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Genetic Profile of DMD/BMD: MLPA and Sanger Sequencing Results from a Single Center

DMD/BMD Hastalarında Genetik Spektrum: Tek Merkezden MLPA ve Sanger Sonuçları

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Abstract

Background: Duchenne and Becker Muscular Dystrophy (DMD/BMD) is an X-linked recessive inherited neuromuscular disease characterised by progressive muscle weakness and wasting due to variants in the *DMD* gene encoding dystrophin protein. This study aimed to evaluate the genetic and clinical characteristics of patients diagnosed with DMD/BMD who were found to have variants in the *DMD* gene by genetic analysis.

Material and Methods: In this retrospective study, we evaluated 34 patients from 32 families diagnosed with dystrophinopathies, including 24 individuals (70.6%) with DMD and 10 individuals (29.4%) with BMD. Clinical, biochemical, and family history data were obtained from patient files. Genetic analyses were performed by multiple ligation-dependent probe amplification (MLPA) and Sanger sequencing. 15 mothers were segregated for carriage.

Results: Exon deletion was detected in 79.4% of cases, and a small variant in 20.6%. The most common deletion sites were between exons 45-52. CK levels were found to be high in all patients. The mean age at diagnosis was 5 years. Segregation analysis showed that 12 of the mothers carried the variant detected in their children. Three new variants that had not been reported in the literature before were found in our cohort.

Conclusions: Genetic analyses are valuable in diagnosis, phenotypic prediction, and family screening in DMD/BMD cases. In this study, the variant types and clinical features of DMD/BMD were analysed to contribute to the reported cases from Türkiye. Effective use of molecular diagnosis is important for early diagnosis and management.

Keywords: DMD, BMD, MLPA, Sanger sequencing, Novel variants

Öz

Amaç: Duchenne ve Becker Musküler Distrofi (DMD/BMD), distrofin proteinini kodlayan *DMD* genindeki değişimlere bağlı olarak gelişen, ilerleyici kas güçsüzlüğü ve kaybı ile seyreden X'e bağlı resesif kalıtılan bir nöromüsküler hastalıktır. Bu çalışmanın amacı, genetik analiz ile *DMD* geninde varyant saptanmış ve DMD/BMD tanısı almış olguların genetik ve klinik özelliklerini değerlendirmektir.

Materyal ve metot: Bu retrospektif çalışmada, *DMD* geni analizi ile moleküler tanısı doğrulanmış 32 aileden 34 erkek DMD/BMD olgusu (DMD tanısı alan 24 olgu (%70,6) ve BMD tanısı alan 10 (%29,4) olgu) değerlendirildi. Klinik, biyokimyasal ve aile öyküsü verileri hasta dosyalarından elde edildi. Hastaların genetik analizleri çoklu ligasyon bağımlı prob amplifikasyonu (MLPA) ve Sanger dizileme yöntemleriyle gerçekleştirilmişti. 15 anneden taşıyıcılığa yönelik segregasyon analizi yapılmıştı.

Bulgular: Olguların %79,4'ünde ekzon delesyonu, %20,6'sında küçük varyant tespit edildi. En sık delesyon bölgeleri 45-52. ekzonlar arasındaydı. CK düzeyleri tüm hastalarda yüksek saptanmıştı. Ortalama tanı yaşı 5'ti. Segregasyon analizi yapılan annelerden 12 tanesinin çocuğunda saptanan varyantı taşıdığı gösterilmişti. Kohortumuzda daha önce literatürde bildirilmemiş 3 yeni varyant saptanmıştır.

Sonuç: DMD/BMD olgularında genetik analizler tanı koymada, fenotipik öngörüde bulunmada ve aile taramasında oldukça değerlidir. Bu çalışmada, Türkiye'den bildirilen olgulara katkı sağlayacak şekilde, DMD/BMD'ye neden olan varyant tipleri ve klinik özellikler ortaya konmuştur. Moleküler tanının etkin kullanımı, erken tanı ve yönetim için önem taşımaktadır.

