

Genotype–phenotype correlations and functional outcomes assessed by the North Star Ambulatory Assessment in ambulatory children with dystrophinopathy

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ABSTRACT

Objective: To evaluate the relationship between genotype characteristics and motor function in ambulatory children with dystrophinopathy using the North Star Ambulatory Assessment (NSAA).

Methods: This retrospective study included 52 ambulatory patients with genetically confirmed dystrophinopathy. Patients were classified according to reading-frame status (in-frame/out-of-frame), mutation type, and mutation localization. Baseline and follow-up NSAA scores, as well as annualized NSAA change, were analyzed. Multivariable regression analysis was performed to identify independent predictors of functional progression.

Results: Although patients with in-frame variants, proximal mutations, and deletion variants tended to have higher NSAA scores, these differences did not reach statistical significance. No significant association was found between genetic characteristics and annualized NSAA progression. In multivariable analysis, older age at first evaluation was independently associated with greater functional decline ($\beta = -0.025$, $p = 0.009$). Corticosteroid treatment was inversely associated with NSAA trajectory ($\beta = -2.020$, $p = 0.008$).

Conclusion: Reading-frame status, mutation type, and mutation localization were not significant predictors of short-term ambulatory functional progression. Although corticosteroid treatment showed an inverse association with NSAA trajectory in this cohort, this finding should be interpreted cautiously because of the potential influence of confounding by indication. Age emerged as the strongest determinant of NSAA decline. These findings suggest that motor outcomes in dystrophinopathy are influenced more by disease stage and other biological modifiers than by primary genetic characteristics alone.

Keywords: dystrophinopathy, Duchenne muscular dystrophy, genotype–phenotype correlation, North Star Ambulatory Assessment, motor function

INTRODUCTION

Duchenne muscular dystrophy (DMD) is the most common inherited neuromuscular disorder of childhood and is caused by pathogenic variants in the dystrophin (DMD) gene located on chromosome Xp21.¹⁻³ The disease is characterized by progressive muscle degeneration

resulting from the absence or severe reduction of functional dystrophin protein. Dystrophin deficiency leads to progressive muscle fiber degeneration, chronic inflammation, fibrosis, and gradual loss of muscle function.⁴ Clinical symptoms usually emerge during early childhood and lead to progressive motor impairment, loss



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of ambulation, respiratory insufficiency, cardiomyopathy, and premature mortality.¹⁻³

The DMD gene is one of the largest genes in the human genome and exhibits substantial mutational heterogeneity. Large exon deletions represent the most common pathogenic variants, accounting for approximately 60–70% of Duchenne and Becker muscular dystrophy (BMD) cases, followed by duplications (5–15%) and smaller sequence variants, including nonsense, splice-site, frameshift, and missense mutations.⁴⁻⁶ Mutation hotspots are predominantly located within exons 2–20 and exons 45–55 of the DMD gene.^{4,7} Advances in molecular diagnostic techniques, particularly multiplex ligation-dependent probe amplification (MLPA) and next-generation sequencing (NGS), have markedly improved mutation detection rates and enabled comprehensive characterization of dystrophin gene variants.^{4,7}

The classical reading-frame rule proposed by Monaco et al. suggests that out-of-frame mutations disrupt dystrophin synthesis and result in the severe Duchenne phenotype, whereas in-frame mutations allow production of partially functional dystrophin and are generally associated with milder clinical manifestations.⁸ Nevertheless, subsequent studies have shown that clinical severity cannot always be predicted solely by reading-frame status, indicating the contribution of additional molecular and genetic factors.⁹⁻¹¹

Growing evidence suggests that disease progression is influenced not only by the primary mutation but also by residual dystrophin expression, mutation localization, alternative splicing mechanisms, corticosteroid exposure, and modifier genes such as SPP1 and LTBP4.^{9,11} In a recent systematic review and meta-analysis, age, baseline functional status, glucocorticoid treatment, and several genetic modifiers were identified as important determinants of disease progression and loss of ambulation.¹² These findings emphasize the complexity of genotype–phenotype relationships in dystrophinopathies and highlight the need for further studies evaluating functional outcomes in genetically characterized cohorts.

