

Online ISSN: 2792-0429

TP Trends in Pediatrics

Volume: **7** Issue: **3** September **2026**



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Volume: **7** Issue: **3** September **2026**

Online ISSN: 2792-0429

Trends in Pediatrics

ISSN (Online): 2792-0429

DOI Prefix: 10.59213

Owner

Owner on behalf of the Aydın Pediatric Society
Prof. Dr. Pınar Uysal, MD (*President*)

Publication Type

International peer-reviewed journal

Publication Frequency and Language

Quarterly (March, June, September, December), English

Abstracting and Indexing

Trends in Pediatrics is abstracted and indexed in Web of Science Emerging Sources Citation Index, Scopus, TR Index, DOAJ (Directory of Open Access Journals), Türkiye Atıf Dizini, J-Gate, Türk Medline, Gale.

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Publisher

Aydın Pediatric Society

Publisher Address

Aydın, Türkiye

Phone: +90 123 456 78 90

E-mail: info@trendspediatrics.com

Web: www.aydinpediatrikderneği.com

Publishing Services

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Address: Kızılay Mah. Gazi Mustafa Kemal Bulvarı No: 23/8 06420 Çankaya/Ankara

Certificate number: 52576

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Clinical outcomes and predictive performance of the Phoenix Sepsis Score in a Vietnamese pediatric sepsis cohort

Nghia Quang Bui¹, Nguyen Phat Nguyen², Thanh Huy Ong², Phuong Minh Nguyen¹, Thi Van Vo¹,
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Cite this article as: Bui NQ, Nguyen NP, Ong TH, Nguyen PM, Vo TV, Truong MAM, et al.. Clinical outcomes and predictive performance of the Phoenix Sepsis Score in a Vietnamese pediatric sepsis cohort. Trends in Pediatrics 2026;7(3):159-166.

This work is part of the Specialist Level II thesis of Nguyen Phat Nguyen at Can Tho University of Medicine and Pharmacy.

ABSTRACT

Background: Pediatric sepsis remains a critical global health concern due to its high morbidity and mortality. Several clinical scoring systems have been employed to assess disease severity and predict outcomes in patients with sepsis. The Phoenix Sepsis Score is a recently proposed pediatric-specific tool for risk stratification, but evidence on its performance in Southeast Asia remains limited.

Objective: To evaluate clinical outcomes and assess the prognostic performance of the Phoenix Sepsis Score in predicting mortality among pediatric patients with sepsis.

Materials and Methods: A cross-sectional analytical study was conducted on 94 pediatric patients (aged 2 months to 16 years) with sepsis admitted to a tertiary referral hospital in the Mekong Delta, Vietnam. Clinical, laboratory, and scoring variables were recorded within the first 6 hours after the diagnosis of sepsis. Prognostic performance was assessed using receiver operating characteristic curve analysis.

Results: Among 94 patients, 67% were under 5 years of age. Lower Glasgow Coma Scale scores (10.96 ± 3.61 vs. 14.19 ± 3.41 ; $p < 0.001$) and higher rates of septic shock (61.5% vs. 20.6%; $p < 0.001$) were observed among non-survivors compared with survivors. The use of vasoactive agents (96.2% vs. 26.5%; $p < 0.001$), mechanical ventilation (92.3% vs. 38.2%; $p < 0.001$), and ≥ 2 antibiotic modifications (80.8% vs. 55.9%; $p = 0.026$) were also associated with mortality. The Phoenix Sepsis Score demonstrated high discriminative ability (area under the curve [AUC], 0.947; 95% confidence interval [CI], 0.90–0.99), similar to that of the pediatric Sequential Organ Failure Assessment (pSOFA) score (AUC, 0.943; 95% CI, 0.898–0.988). At optimal cutoffs of 3.5 and 8.5 points, respectively, the Phoenix Sepsis Score showed higher sensitivity (92.3% vs. 76.9%), whereas pSOFA showed higher specificity (91.2% vs. 83.8%).

Conclusion: The Phoenix Sepsis Score demonstrated prognostic performance similar to that of pSOFA in this cohort, with higher sensitivity but lower specificity. These findings suggest that the Phoenix Sepsis Score may be useful as a supportive tool for early risk stratification in pediatric sepsis, particularly in resource-limited settings. Further multicenter studies are required to validate these findings.

Keywords: pediatric sepsis, Phoenix Sepsis Score, clinical outcomes, predictive performance



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Received: 01.10.2025 Accepted: 24.08.2026

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INTRODUCTION

Sepsis represents a major global health threat, characterized by high incidence and mortality rates, particularly in low- and middle-income countries. The morbidity and mortality of sepsis vary considerably across different regions, with the heaviest burden reported in sub-Saharan Africa, South Asia, East Asia, and Southeast Asia, which includes Vietnam.¹⁻³ Pediatric sepsis constitutes a critical public health problem, responsible for an estimated 3.3 million deaths annually worldwide.³

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection.⁴ In children, the disease often progresses rapidly and may lead to septic shock and multiple organ dysfunction syndrome if not promptly recognized and treated.⁵ Several scoring systems have been developed to assess disease severity and predict outcomes in pediatric sepsis. The recently proposed Phoenix Sepsis Score is a pediatric-specific tool designed to facilitate risk stratification by emphasizing clinical parameters and reducing reliance on laboratory investigations.^{6,7}

However, evidence regarding the performance of the Phoenix Sepsis Score in Southeast Asia remains limited. The Mekong Delta represents a resource-limited health care setting, where access to advanced laboratory testing may be inconsistent, and delays in referral can occur. These characteristics make it a relevant setting for evaluating clinically based scoring systems such as the Phoenix Sepsis Score.

Therefore, this study aimed to (1) evaluate clinical outcomes among pediatric patients with sepsis and (2) assess the prognostic performance of the Phoenix Sepsis Score in predicting mortality in this population.

MATERIALS AND METHODS

Study participants

We conducted a cross-sectional analytical study involving pediatric patients aged 2 months to 16 years who were diagnosed with sepsis and admitted for treatment at Can Tho Children's Hospital, a tertiary pediatric referral center in the Mekong Delta region of Vietnam, between 2024 and 2025.

Patients were enrolled using a consecutive enrollment approach, whereby all eligible patients admitted during the study period were screened consecutively. A total of

128 patients were assessed for eligibility. Of these, 34 were excluded (18 did not meet the inclusion criteria, 10 had incomplete data, and 6 declined participation), and 94 patients were included in the final analysis.

$$n = \frac{Z_{1-\frac{\alpha}{2}}^2 p(1-p)}{d^2}$$

The sample size for the primary objective was calculated using the formula for estimating a single population proportion. With a confidence level of 95% ($Z=1.96$), a margin of error of 0.1, and an estimated sepsis-related mortality rate of 38.9% from a prior regional study,⁸ the minimum required sample size was 92 patients. To account for potential variability, 94 patients were ultimately included.

For the second objective, sample size estimation for diagnostic accuracy was performed using Buderer's formula (1996)⁹, based on expected sensitivity and specificity. Estimates from the Society of Critical Care Medicine Pediatric Sepsis Definition Task Force¹⁰ were used (sensitivity [Se], 84.4%; specificity [Sp], 92.2%). With a margin of error (d) of 10%, a 95% confidence level, and an assumed mortality rate of 54% derived from local institutional data, the minimum required sample sizes were 94 for sensitivity and 61 for specificity. As this estimate was not obtained from a published source, it should be interpreted with caution. Therefore, the final sample size was set at 94 patients.

$$n = \frac{Z^2 \times S_e(1-S_e)}{d^2 \times p} \quad (\text{for sensitivity})$$

$$n = \frac{Z^2 \times S_p(1-S_p)}{d^2 \times (1-p)} \quad (\text{for specificity})$$

Sepsis was defined according to the 2012 Surviving Sepsis Campaign guidelines.¹¹ Patients were required to have suspected or confirmed infection in combination with at least two systemic inflammatory response syndrome (SIRS) criteria, including abnormal temperature or white blood cell count. Additional criteria included age-specific tachycardia or bradycardia, and tachypnea, based on the International Pediatric Sepsis Consensus Conference (IPSCC; 2005).¹² Infection was defined by either microbiological confirmation (positive cultures from sterile sites) or clinically suspected infection supported by laboratory markers (elevated C-reactive protein or procalcitonin) and/or imaging findings consistent with infection. Patients were excluded if they had pre-existing chronic organ dysfunction or if informed consent was not obtained.

Although newer sepsis definitions have been proposed, the 2012 Surviving Sepsis Campaign criteria, in conjunction

with the 2005 IPSCC definitions, remain commonly used in pediatric clinical practice. These criteria rely on readily available clinical parameters and are particularly applicable in resource-limited settings. Therefore, these criteria were used for patient identification in this study.