Anahtar Kelimeler: DMD, BMD, MLPA, Sanger dizileme, Novel varyant

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Introduction

The dystrophin gene, located in the Xp21 region of the X chromosome, spans approximately 2.4 Mb of genomic DNA and, with 79 exons, is recognised as the largest known human gene (1). Its product, dystrophin protein, performs a vital function by linking the cytoskeletal framework within muscle fibres to the extracellular matrix. This structural role is critical for maintaining the integrity and stability of muscle cell membranes during contraction cycles (2).

Duchenne muscular dystrophy (DMD) is a severe and progressive neuromuscular disease characterised by muscle degeneration. Early signs typically appear between the ages of 2 and 3 and include difficulty climbing stairs, a waddling gait, and frequent falls. Most people lose the ability to walk by the age of 10 to 12 and require ventilator support by their twenties. Despite advances in supportive care, the majority of patients succumb to cardiac and/or respiratory complications between the ages of 20 and 40 (3). The disease has an estimated global incidence of approximately 1 in 5,000 live male births (4).

Becker muscular dystrophy (BMD) is typically associated with a milder and more variable phenotype. The pathogenic variants in the dystrophin gene exert less severe effects than those observed in Duchenne muscular dystrophy, as dystrophin production is not completely absent, but rather reduced in quantity or functionality. Skeletal muscle weakness in BMD progresses slowly, with symptom onset generally occurring around the age of 8. In individuals with minimal cardiac involvement, onset may be delayed until around the age of 15. Heart failure remains the leading cause of mortality in patients with BMD (5). The prevalence of BMD is estimated to be 0.26 per 10,000 men (6).

DMD is caused by gene variants that disrupt the open reading frame or introduce premature stop codons, resulting in impaired assembly of the dystrophin-associated protein complex (DAPC) or failure to bind F-actin, thereby preventing the synthesis of functional dystrophin. In contrast, BMD typically involves in-frame deletions that preserve the translational reading frame, allowing the production of a truncated dystrophin protein that retains essential functional domains (7).

In this study, we aim to present the genetic characteristics of 34 patients diagnosed with DMD/BMD from 32 unrelated families. Following the identification of *DMD* gene variants in these patients, segregation analyses were performed in 15 mothers. We also report three previously unreported disease-causing variants in the *DMD* gene.

Materials and Methods

Written informed consent for the use of genetic information for research purposes was obtained from patients who underwent genetic testing at Aydın Adnan Menderes University. As this is a retrospective study, ethical approval was obtained from the Non-Interventional Clinical Research Evaluation Committee of Aydın Adnan Menderes University Faculty of Medicine (approval no: 19, date: January 26, 2025).

In this study, we analysed the results of 34 DMD/BMD patients in whom *DMD* gene variants were identified between 1 January 2015 and 31 December 2024. The study group consisted of 34 patients from 32 unrelated families and 15 mothers from these 32 DMD/BMD families.

Demographic, family history, and genetic data were collected from patient records. Age-, sex-, and pubertal stage-specific reference intervals for CK were based on data from the large population-based study in healthy children and adolescents conducted by Fechner et al. (2024) (8). Blood samples were taken from the participants, and genomic DNA was extracted from EDTA-treated peripheral blood samples. MLPA was performed using the SALSA MLPA P034-B2-0421 DMD Mix1 Kit and the SALSA MLPA P035-B1-0421 DMD Mix2 Kit (MRC-Holland, Amsterdam, The Netherlands) according to the manufacturer's protocol. Amplification products were analysed by capillary electrophoresis on an ABI 3100 Genetic Analyser (Thermo Fisher Scientific, MA, USA). Data were analysed using Genemapper 4.0 and Coffalyser.net software. Dystrophin gene sequence analysis was performed on an APPLIED 3730 DNA Analyzer (48-capillary) automated DNA sequencer according to the manufacturer's protocol. Raw sequence data were analysed using SeqScape analysis software.