Assessment of ambulatory motor performance has become a central component of both natural-history studies and therapeutic trials in DMD. The North Star Ambulatory Assessment (NSAA) is a validated 17-item functional scale specifically developed for ambulatory patients with DMD and is currently one of the most widely used outcome measures in clinical practice and clinical research.^{13,14} The NSAA has demonstrated good reliability, sensitivity to

longitudinal functional change, and strong correlations with other motor outcome measures.^{13,14} More recently, studies investigating longitudinal NSAA trajectories have improved understanding of disease progression and clinically meaningful functional decline during the ambulatory stage.¹⁵

Despite increasing interest in genotype–phenotype relationships, the extent to which mutation characteristics influence NSAA progression remains uncertain. A recent international meta-analysis involving more than 700 ambulatory patients with DMD demonstrated that genotype-related variables explained only a small proportion of variability in one-year NSAA outcomes, whereas clinical characteristics such as age and baseline functional status accounted for substantially greater variability.¹⁶ These findings suggest that the prognostic contribution of genetic factors to ambulatory motor progression remains incompletely understood.

Therefore, the aim of the present study was to investigate genotype–phenotype relationships in ambulatory children aged 3 years and older with genetically confirmed dystrophinopathy. Specifically, we evaluated associations between reading-frame status, mutation localization, and mutation type and baseline NSAA performance, follow-up NSAA scores, and annualized NSAA progression.

MATERIALS AND METHODS

This retrospective observational study was conducted at the Pediatric Neurology Department of Gaziantep City Hospital. Ambulatory patients with genetically confirmed dystrophinopathy who were followed between February 2024 and November 2025 and had at least two NSAA assessments available for review were screened for eligibility. Clinical, genetic, and functional data were retrospectively extracted from electronic medical records and patient files.

Patients were eligible for inclusion if they met all of the following criteria: (1) age ≥ 3 years at the time of assessment, (2) preserved independent ambulation, (3) a genetically confirmed pathogenic DMD gene variant, and (4) at least two NSAA evaluations during follow-up.

Patients were excluded if they had loss of ambulation at baseline, absence of serial NSAA assessments, insufficient clinical data, or lack of genetic confirmation. Loss of ambulation was defined as permanent loss of independent walking ability requiring wheelchair dependence.

Because phenotypic distinction between DMD and BMD may be difficult during the ambulatory stage and could introduce classification bias, all patients were analyzed as a single dystrophinopathy cohort.

Clinical assessment

Demographic and clinical data were obtained from medical records. The following variables were recorded: current age, age at symptom onset, follow-up duration, corticosteroid treatment status, corticosteroid type, age at corticosteroid initiation, serum creatine kinase (CK) level, and first and last NSAA scores. Corticosteroid treatment consisted of prednisolone or deflazacort and was initiated according to current international standards of care.² The decision to start treatment was individualized based on the clinical phenotype, age, ambulatory status, motor function, and overall disease progression.

Genetic analysis

Genetic diagnosis was established using MLPA and/or NGS. Detected DMD gene variants were classified as deletions, duplications, nonsense variants, splice-site variants, small insertions/deletions, or other point mutations. Variants were additionally categorized according to the reading-frame rule as in-frame or out-of-frame mutations.

For mutation localization analysis, deletions and duplications were further categorized as proximal (involving exons 1–44) or distal (involving exons 45–79) according to their location within the DMD gene. The cutoff at exon 45 was selected based on the major distal mutation hotspot of the DMD gene, which encompasses exons 45–55.^{17,18} Point mutations, splice-site variants, and other small sequence variants were not included in the localization analysis.

Only variants classified as pathogenic or likely pathogenic according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guidelines were included in the analysis.¹⁹

Functional assessment

Functional motor performance was evaluated using the NSAA, a validated 17-item functional scale specifically developed for ambulatory patients with DMD and widely used to monitor disease progression in clinical practice and research.^{1,20-23} Each item is scored from 0 to 2, yielding a total score ranging from 0 to 34, with higher scores indicating better motor performance.

NSAA evaluations were performed during routine outpatient follow-up visits by experienced pediatric neurologists. The first NSAA score, last NSAA score, and annualized NSAA change were included in the analysis. Annualized NSAA change was calculated as:

Annualized NSAA change = (Last NSAA score – First NSAA score) / Follow-up duration (years).