Data collection and measures

Data were collected through medical record review and structured interviews with parents or legal guardians. For each patient, all clinical, laboratory, and scoring variables were recorded within the first 6 hours after the initial diagnosis of sepsis, based on the most abnormal values recorded during this period. Collected variables included demographic characteristics (age, sex), risk factors (malnutrition, recent hospitalization), and clinical features such as vital signs, Glasgow Coma Scale (GCS) score, SIRS criteria, clinical presentation (sepsis or septic shock), primary site of infection and evidence of organ dysfunction.

Laboratory parameters included complete blood count, C-reactive protein, procalcitonin, and blood culture results obtained within the same 6-hour window. The Phoenix Sepsis Score (Figure 1) and pSOFA were calculated from the most abnormal values recorded within the same 6-hour window. The pSOFA score incorporated six organ-system subscores (respiratory, coagulation, hepatic, cardiovascular, neurologic, and renal). For both scores, variables not measured within this window were assigned 0 points (treated as normal), consistent with standard scoring conventions. Score calculation was completed from the recorded clinical and laboratory data before analysis of hospital outcomes.

Variables	0 Points	1 Point	2 Points	3 Points		
Respiratory, 0-3 points						
	$\text{PaO}_2/\text{FiO}_2 \geq 400$ or $\text{SpO}_2/\text{FiO}_2 \geq 292^a$	$\text{PaO}_2/\text{FiO}_2 < 400$ on any respiratory support or $\text{SpO}_2/\text{FiO}_2 < 292$ on any respiratory support ^{a,d}	$\text{PaO}_2/\text{FiO}_2$ 100-200 and IMV ^b or $\text{SpO}_2/\text{FiO}_2$ 148-220 and IMV ^b	$\text{PaO}_2/\text{FiO}_2 < 100$ and IMV ^b or $\text{SpO}_2/\text{FiO}_2 < 148$ and IMV ^b		
Cardiovascular, 0-6 points						
	No vasoactive medications ^d	1 Point each (up to 3)	2 Points each (up to 6)			
	Lactate < 5 mmol/L ^e	1 Vasoactive medication ^d	≥ 2 Vasoactive medications ^d			
Age based^f						
	Mean arterial pressure, mm Hg ^g					
< 1 mo	> 30	17-30	< 17			
1 to 11 mo	> 38	25-38	< 25			
1 to < 2 y	> 43	31-43	< 31			
2 to < 5 y	> 44	32-44	< 32			
5 to < 12 y	> 48	36-48	< 36			
12 to 17 y	> 51	38-51	< 38			
Coagulation (0-2 points)^h						
		1 Point each (maximum 2 points)				
	Platelets $\geq 100 \times 10^3/\mu\text{L}$	Platelets $< 100 \times 10^3/\mu\text{L}$				
	International normalized ratio ≤ 1.3	International normalized ratio > 1.3				
	D-dimer ≤ 2 mg/L FEU	D-dimer > 2 mg/L FEU				
	Fibrinogen ≥ 100 mg/dL	Fibrinogen < 100 mg/dL				
Neurological (0-2 points)ⁱ						
	Glasgow Coma Scale score > 10 ; pupils reactive ^j	Glasgow Coma Scale score ≤ 10	Fixed pupils bilaterally			
Phoenix sepsis criteria						
Sepsis	Suspected infection and Phoenix Sepsis Score ≥ 2 points					
Septic shock	Sepsis with ≥ 1 cardiovascular point(s)					

Figure 1. The Phoenix Sepsis Score.

Treatment-related variables, including antibiotic therapy and modifications, fluid resuscitation, use of vasoactive agents, mechanical ventilation, length of hospital stay, and final outcome (survival or death), were recorded throughout hospitalization.

Statistical analysis

Data were analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as frequencies and percentages, whereas continuous variables were presented as means and standard deviations. Group comparisons were performed using the chi-square test or Fisher's exact test for categorical variables and the independent-samples t test for continuous variables, as appropriate. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. A p-value < 0.05 was considered statistically significant.

The prognostic performance of the Phoenix Sepsis Score was evaluated using receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) with 95% CIs was calculated. The optimal cutoff point was determined using the Youden index.

Ethical considerations

This study was conducted in accordance with ethical principles for biomedical research involving human subjects. The study protocol was approved by the Can Tho University of Medicine and Pharmacy Ethics Committee (Approval code: 22.170.HV/PCT-HĐĐĐ) and received authorization from the Board of Directors of Can Tho Children's Hospital. All patient-related information was anonymized and coded to ensure confidentiality. Informed consent was obtained from the parents or legal guardians of all participating children after a thorough explanation of the study's objectives and procedures.

RESULTS

A total of 94 pediatric cases met the inclusion criteria and were included in the analysis. Table 1 presents the general characteristics of the study population. Children under 5 years of age accounted for 67.0% of the study population, while males accounted for 60.6%. There were no statistically significant associations between age group or sex and clinical outcomes. Additionally, 16 of the 94 patients were identified as malnourished, and 32 had a history of prior hospitalization.

Table 1. General characteristics of the study population.

Characteristics		Total n=94 (%)	Non-survivors n=26 (%)	Survivors n=68 (%)	p-value	OR (95% CI)
Age	<5 years	63 (67.0)	18 (69.2)	45 (66.2)	0.778	0.87 (0.33–2.30)
	≥5 years	31 (33.0)	8 (30.8)	23 (33.8)		
Sex	Female	37 (39.4)	14 (53.8)	23 (33.8)	0.076	2.83 (0.90–5.73)
	Male	57 (60.6)	12 (46.2)	45 (66.2)		
Malnourished	Yes	16 (17.0)	4 (15.4)	12 (17.6)	0.794*	1.18 (0.34–4.05)
	No	78 (83.0)	22 (84.6)	56 (82.4)		
History of prior hospitalization	Yes	32 (34.0)	10 (38.5)	22 (32.4)	0.576	0.76 (0.30–1.95)
	No	62 (66.0)	16 (61.5)	46 (67.6)		

p-values were calculated using the chi-square test unless otherwise indicated. *Fisher's exact test. CI, confidence interval; OR, odds ratio.

Table 2. Selected clinical characteristics of pediatric patients with sepsis.

Characteristics		Total Mean ± SD or n (%)	Non-survivors Mean ± SD or n (%)	Survivors Mean ± SD or n (%)	p-value
Sources of infection	Respiratory tract	42 (44.7)	14 (53.8)	28 (41.2)	0.660
	Gastrointestinal tract	44 (46.8)	10 (38.5)	34 (50.0)	
	Skin/soft tissue	1 (1.1)	0 (0.0)	1 (1.5)	
	Other	7 (7.4)	2 (7.7)	5 (7.4)	
Temperature (°C)		38.80 ± 0.72	38.77 ± 1.05	38.81 ± 0.55	0.825
Glasgow Coma Scale score		13.30 ± 3.74	10.96 ± 3.61	14.19 ± 3.41	<0.001
Tachycardia	Yes	72 (76.6)	19 (73.1)	53 (77.9)	0.620
	No	22 (23.4)	7 (26.9)	15 (22.1)	
Tachypnea	Yes	66 (70.2)	18 (69.2)	48 (70.6)	0.900
	No	28 (29.8)	8 (30.8)	20 (29.4)	
Clinical presentation	Septic shock	30 (31.9)	16 (61.5)	14 (20.6)	<0.001
	Sepsis	64 (68.1)	10 (38.5)	54 (79.4)	
Blood culture result	Positive	21 (22.3)	9 (34.6)	12 (17.6)	0.077
	Negative	73 (77.7)	17 (65.4)	56 (82.4)	
Phoenix Sepsis Score	Total score <2	42 (44.7)	1 (3.8)	41 (60.3)	<0.001
	Total score ≥2	52 (55.3)	25 (96.2)	27 (39.7)	

Data are presented as mean ± SD or n (%). SD, standard deviation.

Clinical characteristics

The most commonly identified sources of infection were the gastrointestinal tract (46.8%) and the respiratory tract (44.7%). The mean recorded body temperature was 38.80 ± 0.72 °C, with no significant difference between outcome groups (p=0.825). GCS scores were higher among survivors than among non-survivors (14.19 ± 3.41 vs. 10.96 ± 3.61; p<0.001). For descriptive analysis, the Phoenix Sepsis Score was categorized using a threshold of <2 versus ≥2, consistent with the Phoenix criteria threshold for pediatric sepsis (Table 2).¹⁰

Treatment outcomes in pediatric sepsis

Table 3 summarizes treatment outcomes. The mean length of hospital stay was 13.66 ± 10.73 days, with no statistically significant difference between outcome groups (p=0.206).

Vasoactive agents were used in 43 patients (45.7%), and 50 patients (53.2%) required mechanical ventilation. Both interventions were significantly associated with mortality (p<0.001). Antibiotic therapy was modified two or more times in 59 patients (62.8%); this was more frequent among non-survivors than survivors (80.8% vs. 55.9%; p=0.026).