Variants were classified according to the recommendations of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (9). The Leiden Open Variation Database (LOVD) (10), the Universal Mutation Database (UMD)-DMD database (11), and the ClinVar database (12) were used to determine whether variants had been previously reported. Novel variants were evaluated using multiple in-silico prediction tools (REVEL, SIFT, SpliceAI) and confirmed to be absent or extremely rare in population databases (gnomAD) and variant repositories (ClinVar, LOVD).

Statistical Analysis

Statistical data analysis was performed using Microsoft Excel; otherwise, no statistical analysis program was used.

Results

A total of 34 patients from 32 unrelated families were included in the study (Table 1). The median age at diagnosis was 5 years (range: 10 months - 17 years). The most common initial clinical presentation was incidental elevation of serum creatine kinase (CK) levels (44%, n=15), followed by gait disturbance (26.4%, n=9) and difficulty rising from a sitting position (14.7%, n=5). Among patients with elevated CK levels, many were asymptomatic at diagnosis.

Table 1. Clinical features and genetic results of the cases

Case	Age at diagnosis	First symptom	First CK level	Genetic test	Detected variant	Phenotype	Segregation of mother	Family history	Gene region
C1	4y 11m	Frequent falls, Gowers' sign	27956	*DMD* MLPA + Sanger Sequencing	c.5476 G>T p.(Glu1826*)	DMD	Carrier	Older brother of C2	Central rod domain
C2	6m	Incidental CK elevation	15214	Target region analysis	c.5476 G>T p.(Glu1826*)	DMD	Carrier	Younger brother of C1	Central rod domain
C3	8y	Impaired gait, difficulty ascending stairs	17918	*DMD* MLPA + Sanger Sequencing	c.745 C>T p.(Gln249*)	DMD	Carrier	Negative	Actin-binding domain
C4	4y	Impaired gait	3197	*DMD* MLPA + Sanger Sequencing	c.10033 C>T p.(Arg3345*)	DMD	Carrier	Negative	Cystein-rich domain
C5	3y 4m	Impaired gait	6586	*DMD* MLPA + Sanger Sequencing	c.9947 G>T p.(Cys3316Phe)	DMD	n.k.	n.k.	Cystein-rich domain
C6	4y 6m	Incidental CK elevation	59790	*DMD* MLPA + Sanger Sequencing	c.961-7 T>A	DMD	Negative	Negative	Central rod domain
C7	4y 10m	Impaired gait, quick fatigue	16577	*DMD* MLPA + Sanger Sequencing	c.1514_1515 insAGAGAATTGACCCAAGA p.(Arg506Glufs*3)	DMD	Carrier	Muscle disease history in his maternal uncle	Central rod domain
C8	1y 5m	Incidental CK elevation	6482	DMD MLPA	Exon 3-11 deletion	DMD	Carrier	Muscle disease history in his maternal aunt	Actin-binding and central rod domain
C9	4y 4m		10633	*DMD* MLPA	Exon 8-18 deletion	DMD	n.k.	Muscle disease history in his 2 maternal uncles	Actin-binding and central rod domain
C10	9m	Impaired gait, difficulty ascending stairs	14062	*DMD* MLPA	Exon 8-41 deletion	DMD	n.k.	DMD diagnosis in his maternal uncle	Actin-binding and central rod domain
C11	17y	Incidental CK elevation	2833	*DMD* MLPA	Exon 13-33 deletion	BMD	n.k.	Negative	Central rod domain
C12	8y	Difficulty in ascending stairs and rising from a seated position	13295	*DMD* MLPA	Exon 18-30 deletion	DMD	n.k.	Negative	Central rod domain
C13	4y 6m	Difficulty in rising from a seated position	22675	*DMD* MLPA	Exon 44 deletion	DMD	Carrier	Muscle disease history in his mother's maternal uncle	Central rod domain
C14	13m	Quick fatigue	10701	*DMD* MLPA	Exon 45 deletion	DMD	Negative	n.k.	Central rod domain
C15	3y 10m	Incidental CK elevation	16064	*DMD* MLPA	Exon 45 deletion	DMD	Carrier	Negative	Central rod domain