Ethical approval

The study was approved by the Gaziantep City Hospital Non-Interventional Clinical Research Ethics Committee (approval number: 443/2026; approval date: February 18, 2026). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). The distribution of continuous variables was assessed using the Shapiro–Wilk test. Normally distributed variables were presented as mean ± standard deviation (SD), whereas non-normally distributed variables were expressed as median and interquartile range (IQR). Categorical variables were summarized as frequencies and percentages.

To evaluate genotype–phenotype associations, patients were grouped according to reading-frame status (in-frame vs. out-of-frame), mutation localization (proximal vs. distal), mutation type (deletion vs. non-deletion), and corticosteroid treatment status. Comparisons between two independent groups were performed using the Mann–Whitney U test because most functional outcome variables did not demonstrate a normal distribution. Categorical variables were compared using the chi-square test or Fisher’s exact test when appropriate.

Annualized NSAA change was calculated by dividing the difference between the first and last NSAA scores by the follow-up duration in years. To identify independent predictors of annualized NSAA change, a multivariable linear regression analysis was performed. Age at first evaluation, corticosteroid treatment status, serum CK level, reading-frame status, and mutation type were included in the regression model.

All statistical tests were two-tailed, and a p-value <0.05 was considered statistically significant.

RESULTS

A total of 52 ambulatory patients with genetically confirmed dystrophinopathy were included in the study. Baseline demographic, clinical, genetic, and functional characteristics, including the clinical presentations leading to diagnosis, are summarized in Table 1. The most common reason leading to diagnosis was isolated serum CK elevation (n=17, 32.7%), followed by a positive family history (n=14, 26.9%). Motor-related symptoms, including gait disturbance, difficulty climbing stairs, and frequent falls (n=21, 40.4%).

The mean age at first evaluation was 64.3 ± 25.9 months, while the mean age at last evaluation was 81.5 ± 28.1 months. The median follow-up duration was 15 months (IQR, 12–24 months). The mean first and last NSAA scores were 27.3 ± 3.8 and 26.6 ± 4.1 , respectively. The median annualized NSAA change was -0.48 points/year (IQR, -2.0 to 1.0).

One patient lost ambulation during follow-up. This patient was first evaluated at 8 years of age and lost ambulation at the age of 10 years. Baseline and last NSAA scores were 21

Table 1. Baseline demographic, clinical, and functional characteristics of the study cohort.

Variable	Value
Age at first evaluation, months	64.3 ± 25.9
Age at last evaluation, months	81.5 ± 28.1
Follow-up duration, months	15 (12–24)
Reasons leading to diagnosis, n (%)	
Motor-related symptoms	21 (40.4)
Frequent falls	8 (15.4)
Gait disturbance	7 (13.5)
Difficulty climbing stairs	6 (11.5)
Isolated serum CK elevation	17 (32.7)
Positive family history	14 (26.9)
First NSAA score	27.3 ± 3.8
Last NSAA score	26.6 ± 4.1
Annualized NSAA change (points/year)	-0.48 (-2.0 to 1.0)
CK level (U/L)	$11,326.9 \pm 3,430.1$
Corticosteroid-naïve, n (%)	25 (48.1)
Corticosteroid treatment, n (%)	27 (51.9)
Prednisolone treatment, n (%)	25 (48.1)
Deflazacort treatment, n (%)	2 (3.8)

Data are presented as mean $\pm$ standard deviation, median (interquartile range), or number (%). CK, creatine kinase; IQR, interquartile range; NSAA, North Star Ambulatory Assessment.

Table 2. Genetic characteristics of the study cohort.

Variable	n (%)
Mutation type	
Deletion	44 (84.6)
Duplication	2 (3.8)
Nonsense	3 (5.8)
Splice-site	3 (5.8)
Reading-frame status	
Out-of-frame	35 (67.3)
In-frame	11 (21.2)
Unknown	6 (11.5)
Mutation localization*	
Distal	34 (65.4)
Proximal	12 (23.1)
Not classified	6 (11.5)

*Localization analysis was performed only for deletion and duplication variants.

and 16, respectively. Genetic analysis revealed a deletion involving exons 42–43.