Table 3. Treatment outcomes in pediatric sepsis.

Characteristics		Total Mean $\pm$ SD or n (%)	Non-survivors Mean $\pm$ SD or n (%)	Survivors Mean $\pm$ SD or n (%)	p-value
Length of hospital stay (days)		13.66 $\pm$ 10.73	11.38 $\pm$ 9.14	14.53 $\pm$ 11.22	0.206*
Use of vasoactive agents	Yes	43 (45.7)	25 (96.2)	18 (26.5)	<0.001**
	No	51 (54.3)	1 (3.8)	50 (73.5)	
Mechanical ventilation	Yes	50 (53.2)	24 (92.3)	26 (38.2)	<0.001**
	No	44 (46.8)	2 (7.7)	42 (61.8)	
Antibiotic modification	≥ 2 times	59 (62.8)	21 (80.8)	38 (55.9)	0.026
	<2 times	35 (37.2)	5 (19.2)	30 (44.1)	

*Independent-samples t test.

**Fisher's exact test; unmarked p value, chi-square test. SD, standard deviation.

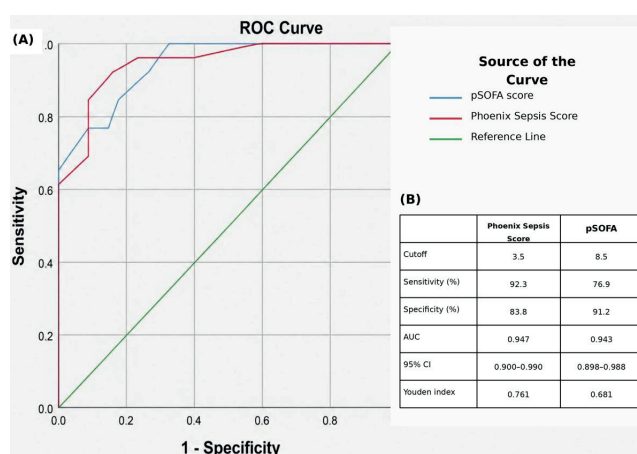


Figure 2. (A) ROC curves of the Phoenix Sepsis Score and pSOFA for predicting mortality in pediatric sepsis. (B) Optimal cutoff, sensitivity, specificity, AUC, 95% confidence interval, and Youden index for each score. AUC, area under the curve; pSOFA, pediatric Sequential Organ Failure Assessment; ROC, receiver operating characteristic.

Prognostic performance of the Phoenix Sepsis Score

The Phoenix Sepsis Score and pediatric SOFA (pSOFA) score demonstrated comparable performance in predicting mortality. The AUC was 0.947 (95% CI, 0.90–0.99) for the Phoenix Sepsis Score and 0.943 (95% CI, 0.898–0.988) for pSOFA. The Phoenix Sepsis Score showed higher sensitivity than pSOFA (92.3% vs. 76.9%), whereas pSOFA showed higher specificity (91.2% vs. 83.8%). ROC analysis using the Youden index identified an optimal cutoff value of 3.5 for the Phoenix Sepsis Score (Figure 2).

DISCUSSION

In our cohort, children younger than 5 years accounted for a large proportion of sepsis-related deaths (69.2%). However, no statistically significant association between age and mortality was observed ($p=0.778$). This finding suggests that although younger children represent a clinically important subgroup, age alone may not predict mortality in this population. Previous large-scale analyses have reported increased vulnerability to severe infection among younger children, likely related to immunological immaturity and limited physiological reserve.³

Among clinical characteristics, GCS scores were lower in non-survivors than in survivors (10.96 ± 3.61 vs. 14.19 ± 3.41 ; $p<0.001$), and septic shock was more frequent in the non-survivor group (61.5% vs. 20.6%; $p<0.001$). This aligns with findings from the SPROUT study by Weiss et al. (2015), which highlighted altered mental status as an independent predictor of mortality in pediatric sepsis.¹³ The association between septic shock and increased mortality has also been emphasized in existing clinical guidelines.¹¹

Treatment-related variables, including the use of vasoactive agents and mechanical ventilation, were more common among non-survivors (96.2% vs. 26.5% and 92.3% vs. 38.2%, respectively; $p<0.001$). These findings likely reflect greater illness severity in this group rather than direct causal effects. Similar observations have been reported in previous studies, where the need for organ support was associated with higher mortality risk in pediatric sepsis.¹⁴

Frequent modification of antibiotic therapy was also more common among non-survivors, with 80.8% requiring two or more modifications compared with 55.9% of survivors ($p=0.026$). This may reflect challenges such as delayed appropriate therapy, treatment refractoriness, or infections caused by resistant pathogens. This aligns with Lane et al., who showed that antibiotic delays beyond 330 minutes independently increased mortality in a large pediatric sepsis cohort.¹⁵ Repeated regimen changes may also indicate clinical instability requiring escalation or de-escalation, as described by Battula et al. in critically ill children with sepsis.¹⁶ A likely underlying driver is antimicrobial resistance: Kalin et al. reported that multidrug-resistant pathogens frequently delay adequate coverage and necessitate repeated empirical escalation.¹⁷

In this study, both the Phoenix Sepsis Score and the pSOFA score demonstrated high discriminative ability for predicting mortality, with AUC values of 0.947 and 0.943, respectively. The Phoenix Sepsis Score showed higher sensitivity (92.3%), whereas pSOFA demonstrated higher specificity (91.2%). These results suggest that both scoring systems may have value in clinical practice, depending on the intended use. The optimal cutoff of 3.5 points identified in our analysis differs from the threshold of ≥ 2 points used to define pediatric sepsis in the Phoenix criteria.¹⁰ This difference reflects the distinct purposes of classification and prognostication: whereas the ≥ 2 threshold is used to identify sepsis, higher cutoffs may provide improved discrimination for predicting mortality.

However, variability in the performance of the Phoenix Sepsis Score across studies has been reported. For example, Hadzhieva-Hristova et al.¹⁸ reported a lower AUC of 0.736. Differences in sample size, patient populations, clinical practices, and particularly the timing of score assessment may contribute to this variability. Inconsistent timing of score calculation, including assessments performed at different points within the first 24 hours, may introduce heterogeneity in disease severity and affect predictive performance. These findings suggest that standardization of the timing of score assessment may improve comparability across studies.

At the optimal cutoff of 8.5 points, the pSOFA score yielded a sensitivity of 76.9% and specificity of 91.2%, with a positive predictive value of 76.9% and negative predictive value of 91.2%. These findings are comparable to those reported by Matics and Sanchez-Pinto, who observed an AUC of 0.912 in their validation cohort, supporting the

consistent performance of pSOFA across different pediatric populations.¹⁹

CONCLUSION

In this study, several clinical and treatment-related factors, including lower GCS scores, septic shock, and the need for organ support, were associated with increased mortality in pediatric sepsis. Although children younger than 5 years accounted for a large proportion of cases, age was not significantly associated with mortality.

The Phoenix Sepsis Score demonstrated high discriminative ability for predicting mortality, with performance comparable to the pSOFA score. The Phoenix Sepsis Score showed higher sensitivity, whereas pSOFA demonstrated higher specificity, suggesting that both scores may have complementary roles depending on clinical priorities. Accordingly, the Phoenix Sepsis Score may be useful as a supportive tool for early risk stratification in pediatric sepsis. Further multicenter studies are needed to validate these findings and to determine its optimal clinical application.

Limitations

This study has several limitations. First, it was conducted at a single center with a relatively small sample size, which may limit generalizability and reduce the ability to detect smaller differences in predictive performance between scoring systems. Although patients were identified using SIRS-based criteria according to the 2012 Surviving Sepsis Campaign and IPSCC definitions, the Phoenix Sepsis Score was subsequently applied for prognostic assessment based on organ dysfunction. The use of these different classification frameworks may have influenced the observed predictive performance. Second, the Phoenix Sepsis Score was assessed only at the time of initial diagnosis, without evaluating dynamic changes over time. Although data were collected within the first 6 hours, variability in clinical practice may have affected the timing and completeness of measurements. In addition, the threshold of ≥ 2 antibiotic modifications was not based on an established clinical cutoff and therefore should be interpreted with caution. While the Phoenix Sepsis Score relies on a relatively limited set of routinely available clinical and laboratory parameters, its applicability may still vary across resource-limited settings. Finally, the mortality estimate used for sample size calculation was derived from local data rather than published sources, which may limit external comparability.

Acknowledgements

The authors would like to express their sincere gratitude to the doctors, nurses, and entire pediatric intensive care team at Can Tho Children's Hospital for their invaluable support and cooperation during this study. We also extend our thanks to Can Tho University of Medicine and Pharmacy for providing academic guidance and institutional support throughout the research process.