Table 1. Continued

C16	8y	Incidental liver enzyme elevation, gastrocnemius hypertrophy, Gowers' sign	3039	*DMD* MLPA	Exon 45-47 deletion	BMD	n.k.	Negative	Central rod domain
C17	7y 6m	Difficulty in rising from a seated position	11352	*DMD* MLPA	Exon 45-47 deletion	BMD	n.k.	Negative	Central rod domain
C18	7y 4m	Incidental CK elevation	7853	*DMD* MLPA	Exon 45-47 deletion	BMD	n.k.	Muscle disease history in his 2 maternal uncles and 2 sons of his maternal aunt	Central rod domain
C19	6y 10m	Incidental CK elevation	6280	*DMD* MLPA	Exon 45-48 deletion	BMD	n.k.	n.k.	Central rod domain
C20	4y 8m	Incidental CK elevation	8517	*DMD* MLPA	Exon 45-49 deletion	BMD	n.k.	Negative	Central rod domain
C21	6y 2m	Incidental CK elevation	1927	*DMD* MLPA	Exon 45-51 deletion	BMD	n.k.	n.k.	Central rod domain
C22	6y	Incidental CK elevation	6612	*DMD* MLPA	Exon 45-51 deletion	BMD	Carrier	Negative	Central rod domain
C23	6y 3m	Incidental CK elevation	8352	*DMD* MLPA	Exon 45-52 deletion	DMD	n.k.	n.k.	Central rod domain
C24	5y	Incidental CK elevation	7657	*DMD* MLPA	Exon 45-54 deletion	DMD	Carrier	Negative	Central rod domain
C25	7y	Incidental CK elevation, Gowers' sign	14450	*DMD* MLPA	Exon 46-50 deletion	DMD	Carrier	Negative	Central rod domain
C26	4y 7m	Impaired gait	23203	*DMD* MLPA		DMD	n.k.	n.k.	Central rod domain
C27	3y 4m	Impaired gait, difficulty ascending stairs	2530	*DMD* MLPA	Exon 46-50 deletion	BMD	n.k.	n.k.	Central rod domain
C28	15y	Incidental CK elevation, gastrocnemius hypertrophy	740	*DMD* MLPA	Exon 48-49 deletion	BMD	n.k.	n.k.	Central rod domain
C29	1y 6m	Impaired gait, difficulty ascending stairs	17382	*DMD* MLPA	Exon 48-51 deletion	DMD	Negative	Negative	Central rod domain
C30	1y 8m	Difficulty in rising from a seated position, gastrocnemius hypertrophy	14515	*DMD* MLPA	Exon 49-50 deletion	DMD	n.k.	Muscle disease history in his mother's maternal uncle	Central rod domain
C31	9y	Incidental liver enzyme elevation, gastrocnemius hypertrophy	6676	*DMD* MLPA	Exon 50 deletion	DMD	n.k.	n.k.	Central rod domain
C32	11m	Difficulty in rising from a seated position	12280	*DMD* MLPA	Exon 51 deletion	DMD	Carrier	Muscle disease history in his mother's maternal uncle	Central rod domain

Table 1. Continued

C33	19m	Incidental CK elevation, gastrocnemius hypertrophy	4394	DMD *MLPA*	Exon 53 deletion	DMD	Carrier	Older brother of C34	Central rod, cystein-rich, and C-terminal domain
C34	10m	Positive family history	17183	*DMD* MLPA	Exon 61-79 deletion	DMD	Carrier	Younger brother of C33	Central rod, cystein-rich, and C-terminal domain

C: Case, CK: Creatin kinase, MLPA: Multiplex ligand-dependent probe amplification, m: Months, n.k.: Not known, y: Years

Serum CK levels at diagnosis ranged from 740 IU/L to 59,790 IU/L, with a median of approximately 12,000 IU/L. Higher CK levels (>20,000 IU/L) were observed in 7 patients, and in particular, the highest CK elevation (59,790 IU/L) was seen in a patient with a splice site variant (c.961-7 T>A) affecting the central rod domain.