The genetic characteristics of the cohort are presented in Table 2. Deletion variants were the most common genetic alteration (84.6%), followed by nonsense variants (5.8%), splice-site variants (5.8%), and duplication variants (3.8%). Reading-frame analysis demonstrated that 67.3% of patients carried out-of-frame variants, whereas 21.2% carried in-frame variants. Among patients with deletion or duplication variants, distal mutations were more common than proximal mutations (65.4% vs. 23.1%).

Reading-frame status and functional outcomes

Comparisons of NSAA outcomes according to reading-frame status are presented in Table 3. Patients carrying in-frame variants demonstrated numerically higher baseline and follow-up NSAA scores than those with out-of-frame variants (29.18 ± 2.89 vs. 27.09 ± 3.84 , $p=0.144$; and 28.82 ± 3.37 vs. 26.37 ± 4.19 , $p=0.070$, respectively). However, annualized NSAA change was comparable between the two groups (-0.18 ± 1.45 vs. -0.33 ± 1.81 points/year, $p=0.735$), indicating no significant association between reading-frame status and functional progression.

Mutation localization and functional outcomes

The association between mutation localization and functional outcomes is presented in Table 4. Baseline and follow-up NSAA scores were comparable between patients

Table 3. Comparison of NSAA outcomes according to reading-frame status.

Variable	In-frame (n=11)	Out-of-frame (n=35)	p value
First NSAA score	29.18 ± 2.89	27.09 ± 3.84	0.144
Last NSAA score	28.82 ± 3.37	26.37 ± 4.19	0.070
Annualized NSAA change (points/year)	-0.18 ± 1.45	-0.33 ± 1.81	0.735

Data are presented as mean ± standard deviation. Comparisons between groups were performed using the Mann–Whitney U test. NSAA, North Star Ambulatory Assessment.

Table 4. Comparison of NSAA outcomes according to mutation localization.

Variable	Proximal (n=12)	Distal (n=34)	p value
First NSAA score	28.33 ± 2.99	27.32 ± 3.94	0.780
Last NSAA score	28.58 ± 2.35	26.38 ± 4.47	0.190
Annualized NSAA change (points/year)	0.28 ± 0.85	-0.50 ± 1.90	0.100

Data are presented as mean ± standard deviation. Comparisons between groups were performed using the Mann–Whitney U test. NSAA, North Star Ambulatory Assessment.

Table 5. Comparison of NSAA outcomes according to mutation type.

Variable	Deletion (n=44)	Non-deletion (n=8)	p value
First NSAA score	27.55 ± 3.79	25.75 ± 3.73	0.050
Last NSAA score	26.91 ± 4.20	24.62 ± 2.83	0.074
Annualized NSAA change (points/year)	-0.29 ± 1.76	-0.94 ± 1.61	0.318

Data are presented as mean ± standard deviation. Comparisons between groups were performed using the Mann–Whitney U test. NSAA, North Star Ambulatory Assessment.

Table 6. Comparison of NSAA outcomes according to corticosteroid treatment.

Variable	Corticosteroid-treated (n=27)	Corticosteroid-naïve (n=25)	p value
First NSAA score	26.81 ± 3.22	27.76 ± 4.36	0.075
Last NSAA score	25.52 ± 3.94	27.68 ± 4.01	0.026
Annualized NSAA change (points/year)	-1.04 ± 1.66	0.31 ± 1.56	0.008

Data are presented as mean ± standard deviation. Comparisons between groups were performed using the Mann–Whitney U test. NSAA, North Star Ambulatory Assessment.

Table 7. Multivariable linear regression analysis for predictors of annualized NSAA change.