Author contributions

Conception and design: N.Q.B., N.P.N.; Data analysis: N.P.N., D.M.T., M.A.M.T., D.M.H.T.; Data interpretation: N.P.N., D.M.T., M.A.M.T., D.M.H.T.; Drafting of the manuscript: N.Q.B., N.P.N., T.H.O., P.M.N., T.V.V., M.A.M.T., D.M.H.T., D.M.T.; Critical revision of the manuscript: N.Q.B., N.P.N., T.H.O., P.M.N., T.V.V., M.A.M.T., D.M.H.T., D.M.T. 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 Ethics Committee of Can Tho University of Medicine and Pharmacy (Date: Not reported, Decision/Protocol No: 22.170.HV/PCT-HĐĐĐ). 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.

Funding

The authors declare that this study received no funding.

Generative AI statement

The authors declare that no generative AI or AI-assisted technologies were used in the writing or preparation of this study.

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Clinical and genetic insights into congenital hypothyroidism with gland-in-situ

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Cite this article as: Dursun F, Eser M, Yazar MH, et al. Clinical and genetic insights into congenital hypothyroidism with gland-in-situ. Trends in Pediatrics 2026;7(3):167-175.

ABSTRACT

Objective: This study aimed to investigate the genetic etiology of congenital hypothyroidism (CH) with gland-in-situ (GIS) by screening 23 candidate genes using next-generation sequencing (NGS) in a cohort of Turkish patients.

Methods: A total of 44 patients diagnosed with GIS-CH were enrolled. Genetic analysis was performed using a targeted gene panel including 23 genes related to thyroid development and function. Clinical and demographic features were compared between permanent CH (PCH) and transient CH (TCH) groups. Patients with thyroid dysgenesis, central hypothyroidism, syndromic features, or insufficient follow-up were excluded.

Results: Genetic variants were identified in 43.1% (19/44) of patients, with similar frequencies in PCH (46.1%) and TCH (38.8%) groups. Ultrasonography revealed hyperplasia in two patients, hypoplasia in 10, and normal-sized gland-in-situ (GIS) in 32 patients. The most frequently identified variant was in the TSHR gene (15.9%), followed by DUOX2 (11.3%), PAX8 (6.8%), TG (4.5%), and DUOX2A2 (2.2%). Homozygous or compound heterozygous variants were consistently associated with PCH. In contrast, heterozygous variants showed variable clinical outcomes, ranging from transient disease to persistent hypothyroidism requiring ongoing L-thyroxine therapy. An oligogenic variant (DUOX2+TG) was identified in one patient and was associated with a milder clinical course. Thyroid hypoplasia was predominantly observed in patients with TSHR and PAX8 variants and was strongly associated with the need for continued treatment, even in heterozygous cases.

Conclusions: The most common genetic causes of GIS-CH in our cohort were mono- and biallelic TSHR, DUOX2, and monoallelic PAX8 variants. Thyroid hypoplasia was observed predominantly in patients with TSHR and PAX8 variants and was consistently associated with the continued requirement for L-thyroxine therapy, even among heterozygous cases. The main limitations of this study include the relatively small sample size and lack of functional validation of identified variants.

Keywords: transient, permanent, congenital hypothyroidism, gland-in-situ

INTRODUCTION

Congenital hypothyroidism (CH) is the most prevalent endocrine disorder in neonates and has historically been attributed predominantly to thyroid dysgenesis, which was estimated to account for approximately 80% of cases.¹ Based on pathophysiological classification, most cases of

CH are categorized as either thyroid dysgenesis (TD) or dyshormonogenesis (DH). However, recent studies indicate that the apparent rise in the incidence of CH, currently estimated at approximately 1 in 1500 live births, is largely due to increased detection of CH cases with eutopic glands in situ (GIS).



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Received: 26.11.2025 Accepted: 10.04.2026

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Accordingly, the proportion of patients with GIS has increased to about 35–40%, and in some studies, CH with a eutopic thyroid gland accounts for nearly two-thirds of newly diagnosed cases.^{2,3} Possible explanations for the increased frequency of GIS-CH include lower TSH screening thresholds, increased functional thyroid defects due to iodine deficiency or excess, exposure to thyroid-disrupting chemicals, and a higher incidence of premature births.⁴ In addition, as molecular studies accelerate, new genes and mechanisms are being discovered, and the etiology of CH appears to be more complex than previously thought.⁵ It has previously been estimated that the genetic variants in the candidate genes for both TD and DH can be detected in approximately 20% of patients with CH.^{6,7} Recent studies have reported an increased rate of variant detection with the application of next-generation sequencing (NGS); however, findings have varied across studies, and the pathogenic significance of some identified variants remains unclear.^{8,9} Nevertheless, NGS has been demonstrated to be both cost-effective and robust, thus indicating that traditionally goiter-associated candidate genes, such as *DUOX2* and thyroid peroxidase (TPO), may also be implicated in TD. Consequently, these findings suggest an increasingly complex genotype-phenotype relationship in CH.^{10,11} The increasing identification of genetic etiology in CH is attributed to several factors, including the widespread use of advanced genetic analysis methods such as NGS, the diagnosis of a greater number of patients through neonatal CH screening programs, and the higher contribution of genetic factors, particularly in patients with GIS. With advances in technology, it has become evident that the genetic basis of CH is highly complex.⁵

In this study, the aim was to screen 23 candidate genes using NGS in a cohort of Turkish children diagnosed with gland *in situ* CH.

MATERIALS AND METHODS

The study was conducted on patients who were diagnosed with CH and started treatment in the pediatric endocrinology clinic. Between June 2024 and June 2025, 175 patients with CH who were regularly followed in the pediatric endocrinology outpatient clinic were reviewed. Among these patients, 59 with CH and GIS who underwent genetic analysis were identified. Of these, 44 patients with complete clinical and genetic data were included in the study. Patients with an identified thyroid dysgenesis (agenesis, hemiagenesis, or ectopy), patients with syndromes like

Down syndrome, patients with central hypothyroidism, and patients with thyroid dysfunction due to prematurity or maternal antithyroid drugs were excluded from the study. Furthermore, patients with transient hypothyroidism who required less than two years of treatment and had not yet reached the adequate follow-up period of two years were excluded from the study.

The study was performed according to the Helsinki II declaration and approved by the local Ethical Committee of our hospital (approval number: 20/05/2024-244120985).

The national screening program for CH has been implemented in Turkey since 2006. Dry blood samples are collected between days 2 and 5, and infants with TSH levels >5.5 $\mu\text{U/mL}$ are referred to hospitals for serum TSH and free thyroxine (FT4) levels.¹² Primary hypothyroidism was diagnosed with elevated TSH and low/normal FT4 levels, using reference values. Thyroid function tests were measured in the early morning fasting blood samples using the chemiluminescent immunometric method (Architect i4000, Abbott Laboratories Diagnosis Division, IL, USA) in the biochemistry laboratory of our hospital, where the reference range for serum TSH concentrations was 0.69–5.8 mIU/L and for FT4 was 0.85–1.7 ng/dL.

All patients in this study with low serum FT4 and/or persistently elevated TSH levels (>10.0 $\mu\text{U/mL}$) were treated with L-thyroxine (LT4).

The study was designed as a retrospective analysis based on a review of patients' medical records and follow-up data, including treatment requirements beyond 2 years. The patients were initially classified into two diagnostic categories-Permanent Congenital Hypothyroidism (PCH) and Transient Congenital Hypothyroidism (TCH)-based on findings at presentation, throughout follow-up, or upon re-evaluation after the age of 2–3 years. PCH was defined as the necessity to augment the dosage of LT4 in cases of elevated TSH levels on subsequent follow-ups and the requirement to reinstitute medication in instances where TSH levels exceeded 10 $\mu\text{U/mL}$ following the cessation of treatment. TCH was defined as initiation of LT4 treatment during the neonatal period, followed by discontinuation during the subsequent follow-up period, without the need to restart treatment. This definition was applied to infants in whom FT4 was normal and TSH was normal or <10 $\mu\text{U/mL}$ six months after the cessation of treatment. Transient cases requiring at least two years of LT4 treatment were included in the genetic analyses, given the predominance of environmental factors in the etiology of this group.^{13–17}

A comparative analysis was conducted of permanent CH (PCH) and transient CH (TCH) patients with respect to demographic characteristics and genetic variants.

Thyroid ultrasonography (USG) was performed by an experienced pediatric radiologist. All examinations were conducted with patients in the supine position and the neck in slight hyperextension. High-resolution linear transducers with frequencies of 4.8–11 MHz or 5–14 MHz were used (Toshiba Aplio 500, Aplio 300; Toshiba Medical Systems, Tokyo, Japan; Siemens Acuson S3000, Siemens Medical Solutions, USA). Both transverse and longitudinal scans were obtained using real-time grayscale and color Doppler imaging. Thyroid volume was calculated using the formula (Thyroid volume = depth × length × width × 0.479), with age-matched references for Turkish children. Standard deviation scores (SDS) were computed using the CHILD METRICS software developed by the Pediatric Endocrine and Diabetes Association.¹⁸ Glands with SDS values below –2 were classified as hypoplastic, between –2 and +2 as normal, and above +2 as hyperplastic. In this study, GIS was defined as the presence of a eutopic thyroid gland located in its normal anatomical position on ultrasonography, regardless of gland size. Thus, GIS included all patients with a normally located thyroid gland, irrespective of size variations. Patients with thyroid agenesis and ectopic thyroid were excluded from the study.