Molecular analysis revealed that 79.4% (n=27) of the patients carried large deletions detected by MLPA, while 20.6% (n=7) carried small nucleotide variants identified by Sanger sequencing (Figure 1). Among the exon deletions, exon 45 was the most frequently affected. 11 patients (32.3%) had deletions involving exon 45. The most common deletion pattern was the deletion of exons 45-47, which was detected in 3 cases.

In our cohort, the distribution of dystrophin gene variants across protein domains revealed that the majority of pathogenic variants (26 out of 34; 76.5%) were localised within the central rod domain. This was followed by variants in the cysteine-rich domain in 2 patients (5.9%) and the actin-binding domain in 1 patient (2.9%). In addition, 3 patients (8.8%) had variants involving both the actin-binding and central rod domains, while 2 patients (5.9%) had variants involving a combination of the central rod, cysteine-rich, and C-terminal domains.

Regarding the clinical phenotype, 24 patients (70.6%) were diagnosed with DMD and 10 patients (29.4%) with BMD (Figure1). All patients with nonsense or frameshift variants had a DMD phenotype, consistent with the severe nature of these variants.

Family studies showed that 37.5% (n=12) of mothers were confirmed carriers, 53.1% (n=17) had unknown carrier status due to lack of testing or inconclusive results, and 9.4% (n=3) were reported as non-carriers. A positive family history of neuromuscular disease was present in 35.3% (n=12) of cases, predominantly through the maternal line.

Among the small nucleotide variants, three variants were considered novel, as they were absent from population databases (gnomAD), unreported in ClinVar, UMD-DMD, and LOVD, and had not been described in the published literature (Table 2). These include NM_004006.2: *DMD* c.9947G>T p.(Cys3316Phe), *DMD* c.961-7T>A and *DMD* c.1514_1515insAGAGAATTGACCCAAGA p.(Arg506Glufs*3). All were classified as likely pathogenic according to ACMG criteria. Segregation analysis of the parents of C6 showed that the parents were not carriers of the c.961-7 T>A variant, and the variant was considered to be de novo.

Discussion

In this study, we analysed the genetic and clinical features of 34 DMD/BMD patients, focusing on the variant spectrum in the dystrophin gene.