Variable	β coefficient	95% CI	p value
Age at first evaluation (months)	-0.025	-0.044 to -0.007	0.009
Corticosteroid treatment	-2.020	-3.485 to -0.555	0.008
CK level	0.0002	-0.00005 to 0.0004	0.122
Out-of-frame mutation	-0.733	-1.826 to 0.361	0.183
Deletion mutation	-0.767	-2.912 to 1.377	0.474

Model statistics: R²=0.383; Adjusted R²=0.306; F=4.98; p=0.001. CI, confidence interval; CK, creatine kinase; NSAA, North Star Ambulatory Assessment.

with proximal and distal mutations (28.33 ± 2.99 vs. 27.32 ± 3.94, p=0.780; and 28.58 ± 2.35 vs. 26.38 ± 4.47, p=0.190, respectively). Although patients with proximal mutations showed a trend toward a more favorable annualized NSAA

change than those with distal mutations (0.28 ± 0.85 vs. -0.50 ± 1.90 points/year), the difference did not reach statistical significance (p=0.100).

Mutation type and functional outcomes

Comparisons according to mutation type are presented in Table 5. Patients harboring deletion variants demonstrated higher baseline NSAA scores than those with non-deletion variants (27.55 ± 3.79 vs. 25.75 ± 3.73 , $p=0.050$). Follow-up NSAA scores also tended to be higher in the deletion group (26.91 ± 4.20 vs. 24.62 ± 2.83), although the difference did not reach statistical significance ($p=0.074$). Annualized NSAA change was comparable between the two groups (-0.29 ± 1.76 vs. -0.94 ± 1.61 points/year, $p=0.318$).

Corticosteroid treatment and functional outcomes

The association between corticosteroid treatment and functional outcomes is presented in Table 6. Baseline NSAA scores were comparable between corticosteroid-treated and corticosteroid-naïve patients (26.81 ± 3.22 vs. 27.76 ± 4.36 , $p=0.075$). However, patients receiving corticosteroid treatment had lower follow-up NSAA scores (25.52 ± 3.94 vs. 27.68 ± 4.01 , $p=0.026$) and a less favorable annualized NSAA change (-1.04 ± 1.66 vs. 0.31 ± 1.56 points/year, $p=0.008$) compared with corticosteroid-naïve patients.

Multivariable analysis

Results of the multivariable linear regression analysis are presented in Table 7. The regression model evaluating predictors of annualized NSAA change was statistically significant ($R^2=0.383$, adjusted $R^2=0.306$; $F=4.98$, $p=0.001$). Older age at first evaluation was independently associated with a less favorable annualized NSAA change ($\beta=-0.025$, 95% CI -0.044 to -0.007 ; $p=0.009$). Corticosteroid treatment was also independently associated with poorer annualized NSAA change ($\beta=-2.020$, 95% CI -3.485 to -0.555 ; $p=0.008$). In contrast, serum CK level ($p=0.122$), reading-frame status ($p=0.183$), and mutation type (deletion vs. non-deletion; $p=0.474$) were not independently associated with functional progression.

DISCUSSION

In the present study, we investigated genotype–phenotype relationships in ambulatory children with genetically confirmed dystrophinopathy aged ≥ 3 years using NSAA-based functional outcomes. Although patients carrying in-frame variants, proximal mutations, and deletion variants tended to show more favorable motor performance, we found no statistically significant association between genotype-related variables and annualized NSAA

progression. In contrast, older age at evaluation emerged as an independent predictor of functional decline.

The genetic distribution observed in our cohort was consistent with previous epidemiological studies of dystrophinopathies. Deletions represented the most common mutation type (84.6%), followed by nonsense, splice-site, and duplication variants. Similar mutation frequencies have been reported in large international registries and population-based studies, where deletions account for approximately 60–75% of DMD mutations.^{4-6,24} Therefore, the mutation spectrum in our cohort appears representative of the broader dystrophinopathy population.

The reading-frame rule remains the fundamental framework for interpreting genotype–phenotype relationships in dystrophinopathies. According to the Monaco et al. hypothesis, out-of-frame mutations generally result in complete absence of dystrophin and a Duchenne phenotype, whereas in-frame mutations permit production of partially functional dystrophin and are more commonly associated with Becker muscular dystrophy.⁸ However, increasing evidence indicates that this relationship is not absolute. Previous studies have demonstrated exceptions to the reading-frame rule in approximately 10–15% of patients, suggesting that additional molecular mechanisms contribute to phenotypic variability.^{9,10} In our cohort, patients with in-frame variants exhibited numerically higher baseline and follow-up NSAA scores than those with out-of-frame variants; however, these differences were not statistically significant and did not translate into differences in annualized NSAA progression. These findings suggest that reading-frame status alone may have limited predictive value for short-term ambulatory motor outcomes.

