator of the proximal D4Z4 repeat methylation status on 4q35ter. J Neurochem. 2024;168:3209–20.
30. Nguyen K, Walrafen P, Bernard R, Attarian S, Chaix C, Vovan C et al. Molecular combing reveals allelic combinations in facioscapulohumeral dystrophy. Ann Neurol. 2011;70:627-33.
31. Chun Pang AW, Hastie A, Chaubey A. Identification of structural variation in constitutional disorders by optical genome mapping. Mol Genet Metab. 2021;132:S281.
32. Mitsuhashi S, Nakagawa S, Takahashi Ueda M, Imanishi T, Frith MC, Mitsuhashi H. Nanopore-based single molecule sequencing of the D4Z4 array responsible for facioscapulohumeral muscular dystrophy. Sci Rep. 2017;7:14789.
33. Hangül C, Karaüzüm SB, Akkol EK, Demir-Dora D, Çetin Z, Saygılı Eİ et al. Promising perspective to facioscapulohumeral muscular dystrophy treatment: nutraceuticals and phytochemicals. Curr Neuropharmacol. 2021;19:2276-95.
34. Akpınar A, Gözke E. Evaluation of the relationship between Fascioscapulohumeral dystrophy and noonan syndrome. Neurol Sci Neurophysiol. 2025;42:69–71.
35. Hangül C, Yücel OK, Toylu A, Uysal H, Karaüzüm SB. A novel coincidence: essential thrombocythemia with facioscapulohumeral muscular dystrophy. Turk J Haematol. 2020;37:306-7.
36. Hangul C, Tokta O, Karauzum SB, Akkaya B, Yildirim H, Kupesiz FT et al. Analysis of dux4 expression in bone marrow and re-discussion of dux4 function in the health and disease. Turk Patoloji Derg. 2021;38:219–26.
37. Ragozzino E, Bortolani S, Di Pietro L, Papait A, Parolini O, Monforte M et al. Muscle fibrosis as a prognostic biomarker in facioscapulohumeral muscular dystrophy: a retrospective cohort study. Acta Neuropathol Commun. 2023;11:165.