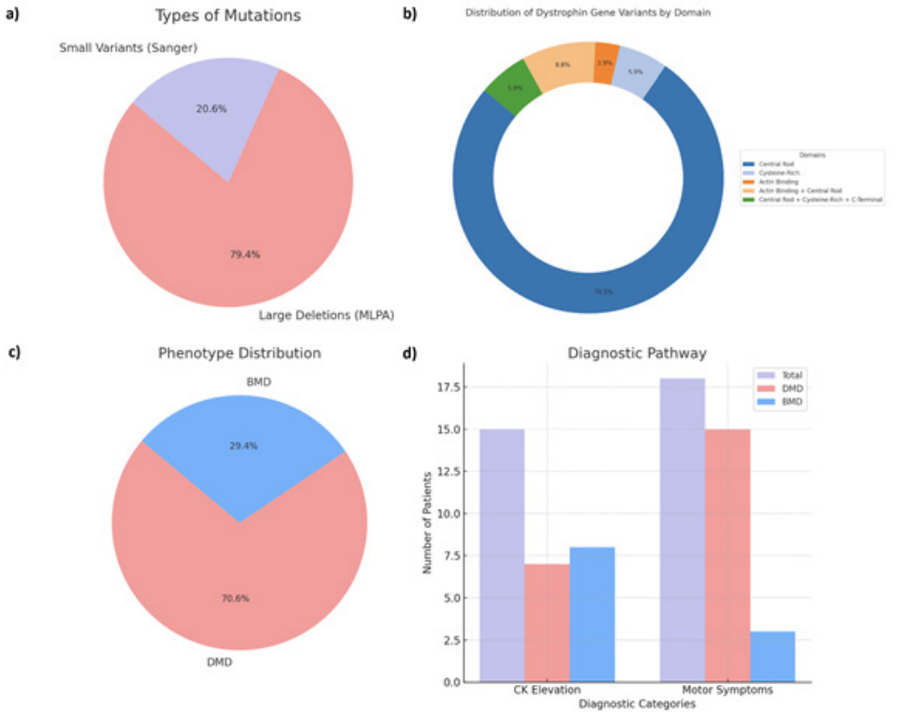

Figure 1. Genetic and clinical characteristics of DMD/BMD patients. (a) Types of variants identified: The majority of variants detected in the cohort were large deletions identified by MLPA (79.4%), while small variants detected by Sanger sequencing accounted for 20.6% of cases. (b) Distribution of dystrophin gene variants by protein domain: Most pathogenic variants (76.5%) were located in the central rod domain of the dystrophin protein, followed by combinations involving actin-binding, cysteine-rich, and C-terminal domains. (c) Phenotype distribution: Among the patients, 70.6% were clinically diagnosed with DMD and 29.4% with BMD. (d) Diagnostic pathways: Initial diagnostic clues were categorised as either elevated CK levels or motor symptoms. Most DMD cases were diagnosed based on motor symptoms, whereas CK elevation contributed significantly to the diagnosis of both DMD and BMD.

DMD/BMD: Duchenne and Becker Muscular Dystrophy, CK: Creatin kinase, MLPA: Multiple ligation-dependent probe amplification

Table 2. Phenotype-genotype correlation of small genomic changes

Case	Phenotype	Variant	Pathogenicity and ACMG evidence	Known/ novel variant
C1-C2	DMD	c.5476 G>T p.(Glu1826*)	Pathogenic (PS4, PVS1, PM2)	Known
C3	DMD	c.745 C>T p.(Gln249*)	Pathogenic (PS4, PVS1, PM2)	Known
C4	DMD	c.10033 C>T p.(Arg3345*)	Pathogenic (PS4, PVS1, PM2)	Known
C5	DMD	c.9947 G>T p.(Cys3316Phe)	Likely Pathogenic (PP3, PM1, PM2)	Novel
C6	DMD	c.961-7 T>A	Likely Pathogenic (PM2, PM6, PP4)	Novel
C7	DMD	c.1514_1515 insAGAGAATTGACCCAAGA p.(Arg506Glufs*3)	Likely Pathogenic (PVS1, PM2)	Novel

DMD: Duchenne Muscular Dystrophy

Comparative Analysis with the Turkish Population

Our findings were compared with three recent Turkish studies - Ozkalayci et al. (13), Öztürk et al. (14), and Çavdarlı et al. (15)- to contextualise our results within the national landscape.

The deletion frequency in our cohort (79.4%) is consistent with the rates reported in these three larger Turkish cohorts. Ozkalayci et al. (13) reported a deletion rate of 87.7% and a duplication rate of 12.3% (13), Öztürk et al. (14) observed 89.9% deletions and 10.1% duplications, while Çavdarlı et al. (15) also identified deletions as the most common variant type in their

single-centre analysis. The predominance of deletions in all studies reinforces the central role of MLPA as a first-line genetic test for dystrophinopathies.

Consistent with these studies, exon 45 was the most frequently affected region in our data set. Exons 45-52 were also a major hotspot in the cohort of Öztürk et al. (14), while Ozkalayci et al. (13), highlighted introns 43-55 as a breakpoint cluster. The Çavdarlı et al. (15) study confirmed this by identifying exons 44-53 as major deletion hotspots, corresponding to the globally recognised variant-prone zones in the dystrophin gene.