Similarly, mutation localization was not significantly associated with NSAA outcomes. Although proximal mutations were associated with a trend toward more favorable annualized NSAA trajectories, no significant differences were observed in baseline NSAA, follow-up NSAA, or annualized NSAA change. Previous investigations have suggested that disruption of specific dystrophin domains may influence disease severity independently of reading-frame status.⁹ Nevertheless, our results support the notion that mutation position alone is insufficient to explain variability in ambulatory motor function.

The lack of a strong genotype effect in our study is consistent with findings from the recent multi-institutional meta-analysis by Muntoni et al., which evaluated more than 1,600 patient-years of follow-up from over 700 ambulatory

patients with DMD across six international natural-history datasets.¹⁶ Importantly, that study demonstrated that genotype class explained only approximately 2% of the variation in one-year NSAA outcomes after adjustment for baseline prognostic factors, whereas non-genetic clinical variables explained more than 30% of the observed variability. Furthermore, genotype-related differences in annual NSAA change were smaller than clinically meaningful NSAA differences.¹⁶ These observations closely parallel our findings and support the growing view that genotype contributes relatively little to short-term ambulatory functional progression compared with other prognostic factors.

When mutation types were grouped as deletion vs. non-deletion variants, deletion mutations were associated with slightly higher baseline NSAA scores, although this association was not maintained in longitudinal analyses. Similar findings have been reported in previous genotype–phenotype studies, which demonstrated considerable heterogeneity in motor performance even among patients sharing identical mutation classes.^{10,16} Collectively, these observations suggest that mutation category alone is unlikely to serve as a reliable predictor of ambulatory disease progression.

Age was identified as the most important predictor of annualized NSAA decline in our cohort. This finding is biologically plausible and aligns closely with the natural history of DMD. Longitudinal studies have consistently demonstrated that NSAA performance improves during early childhood, reaches a plateau around 6–7 years of age, and subsequently declines with progressive muscle degeneration.^{14,15} In addition, a recent systematic review and meta-analysis identified lower baseline NSAA scores and other markers of advanced disease stage as major predictors of earlier loss of ambulation.¹² Therefore, the association between increasing age and functional decline observed in our cohort likely reflects the expected progression of dystrophinopathy rather than genotype-specific effects.

Another notable observation was the association between corticosteroid treatment and less favorable longitudinal NSAA outcomes. This finding should be interpreted with considerable caution and should not be considered evidence of a detrimental effect of corticosteroid therapy. Current international standards of care and multiple natural-history studies have consistently demonstrated that corticosteroids delay disease progression and prolong ambulation in DMD.¹ Indeed, the recent systematic review and meta-

analysis by Landfeldt et al. reported an approximately 56% reduction in the hazard of loss of ambulation among glucocorticoid-treated patients (hazard ratio [HR] 0.44, 95% CI 0.40–0.48).¹² In our cohort, corticosteroid therapy was prescribed according to routine clinical practice rather than by random allocation, taking into account the overall clinical assessment. Although baseline NSAA scores were comparable between corticosteroid-treated and corticosteroid-naïve patients, treatment decisions were based on multiple clinical factors beyond baseline NSAA alone. Therefore, corticosteroid treatment in our cohort most likely reflects underlying disease severity rather than a causal adverse effect of therapy. Moreover, because corticosteroids are routinely initiated earlier and more frequently in patients with DMD than in those with BMD, the inclusion of both phenotypes may have further contributed to this treatment-selection bias. Consequently, the observed inverse association is most likely explained by confounding by indication and should therefore be interpreted with caution.

Our findings should also be interpreted in the context of the inherent variability of NSAA measurements. Recent methodological studies have demonstrated substantial heterogeneity in longitudinal NSAA trajectories among ambulatory patients with DMD.^{14,15} Muntoni et al. reported that a change of approximately 2.8 NSAA points is required to distinguish true functional change from measurement variability with high confidence.¹⁵ Furthermore, the item-level NSAA analysis reported by Muntoni et al. demonstrated considerable inter-individual variability in disease progression when loss of individual NSAA skills was examined rather than change in the total score.¹⁴ Therefore, subtle genotype-related effects may be difficult to detect in relatively small cohorts using conventional NSAA change scores alone.