Genetic analysis

Peripheral blood samples were obtained from all participants after written informed consent was secured. Genomic DNA was isolated from EDTA-anticoagulated whole blood using automated extraction systems (Qiagen), following the manufacturer's protocols. DNA concentration and purity were evaluated using Qubit fluorometry and UV spectrophotometry (260/280 and 260/230 ratios). Library preparation for next-generation sequencing (NGS) was performed using the Sophia Genetics Inherited Disease Panel, including a custom 23-gene panel related to congenital hypothyroidism (DUOX2, DUOX2A2, GABRD, GLIS3, IYD, KCNAB2, KDM6A, KMT2D, NKX2-1, NKX2-5, PAX8, PDE4D, PRDM16, PRKAR1A, SKI, SLC26A4, SLC5A5, TG, THRA, THRB, TPO, TSHB, TSHR). The sequencing was carried out on the Illumina NextSeq 500 platform.

Bioinformatic and variant interpretation

Raw sequencing data were analyzed using the Sophia DDM® platform. Sequence alignment and variant calling were performed via the proprietary Pepper® algorithm,

based on the GRCh37/hg19 reference genome. Variant annotation was conducted using MOKA® software and included data on mutation type (e.g., missense, nonsense), population frequency (from 1000 Genomes, gnomAD, ExAC), and in silico predictions (e.g., SIFT, PolyPhen). Mean sequencing coverage was approximately 90% at an average depth of 30×. A minimum read depth threshold of 30 reads was applied to ensure reliable variant calling. Variants were filtered using a variant allele fraction (VAF) cutoff of ≥15%, thereby prioritizing those with sufficient representation for downstream analysis.

Copy number variation (CNV) analysis was conducted using Sophia's MUSKAT® software. Variant pathogenicity was interpreted according to the American College of Medical Genetics and Genomics (ACMG) guidelines, incorporating ClinVar annotations and published evidence, including criteria proposed by Maxwell et al. For evidence codes such as PP2, BP1, and PVS1, gene-specific mechanisms and functional consequences were considered. Rare variants were filtered using a minor allele frequency (MAF) threshold of <0.0001 for PM2 classification.¹⁹⁻²⁷

Statistical analysis

All statistical analyses were performed using SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Normality of continuous variables was assessed using the Kolmogorov–Smirnov test. Descriptive data are presented as mean ± standard deviation (SD) for normally distributed variables and as median (interquartile range, IQR) for non-normally distributed variables. Between-group comparisons were conducted using the Student's t-test or Mann–Whitney U test, as appropriate. Categorical variables were compared using the Chi-square test. A p-value of <0.05 was considered statistically significant.

RESULTS

In this study of 44 patients (23 female, 21 male) diagnosed with CH, 18 had TCH and 26 had PCH. The mean ages of patients with TCH and PCH were 22.5 ± 13 and 20 ± 19 days, respectively. Parental consanguinity was present in 23.3% of the patients. All but two siblings were from different families. The prevalence of hypothyroidism in the families of both groups did not exhibit a statistically significant difference (61.5% in PCH, 38.5% in TCH, p=0.68). Ultrasonography revealed hyperplasia in two patients, hypoplasia in 10, and normal-sized gland-in-situ (GIS) in 32 patients. Genetic variants were detected in 19 of 44

patients (43.1%), with no significant difference between the two groups ($p=0.86$), as detailed in Table 1.

Genetic results

Among the 19 patients with detected genetic variants, nine were homozygous and nine were heterozygous. One patient had an oligogenic variant (DUOX2+TG). In total, 17 different variants were identified in 18 families (Table 2). Two siblings from one family were found to have a homozygous TSHR variant (c.1349G>A p. Arg450His). The most frequently affected gene was TSHR (15.9%), followed by DUOX2 (11.3%), PAX8 (6.8%), TG (4.5%), and DUOX2A2 (2.2%), respectively. In addition, one patient was found to have an oligogenic variant involving DUOX2 and TG. All patients with homozygous genetic variants were diagnosed with PCH, whereas the clinical diagnosis of heterozygous variant carriers was variable. Two of the three patients with TSHR heterozygous variants had PCH, while one of the three patients with PAX8 heterozygous variants had PCH. All three patients with PCH due to heterozygous variants had hypoplastic thyroid glands (TSHR heterozygous variant in two patients and PAX8 heterozygous variant in one patient).

Based on long-term clinical follow-up, biochemical findings, and the absence of hypothyroidism after supervised LT4 withdrawal, treatment was discontinued and not restarted in six patients (one with TSHR heterozygosity, one with DUOX2 heterozygosity, one with DUOX2+TG oligogenic

disease, one with DUOX2A2 heterozygosity, and two with PAX8 heterozygosity), rather than solely on genetic results (Table 3).

TSHR variants

All patients with homozygous variants in the TSHR gene had PCH. Their thyroid volumes were compatible with hypoplasia. Treatment for three patients with heterozygous variants in the TSHR gene was discontinued after the genetic results; however, it was restarted for two of them (Table 3). After cessation of LT4, one had a TSH level of 27 mIU/L and a concomitant secondary amenorrhea, which improved after normalization. The other had no symptoms, but treatment was restarted because her TSH level had risen to 25 mIU/L. Both had hypoplasia of the thyroid gland.

DUOX2 variants

Four patients harbored homozygous or compound heterozygous DUOX2 variants and were diagnosed with PCH. Thyroid USG revealed hyperplasia in two patients, while thyroid volumes were normal in the other two (Table 3). One patient developed a nodule in the thyroid gland (patient 42) and underwent a total thyroidectomy with a fine-needle aspiration biopsy (FNAB) result of atypia of unknown etiology (AUS) (Bethesda III). The pathology result was interpreted as benign. In the other three patients, iodine treatment was initiated in conjunction with L-thyroxine therapy after the genetic result. A heterozygous

Table 1. Clinical features and laboratory findings of patients with transient and permanent congenital hypothyroidism at diagnosis and at follow-up

Parameters	Transient n=18	Permanent n=26	p
Gender: F/M (n)	10/8	13/13	0.950 ^a
Age at diagnosis (day)	22.5±13	20±19	0.660 ^b
Gestational age (week)	40 (29-42)	40 (29-42)	0.200 ^c
Consanguinity (%)	11	32	0.110 ^a
Birth weight (g)	2845±744	3145±756	0.210 ^b
Family history for CH (%)	38.5	61.5	0.680 ^a
First TSH (NR: 0.69-5.8mIU/L)	55 (7-216)	91 (13-500)	*0.050 ^c
First FT4 (NR: 0.85-1.7 ng/dL)	0.9±0.29	0.64±0.28	*0.050 ^b
Thyroid volume SDS	-0.89 (-1.9-1.3)	-0.43 (-2.8-2.7)	0.370 ^c
LT4 dose at diagnosis (µg/kg/day)	8.7±2.7	9.7±3.3	0.380 ^b
LT4 dose at 24 months (µg/kg/day)	1.6±0.43	2.9±0.9	*0.010 ^b
Height at last visit (SDS)	-0.54±0.9	-0.19±0.1	0.270 ^b
Genetic etiology: yes/no (n)	7/11	12/14	0.860 ^a

F, female; M, male; CH, congenital hypothyroidism; TSH, thyroid-stimulating hormone; FT4, free thyroxine; NR, normal range; SDS, standard deviation score; L-T4, L-thyroxine; n, number. * $p<0.05$, a: Chi-square test, b: Independent samples T test, c: Mann Whitney U test.