Although duplications were not observed in our small cohort, both Öztürk et al. (14) and Çavdarlı et al. (15) reported duplication frequencies close to 10%. Interestingly, the most common duplicated exon differed slightly between studies. This may be due to regional or methodological differences.

Our study also contributes novel findings, including three small nucleotide variants not previously reported in the literature. One of these, an intronic variant (c.961-7T>A), was associated with a particularly severe biochemical phenotype, suggesting clinical significance. Ozkalayci et al. (13) also identified five novel variants by sequencing, highlighting the importance of ongoing regional data collection for variant databases.

In terms of phenotype-genotype correlation, our study supports the reading frame rule: all patients with out-of-frame variants had the DMD phenotype. However, Ozkalayci et al. (13) found that 7.6% of their cases did not follow this rule, particularly in patients with complex or novel rearrangements, suggesting that this rule may not be universally reliable.

Our maternal carrier detection rate (37.5%) was lower than that reported by Ozkalayci et al. (13) (63.6%), possibly due to differences in sample size or testing practices. Notably, their study also documented cases of germline and gonosomal mosaicism, a reminder of the complexity of genetic counselling for these conditions.

Overall, our results are consistent with national data and highlight the value of MLPA as a diagnostic tool. The common identification of variant hotspots across Turkish studies, particularly exons 45-55, supports the strategic targeting of exon skipping therapies and reinforces the potential for national registries and collaborative research to optimise DMD/BMD management in Türkiye.

Comparative Analysis with Other Populations

The prevalence of large deletions in our cohort (79.4%) is in close agreement with findings from international studies. For example, Mathur et al. (4) reported deletions in 74% of Indian patients using MLPA and whole-exome sequencing (WES), reinforcing the utility of MLPA as a first-line tool for detecting copy number variations in the *DMD* gene (4). Similarly, Guo et al. (16) analysed 613 Chinese patients and identified deletions in 71.1% and duplications in 8.6% of cases, with the remainder harbouring small nucleotide variants.

Consistent with these studies, we found that exon 45 was the most commonly affected region. This is supported by the findings of Viggiano et al. (17), who reported that exons 45-55 were a major hotspot in their southern Italian cohort of 750 DMD/BMD patients. Vieitez et al. (18) also identified exon 45 as the most frequently deleted exon in 284 Spanish patients.

The majority of pathogenic variants in our patients (76.5%) were located in the central rod domain, mirroring the distribution patterns reported by Zamani et al. (19), who found this region to be disproportionately affected in their Iranian cohort of 314 patients. Interestingly, this domain is also a target region for exon skipping therapies, highlighting the therapeutic relevance of our findings.

Three small nucleotide variants identified in our study were novel. One of them, the intronic variant c.961-7T>A, was associated with the highest serum CK level observed in our cohort (59,790 IU/L), suggesting a severe functional impact. Similar efforts to report novel variants have been made by Amr et al. (20), who identified 30% previously unreported variants in 1,250 DMD patients, including six novel variants.

Our results also confirmed the reading frame rule: all patients with frameshift or nonsense variants showed a DMD phenotype. This genotype-phenotype correlation has been validated in different populations, although exceptions have been documented, particularly in patients with complex variants or partial exon skipping (17).

Finally, the maternal carrier rate in our study (37.5%) was lower than that reported in other international cohorts, such as 63.6% in Iran (19), which may reflect differences in sample size, testing strategy, or genetic counselling practices.

In conclusion, our results confirm global findings while introducing novel pathogenic variants, highlighting the continued need for regional data collection and contribution to global variant databases. The recurrent detection of variants around exon 45 supports the rationale for targeted therapies and highlights a critical mutational hotspot in dystrophinopathies.