The biological complexity of dystrophinopathies may further explain the absence of strong genotype–phenotype associations. Clinical severity is influenced not only by the primary DMD mutation but also by residual dystrophin expression, alternative splicing patterns, modifier genes, inflammatory pathways, rehabilitation interventions, and treatment exposure.^{9,11} Modifier loci such as SPP1, LTBP4, CD40, and TCTEX1D1 have all been associated with variability in ambulation outcomes and disease progression.^{11,12} Consequently, motor performance likely reflects the cumulative effect of multiple interacting biological and environmental factors rather than mutation type alone.

Several limitations should be acknowledged. First, this retrospective single-center study may have introduced selection bias. Second, subgroup analyses were limited by the relatively small number of patients with non-deletion and in-frame variants, as well as the imbalance between the in-frame and out-of-frame groups. Together with the considerable inter-individual variability in NSAA outcomes, these factors may have limited the statistical power to detect modest but clinically meaningful genotype-related differences. Therefore, the absence of statistically significant associations should be interpreted with caution. Third, follow-up duration may not have been sufficient to detect genotype-related differences that become more apparent later in the disease course. Fourth, dystrophin protein expression and modifier gene analyses were unavailable. Finally, the inclusion of both patients with DMD and patients with BMD may have increased clinical heterogeneity because these conditions differ substantially in disease progression and functional decline. This may have attenuated genotype–phenotype associations. Future multicenter studies with larger cohorts and phenotype-specific analyses are warranted to better define the relationship between genotype and motor outcomes.

Despite these limitations, the present study provides real-world evidence regarding genotype–phenotype relationships in ambulatory patients with dystrophinopathy assessed using serial NSAA measurements. Consistent with recent large-scale natural-history studies and meta-analyses, our findings suggest that genotype alone has limited prognostic value for short-term ambulatory functional progression, whereas age and disease stage remain the principal determinants of motor decline.^{12,14–16} These observations may contribute to future risk stratification strategies and the design of longitudinal natural-history and therapeutic studies.

CONCLUSION

In this cohort of ambulatory patients with dystrophinopathy, reading-frame status, mutation localization, and mutation type were not significantly associated with annualized NSAA progression. Although in-frame variants, proximal mutations, and deletion variants tended to be associated with more favorable functional outcomes, these differences did not remain significant after statistical adjustment. Corticosteroid treatment also showed an inverse association with NSAA trajectory; however, this finding should be interpreted cautiously because of the potential influence of confounding by indication. Older

age emerged as the strongest predictor of functional decline. These findings suggest that short-term ambulatory motor outcomes are influenced by factors beyond the primary genetic defect, including disease stage and other biological modifiers. Larger multicenter studies with more balanced genotype distributions, longer follow-up, and comprehensive molecular characterization are needed to further clarify genotype–phenotype relationships in dystrophinopathies.

Author contributions

Conception and design: M.Y., R.K.K.; Data acquisition: M.Y., İ.B., N.Ö.; Data analysis: M.Y., R.K.K., N.Ö.; Data interpretation: M.Y., R.K.K., N.Ö.; Drafting of the manuscript: M.Y. All authors reviewed the results, approved the final version of the manuscript, and agreed to be accountable for all aspects of this study.

Ethical approval

This study was approved by the Gaziantep City Hospital Non-Interventional Clinical Research Ethics Committee (Date: February 18, 2026, Decision/Protocol No: 443/2026). Informed consent was obtained from all participants involved in this study.

Data availability statement

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

Conflict of interest

The authors declare that this study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Generative AI statement

The authors declare that during the preparation of this study, the following AI-assisted technology was used: ChatGPT (OpenAI) on May 2026. Extent of Use: Language editing and improvement of manuscript readability. The authors confirm that they have critically reviewed and

edited any AI-generated content and take full responsibility for the integrity, accuracy, and originality of the publication. The authors certify that the original human contribution is maintained and that AI-assisted tools are not listed or cited as authors.

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