Table 2. The variants and proteins in the genes associated with congenital hypothyroidism

Case	CF	Gene	Zygosity	Variant	Protein	ACMG classification
#9	yes	TSHR	HT	c.484C>G	Pro162Ala	VUS (PM2, PP3)
#10	No	TSHR	HM	c.1422 C>A	Asp474Glu	VUS (PM2, PP3)
#13	No	TSHR	HT	c.1422C>A	Asp474Glu	VUS (PM2, PP3)
#15	Yes	TSHR	HM	c.434C>T	Thr145Ile	VUS (PM2, PP3)
#21	Yes	TSHR	HM	c.1349G>A	Arg450His	VUS (PM2, PP3, PP5)
#22	Yes	TSHR	HM	c.1349G>A	Arg450His	VUS (PM2, PP3, PP5)
#44	No	TSHR	HT	c. 351A>G	Ile117Met	VUS (PM2, PP3)
#23	Yes	DUOX2	HT	c.1464 del	Leu489Serfs*97	Likely Pathogenic (PVS1, PM2)
#27	Yes	DUOX2	HM	c.3416-2A>C	splice acceptor	Likely Pathogenic (PVS1, PM2)
#41	Yes	DUOX2	CHT	c.2895_2898del c.3968C>A	Phe966Serfs*29 (Ala1323Glu)	Pathogenic (PVS1, PM2, PP5) VUS (PM2, PP3)
#42	Yes	DUOX2	HM	c.1300 C>T	Arg434*	Pathogenic (PVS1, PM2, PP5)
#43	No	DUOX2	HM	c. 3251G>A	Arg1084Gln	Likely Pathogenic (PM1, PP3)
#6	No	PAX8	HT	c.1109C>T	Thr370Met	VUS (PM2, PP3)
#37	No	PAX8	HT	c.97C>T	Arg33Cys	VUS (PM2, PP3)
#39	No	PAX8	HT	c.161G>A	Ser54Asn	VUS (PM2, PM5, PP3)
#30	Yes	TG	HM	c.886C>T	Arg296*	Pathogenic (PVS1, PM2, PP5)
#32	No	TG	HT	c.886C>T	Arg296*	Pathogenic (PVS1, PM2, PP5)
#26	No	DUOX2+TG	HT/HT	c.2048G>A/ c. 3149G>T	Arg683His/Trp1050Leu	VUS (PM2) VUS (PP3)
#8	No	DUOXA2	HT	c.33C>A	(Tyr11*)	Likely Pathogenic (PVS1, PM2)

HT, heterozygous; HM, homozygous; CHT, compound heterozygous; CF, consanguineous family; F, female; M, male; *, stop codon

variant in DUOX2 was identified in one patient, and a DUOX2+TG oligogenic variant was detected in one patient (Table 3). After genetic results, L-thyroxine treatment was discontinued in both patients. The TSH level increased to 14 mIU/L in the patient with the DUOX2 variant; treatment was not restarted, and no symptoms were observed. In the oligogenic patient, TSH levels remained within normal limits following the cessation of treatment.

After the detection of a heterozygous variant in DUOXA2 in one patient, L-thyroxine replacement was withdrawn following variant interpretation.

PAX8 variants

A heterozygous PAX8 variant was detected in three patients (Table 2). Treatment was discontinued in two patients after genetic analysis, while treatment was continued in one patient (patient 39). The result of this patient showed a heterozygous c.161G>A (p.Ser54Asn) variant, but the thyroid was hypoplastic (TV SD: -2.1 SD). At the age of 2 years, this patient was able to maintain normal TSH levels with an L-T4 dose of 5 mcg/kg/day.

Thyroglobulin variants

A homozygous variant in the TG gene was found in one patient and a heterozygous variant in one patient (Table 2). While treatment of the heterozygous patient could be discontinued before the age of 2 years, the homozygous patient had multiple admissions with very high TSH levels (TSH >200 mIU/mL, FT4 <0.5 ng/dL).

DISCUSSION

Using next-generation sequencing (NGS), we screened 23 candidate genes in 44 Turkish CH patients. Genetic etiology was identified in 19 patients (43.1%); 7 of 18 patients (38.8%) with TCH and 12 of 26 patients (46.1%) with PCH. Among the 19 patients with variants, nine were heterozygous, nine homozygous or compound heterozygous, and one had an oligogenic variant (DUOX2+TG). The most frequently identified variant was in the TSHR gene (15.9%), followed by DUOX2 (11.3%), PAX8 (6.8%), TG (4.5%), and DUOXA2 (2.2%). Targeted candidate-gene analysis using NGS for CH has been reported with increasing frequency. However,

Table 3. Phenotypic features and thyroid dysfunction status in patients with identified genetic variations associated with congenital hypothyroidism

Case	Gender	Gene	Zygosity	Thyroid Volume (SDS)	Structure	Treatment	Age at last visit (year)	LT4 dose µg/kg/d	Last TSH mIU/L	Last FT4 ng/dL
#9	F	TSHR	HT	-1.96	Hypoplasia	Yes	5.5	1.8	27 ^a 4 ^b	1.0 ^a 1.6 ^b
#10	F	TSHR	HM	-2.01	Hypoplasia	Yes	11	3.1	1.4	0.97
#13	F	TSHR	HT	-1.81	Normal	No*	8.5	-	8.9	1.3
#15	F	TSHR	HM	-1.98	Hypoplasia	Yes	9.0	2.0	5.1	1.4
#21	F	TSHR	HM	-2.01	Hypoplasia	Yes	11.0	3.0	23	0.8
#22	M	TSHR	HM	-1.99	Hypoplasia	Yes	8.8	5.0	6.5	1.1
#44	F	TSHR	HT	-2.81	Hypoplasia	Yes	17	1.1	25 ^a 6.8 ^b	0.9 ^a 1.1 ^b
#23	M	DUOX2	HT	-1.48	Normal	No*	12	-	14.5	1.2
#27	M	DUOX2	HM	1.44	Normal	Yes	5.8	1.8	2.4	1.5
#41	F	DUOX2	CHT	2.50	Hyperplasia	Yes	11.5	2.0	3.8	1.4
#42	M	DUOX2	HM	2.71	Hyperplasia	Yes	8.5	3.0	4.2	1.5
#43	F	DUOX2	HM	-0.37	Normal	Yes	5.2	3.1	6.5	1.3
#6	M	PAX8	HT	-0.61	Normal	No*	14.0	-	6.08	1.4
#37	M	PAX8	HT	-1.12	Normal	No *	6.0	-	5.6	1.2
#39	M	PAX8	HT	-2.11	Hypoplasia	Yes	2.0	5.0	21 ^a 4.5 ^b	1.6
#30	M	TG	HM	1.67	Normal	Yes	13.5	2.0	16	1.7
#32	F	TG	HT	0.46	Normal	No*	3.5	-	8.0	1.1
#26	M	DUOX2+TG	HT/HT	1.30	Normal	No*	5.5	-	4.1	1.2
#8	F	DUOX2	HT	-0.76	Normal	No*	4.8	-	9.1	1.2

HT, heterozygous; HM, homozygous; CHT, compound heterozygous; F, female; M, male; TSH, thyroid-stimulating hormone; FT4, free thyroxine; SDS, standard deviation score; y, year; d, day

* Discontinued after genetic analysis,

a: TSH and FT4 levels measured after the discontinuation of L-thyroxine therapy; b: TSH and FT4 levels measured during ongoing L-thyroxine treatment.

detection rates vary widely due to differences in patient selection, variant filtering strategies, and pathogenicity assessment. Recent studies have identified genetic variants in 33.0% to 51.5% of CH cases, and the results of the present study are consistent with these previously reported ranges.²⁸⁻³²

Yamaguchi et al.⁷, in a study of 167 patients including dyshormonogenesis (57 patients), dysgenesis (32 patients), and CH patients of unknown etiology (78 patients), detected 66.5% variants by NGS. Of these variants, 2.6% were biallelic, 18% were oligogenic, and 35.9% were monoallelic. Kara et al.¹³ studied 48 patients with CH, and a genetic etiology was found in 65% of patients, which was higher than in our study. This difference can be attributed to the selection of GIS cases in our study. PAX8 mutations are typically inherited in an autosomal dominant (AD) manner,

and autosomal recessive cases have not been reported to date. These AD variants are generally associated with PCH.¹⁷ A single case of TCH has been reported in association with a heterozygous missense mutation in PAX8, a key thyroid transcription factor essential for both thyroid morphogenesis and the maintenance of normal thyroid structure and function in adulthood.³³ Consistent with the case reported in the literature, treatment was successfully discontinued in two of the three patients harboring PAX8 variants in our cohort. One patient presented with thyroid hypoplasia, and two patients had normal thyroid volume with congenital hypothyroidism. These findings suggest that additional, yet unidentified epigenetic, environmental or unidentified factors may contribute to the phenotypic variability observed in TCH or PCH beyond genetic mutations.