Novel Variants

Importantly, three novel variants were identified in our cohort: c.9947 G>T p.(Cys3316Phe), c.961-7 T>A, and c.1514_1515insAGAGAATTGACCCAAGA p.(Arg506Glufs*3). All of these were classified as likely pathogenic according to ACMG criteria and have not been previously reported in the literature. These novel findings contribute to the expanding variant landscape of the *DMD* gene and may have implications for future genotype-phenotype correlation studies. The REVEL score of the c.9947 G>T variant is 0.98, supporting the pathogenicity of this variant (21). Notably, the highest recorded CK level (59,790 IU/L) in our cohort was associated with the c.961-7T>A variant,

suggesting a potentially severe impact on protein function. Analysis using the Human Splicing Finder (HSF) indicated that the variant could affect normal splicing. Specifically, the variant was predicted to activate a cryptic acceptor splice site, which could lead to aberrant splicing by activating a cryptic exon. Furthermore, MaxEnt analysis revealed disruption to the wild-type acceptor site, which is likely to affect normal splicing. Both the HSF and MaxEnt algorithms consistently predicted the activation of a novel cryptic acceptor site, further reinforcing the likelihood of a splicing defect associated with this intronic change. Considering the clinical presentation of the case (rapid fatigue, the Gowers sign, and gastrocnemius hypertrophy), and the fact that parental segregation analysis showed the variant to be de novo, it is hypothesised that the variant may be associated with the disease. However, functional studies are needed to draw definitive conclusions about its pathogenicity (22).

Genetic Counselling

In the context of DMD/BMD, genetic counselling plays a critical role in informing families about the genetic basis of the disease and in accurately assessing the risk of relapse. Germline mosaics, even if the mother is not identified as a carrier, can significantly increase the risk of relapse and should therefore be carefully considered during prenatal counselling (23). Recent practice guidelines emphasise the importance of integrating clinical, familial, and molecular data to provide comprehensive and individualised counselling (24). In addition, women at risk of being carriers can use genetic information as a means of empowerment, especially when supported by effective communication and psychosocial counselling (25). On a broader scale, population-based carrier screening programmes have demonstrated the value of early detection and public awareness in reducing the long-term incidence of hereditary neuromuscular disorders (26). Taken together, these findings highlight the need for a personalised, multidimensional approach to genetic counselling in DMD/BMD cases, especially when designing reproductive and preventive strategies.

Limitations of the Study

One limitation of our study is that segregation analysis was only performed on 15 of the 32 mothers, primarily due to parental sample unavailability, an inability to contact family members for testing, parental refusal, or a decision to undergo testing at another medical centre where treatment would continue. CK measurements were obtained at different time points in the disease course, which may limit direct comparability between patients. The relatively small number of BMD cases limited the statistical power for subgroup analyses. Furthermore, genotype-phenotype correlation was limited for rare variants, as comparable cases are scarce in the literature. Finally, the short follow-up duration for some patients prevented a comprehensive evaluation of long-term disease progression and complications.

Conclusion

In conclusion, our study confirms that exon deletions, especially involving exon 45, are the most common variant type in Turkish DMD/BMD patients. The predominance of variants within the central rod domain supports its role as a critical variant hotspot. The identification of three novel variants, including a de novo splice site variant, expands the variant spectrum of DMD and highlights the need for regional data contribution to global variant databases. MLPA, complemented by sequencing, remains the most effective diagnostic strategy. Further integration of genetic, clinical, and biochemical data will improve our understanding of disease heterogeneity and optimise patient management.

Future Perspectives

Further studies with larger cohorts and functional analysis of novel variants are essential to elucidate their pathogenicity and impact on dystrophin function. The consistent identification of variants within exons 45-55 highlights the potential for exon skipping therapies targeting these regions. National registries and collaborative research initiatives should be strengthened to facilitate genotype-phenotype correlations and advance personalised therapeutic strategies in DMD/BMD patients. In addition, expanding maternal carrier testing and incorporating next-generation sequencing approaches may improve diagnostic accuracy and genetic counselling outcomes.

Ethical Approval: *This study was approved by the Non-Interventional Clinical Research Ethics Committee of Aydın Adnan Menderes University Faculty of Medicine (Approval No: 19, Date: January 26, 2025).*

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