Previous studies have shown that the mutation detection rate in cases of thyroid dysgenesis (TD) is approximately 5%, mainly involving the PAX8 and TSHR genes.^{17,34} However, TSHR and PAX8 variants, which may present with either thyroid hypoplasia or normal thyroid volume, can be observed in both thyroid dysgenesis and gland-in-situ (GIS). Therefore, the identification of TSHR or PAX8 variants in patients with GIS is not unexpected.³⁵ In the present cohort, variants were most frequently identified in TSHR (7/19), followed by DUOX2 (5/19) and PAX8 (3/19). Yamaguchi et al.⁷ found variants most frequently in DUOX2, followed by TG. In the study by Kara C et al from Turkey¹³, the most common variants were in the TG, TSHR, and DUOX2. In the study by Nicholas et al.² in CH patients with GIS, the most frequent variants were in TG, followed by TPO, whereas DUOX2 variations were relatively rare compared to the previous literature. DUOX2 mutations appear to be more common in individuals of Asian origin.²

Thyroid glands tend to be hypoplastic in patients with TSHR variants. All homozygous TSHR variants were found to be associated with persistent hypothyroidism, while heterozygous TSHR variants tended to require treatment when the patient had hypoplastic Tenenbaum-Rakover et al.³⁶ showed that in individuals heterozygous for loss-of-function TSHR mutations represent a stable, compensated state, which does not necessitate replacement therapy. However, there were no patients with thyroid hypoplasia in their study, while some patients did not even have US findings. Treatment cessation could not be made in one of three patients with a PAX8 variant (patient 39), who had a heterozygous variant and a hypoplastic thyroid gland. By the age of two, this patient could maintain normal TSH levels with an average L-T4 dose of 5 mcg/kg/day. Stoupa et al.³⁵ stated that thyroid morphology and the severity of biochemical hypothyroidism exhibit considerable variability in PAX8 variants, with cases ranging from severe congenital hypothyroidism to mild hyperthyrotropinemia observed even within the same family.

Reassessment is deemed unnecessary for patients with CH presenting with ectopic thyroid or athyreosis. However, current guidelines provide no definitive recommendations regarding reassessment in cases of thyroid hypoplasia.¹⁴ Rabbiosi et al. have shown that thyroid hypoplasia on US was correlated with PCH, and that no patients with hypoplastic thyroid harbored TCH.¹⁶ Our study showed that heterozygous variants can cause hypoplastic gland and suggested that although the variant is heterozygous, having a hypoplastic thyroid gland is more important in PCH-TCH differentiation.

In our study, when TCH and PCH were compared in terms of demographic characteristics, TSH levels at presentation were significantly higher in PCH patients. Among the studies in literature, Scavone et al. also found a significantly different baseline TSH levels of 24.2 (52.4) uIU/L in TCH patients and 73.3 (276.5) uIU/L in PCH patients (p:0.013).³⁷

The prevalence of DUOX2 mutations among CH patients is highly variable and generally high, with 44% in the Netherlands³⁸, 43% in Japan⁶, 30-45% in Italy³⁹ and 29-83% in China.⁴⁰ While this difference may be attributed to differences in the methodologies of the studies, genetic diversity among different ethnic groups may also play a contributing role. In the current study, we identified six patients with DUOX2 variation, four with homozygous or compound heterozygous variants, and two with heterozygous variants. Those homozygous for DUOX2 were observed to have PCH, whereas those heterozygous for DUOX2 were observed to have TCH or mild subclinical hypothyroidism, which did not require treatment.

Study limitations

The primary limitation of our study was its hospital-based, regional design, which resulted in a relatively small sample size. To validate our findings on the genetics of CH, additional studies with larger cohorts are necessary. Another notable limitation was the exclusive *in silico* analysis of the pathogenicity of the variants, which lacks experimental validation. Another limitation of this study is that patients with thyroid agenesis were not included; therefore, the phenotypic spectrum associated with severe PAX8 mutations may not be fully represented. A major limitation of this study is the exclusion of patients with insufficient follow-up and the selective application of genetic testing, which may have influenced variant detection rates and the observed phenotype distribution.

CONCLUSION

In this study, the most common genetic causes of GIS-CH in our cohort were mono- and biallelic TSHR, DUOX2, and monoallelic PAX8 variants. While homozygous mutations cause PCH, we found that the clinical presentation of heterozygous mutations can be very variable. We found that heterozygous TSHR and PAX8 variants may require treatment at a higher rate compared to studies in the literature. We observed that the need for treatment persists when thyroid hypoplasia is present in heterozygous variants. Thyroid volume appears to be an important criterion for the persistent need for thyroid hormone replacement therapy

in patients with heterozygous variants, which needs to be confirmed in larger patient groups.

Author contributions

Conception and design: F.D., M.E.; Data acquisition: A.Y., E.E.A., Ş.E.U., F.G.A., İ.D.S.; Data analysis: F.D., M.E., M.H.Y.; Data interpretation: F.D., M.E., M.H.Y.; Drafting of the manuscript: F.D., M.E., H.K. 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 local Ethical Committee of University of Health Sciences, Umraniye Training and Research Hospital (Decision/Protocol No: 20/05/2024-244120985). 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.

Funding

The authors declare that this study received no funding.

Generative AI statement

The authors declare that no generative AI or AI-assisted technologies were used in the writing or preparation of this study.

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Predictors of intravenous immunoglobulin therapy in juvenile idiopathic inflammatory myopathies

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Cite this article as: Özçelik E, Uğur Es Y, Öztürk D, et al. Predictors of intravenous immunoglobulin therapy in juvenile idiopathic inflammatory myopathies. Trends in Pediatrics 2026;7(3):176-183.

ABSTRACT

Objective: Juvenile idiopathic inflammatory myopathies (JIIM) exhibit heterogeneous presentation, and intravenous immunoglobulin (IVIG) is often required in refractory cases. This study aimed to identify the clinical characteristics associated with IVIG use.

Methods: This retrospective study included patients aged 0–18 years who fulfilled the Bohan and Peter and/or 2017 EULAR/ACR criteria for JIIM and were followed between March 2009 and August 2025. Demographic, clinical, laboratory, and treatment data were recorded.

Results: Thirty-eight patients were included; 29 (76.3%) had juvenile dermatomyositis. Sixteen patients received IVIG. Severe or unresponsive muscle weakness was the main indication (56.3%). IVIG-treated patients had significantly lower Childhood Myositis Assessment Scale (CMAS) scores at diagnosis and at the third month, higher AST levels, more frequent nailfold capillaroscopy abnormalities, and lower remission rates. Each one-point decrease in CMAS increased the odds of IVIG use. Additionally, the third-month CMAS score was the only independent predictor for IVIG therapy.

Conclusion: In conclusion, IVIG is an effective treatment option for patients with more severe and refractory JIIM. These patients are characterized by lower CMAS scores and marked microvascular involvement. Earlier use of IVIG as a steroid-sparing strategy may help restrict adverse effects.

Keywords: juvenile dermatomyositis, myositis, intravenous immunoglobulins, capillaroscopy

INTRODUCTION

Juvenile idiopathic inflammatory myopathies (JIIM) are rare pediatric autoimmune conditions characterized by immune-mediated myositis and a prominent microvascular vasculopathy.¹ The annual incidence of JIIM is estimated to be 2-4 per million children.² Various subtypes have been identified within JIIM, including juvenile dermatomyositis (JDM), juvenile polymyositis, inclusion body myositis, and amyopathic dermatomyositis.³ JDM, which accounts for approximately 80–85% of all JIIM cases.⁴

The first-line treatment of JIIM usually consists of immunosuppressants such as glucocorticoids and methotrexate. Some patients may not achieve disease control with those and may require additional treatments.⁵ IVIG is often used in combination with disease-modifying antirheumatic drugs (DMARDs) to induce and maintain remission.^{6,7} Current studies have demonstrated that IVIG is an effective and safe treatment option for patients with severe or treatment-refractory JDM.^{8,9}

The pathogenesis of JIIM is complex and multifactorial, driven predominantly by type I interferon activation,



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Received: 02.12.2025 Accepted: 03.05.2026

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capillary dropout, complement deposition, and endothelial activation, which together contribute to a characteristic vasculopathy.⁵ The therapeutic effects of IVIG are considered to result from the neutralization of autoantibodies, the attenuation of microangiopathy, and the suppression of the type I interferon-mediated inflammatory response in JDM.¹⁰

The present study aims to describe the clinical, laboratory, and treatment characteristics of children with JIIM who received IVIG therapy and to identify the clinical factors that warranted IVIG treatment.

MATERIALS AND METHODS

Patient selection

The study included the data on patients aged 0–18 years who met the Bohan and Peter classification criteria and/or the 2017 European Alliance of Associations for Rheumatology/American College of Rheumatology (EULAR/ACR) classification criteria for JIIM retrospectively and who were followed up in the pediatric rheumatology department between March 2009 and August 2025.^{11–13} Patients with missing data and a follow-up duration less than 12 months were excluded.

Data collection

Demographic characteristics, clinical manifestations, laboratory results, treatment, and disease outcomes were collected from patient records. Patient data for IVIG therapy were recorded in detail.

Definitions

Disease activity and treatment response were evaluated through clinical examination and serial measurements of serum muscle enzyme levels. The Childhood Myositis Assessment Scale (CMAS) was used to assess muscle strength during follow-up.¹⁴

Clinically inactive disease was defined according to the PRINTO criteria, requiring at least three of the following: creatine kinase ≤ 150 U/L, CMAS ≥ 48 , Manual Muscle Testing of eight muscle groups (MMT8) ≥ 78 , and physician's global assessment ≤ 0.2 cm on a 10-cm visual analog scale.¹⁵

Refractory JIIM was defined as patients with an inadequate clinical response to first-line treatments such as corticosteroids and methotrexate or another immunosuppressive agent.¹⁶

Treatment protocol

In our clinic, IVIG therapy was administered to patients with JIIM who had ongoing active disease despite standard treatment with corticosteroids and/or conventional DMARDs. The decision to initiate IVIG therapy was guided by the clinician's overall evaluation of disease activity and accompanying clinical findings. In these patients, IVIG was administered in combination with conventional and/or biologic DMARDs at a total monthly dose of 2 g/kg, with a maximum single dose of 70 g.

Ethical approval

The study was approved by the local ethics committee (approval no: TABED 2-25-1672). It was conducted in accordance with the ethical standards of the institutional and national research committees and the principles of the Declaration of Helsinki.

Statistical analysis

The research data were evaluated using the Statistical Package for the Social Sciences (SPSS) version 23, IBM Corp., Armonk, NY, USA, a statistical package program. In the descriptive statistics section, categorical variables were presented in tables by giving numbers and percentages, and continuous variables were presented with the median (IQR). The normality of continuous variables was evaluated using visual methods (histograms and probability plots) and analytical methods (Kolmogorov–Smirnov/Shapiro–Wilk tests). The Mann–Whitney U test was used to compare continuous variables that did not conform to a normal distribution between two groups. The chi-square test was used to compare categorical variables. The statistical significance level was accepted as $p < 0.05$. Potential collinearity between explanatory variables was assessed prior to regression analyses. Variance inflation factors (VIFs) and correlation matrices were examined to identify overlapping effects. When variables exhibited high collinearity ($VIF > 5$ or strong pairwise correlations), only the clinically more relevant variable was retained in the multivariate model to avoid instability of the estimates. Variables with a p-value less than 0.25 in the bivariate comparisons were included in the bivariate regression analysis. Variables that were significant in the bivariate regression analysis were then entered into the multivariate logistic regression model. We used the “backward” selection method to identify the most significant variables in the final model.

RESULTS

A total of 38 patients diagnosed with JIIM were included in the study, of whom 23 (60.5%) were female. The median age at symptom onset was 8.5 years (IQR: 6.8–11.3), and the median age at diagnosis was 9 years (IQR: 6.0–12.3), respectively. The median interval between symptom onset and diagnosis was 2 months (IQR: 1.0–6.5), and the median follow-up period was 31 months (IQR: 13.3–59).

Of the 38 patients, 29 (76.3%) had JDM. Five patients (13.2%) had the amyopathic type, three patients (7.9%) had the hypomyopathic type, and one patient (2.6%) had immune-mediated necrotizing myopathy.

Muscle weakness was present in 31 patients (81.6%), and cutaneous manifestations in 35 (92.1%). Among the cutaneous findings, Gottron's papules were observed in 25 patients (65.8%), heliotrope rash in 27 (71.1%), V-sign in 10 (26.3%), shawl sign in 8 (21.1%), and ulcerative lesions in 6 (15.8%) patients. Extramuscular manifestations

were arthritis (13, 34.2%), gastrointestinal involvement (n=9, 23.7%), dysphagia (n=7, 18.4%), dysphonia (n=5, 13.2%), cardiac involvement (n=1, 2.6%), and pulmonary involvement (n=1, 2.6%) (Table 1).

The median CMAS score at the initial presentation was 32.5 (IQR: 21.0–45.3). By the 3-month follow-up, this had increased to 43.0 (IQR: 38.3–46.5). It further improved to 46.0 (IQR: 42.5–50.0) at 6 months and to 51.0 (IQR: 50.0–52.0) by the final follow-up (Table 2).

Myositis-specific autoantibody positivity (MSA) was present in 21 patients (55.3%); 8 patients (21.1%) were negative, and 9 patients (23.7%) were not tested. Among the 21 patients with MSA positivity, NXP2 was the most frequently detected antibody in 9 patients (31.0%). This was followed by the presence of TIF1 antibodies in 5 patients (17.2%) and MDA5 antibodies in 3 patients (10.3%). Antinuclear antibody (ANA) positivity was present in 18 patients (47.4%).

Table 1. The comparison of the demographic and clinical characteristics of JIIM patients according to IVIG therapy

	Total (n=38)	IVIG group (n=16)	Non-IVIG group (n=22)	p
Age, years ^b	15 (10-18)	14 (10-15.8)	17 (11.5-18)	0.375 [¥]
Sex, female [§]	23 (60.5)	9 (56.3)	14 (63.7)	0.901 ^α
Age at symptom onset, years ^b	8.5 (6.8-11.3)	8.5 (6.3-11.8)	8.5 (6.8-11.5)	0.965 [¥]
Age at diagnosis, years ^b	9 (6-12.3)	9 (6.3-11.8)	9.5 (6-13.5)	0.872 [¥]
Time from symptom onset to diagnosis, months ^b	2 (1-6.5)	2 (1-7.8)	2.5 (1-7.5)	0.549 [¥]
Follow-up duration, months ^b	31 (13.3-59)	27.5 (8-48)	36 (14-77.5)	0.438 [¥]
Clinical findings[§]				
Muscle weakness	31 (81.6)	16 (100)	15 (68.2)	N/A
Constitutional symptoms	7 (18.4)	4 (25.0)	3 (13.6)	0.425 ^α
Cutaneous involvement				
Rash	33 (86.8)	14 (87.5)	19 (86.4)	>0.999 ^α
Gottron's papule	25 (65.8)	11 (68.8)	14 (63.6)	0.743 ^α
Heliotrope rash	12 (31.6)	7 (43.8)	5 (22.7)	0.306 ^α
V-sign	2 (5.3)	1 (6.3)	1 (4.5)	0.816 ^α
Shawl sign	3 (7.9)	2 (12.5)	1 (4.5)	0.562 ^α
Arthritis [§]	13 (34.2)	6 (37.5)	7 (31.8)	0.742 ^α
Cardiac involvement	1 (2.6)	1 (6.3)	0 (0.0)	N/A
Pulmonary involvement	1 (2.6)	1 (6.3)	0 (0.0)	N/A
Gastrointestinal involvement	9 (23.7)	5 (31.3)	4 (18.2)	0.450 ^α
Dysphagia	7 (18.4)	3 (18.8)	4 (18.2)	>0.999 ^α
Dysphonia	5 (13.2)	3 (18.8)	2 (9.1)	0.632 ^α
Subcutaneous edema	5 (13.2)	4 (25.0)	1 (4.5)	0.141 ^α
Lipodystrophy	1 (2.6)	1 (6.3)	0 (0.0)	N/A

^bMedian (IQR), IQR: interquartile range, ^αChi-square test, [¥]Mann-Whitney U test, [§]n (%)

	Total	IVIG group	Non-IVIG group	p
AST, U/Lb	96.0 (35.0–210.8)	174.0 (86.3–431.5)	84.5 (32.5–157.5)	0.029 [‡]
LDH, U/Lb	614.5 (360.3–781.3)	662.5 (571.5–868.3)	508.0 (351.5–781.3)	0.162 [‡]
CK, U/Lb	1216.0 (377.8–4220.8)	1355.0 (528.5–6904.5)	1216.0 (204.5–3191.0)	0.589 [‡]
MSA positivity	21 (72.4)	11 (73.3)	10 (71.4)	>0.999 [‡]
Anti-NXP2	9 (31.0)	5 (33.3)	4 (28.6)	>0.999
Anti-SRP	2 (6.9)	1 (6.7)	1 (7.1)	>0.999
Anti-TIF1-γ	5 (17.2)	3 (20.0)	2 (14.3)	>0.999
Anti-Mi-2	2 (6.9)	1 (6.7)	1 (7.1)	>0.999
Anti-MDA5	3 (10.3)	2 (13.3)	1 (7.1)	>0.999
Anti-SAE	1 (3.4)	0 (0.0)	1 (7.1)	N/A
Anti-PL-7	2 (6.9)	1 (6.7)	1 (7.1)	>0.999
Anti-OJ	2 (6.9)	2 (13.3)	0 (0.0)	N/A
Disease Activity^b				
CMAS at presentation	32.5 (21.0–45.3)	26 (12–36.3)	44 (25.5–48)	0.021 [‡]
CMAS at 3 months	43.0 (38.3–46.5)	38.0 (29.0–44.3)	45.0 (42.3–50.5)	0.011 [‡]
CMAS at 6 months	46.0 (42.5–50.0)	44.0 (39.8–48.5)	48.0 (45.0–51.5)	0.109 [‡]
CMAS at last visit	51.0 (50.0–52.0)	52.0 (47.5–52.0)	51.0 (50.0–52.0)	0.753 [‡]
Nailfold capillary abnormalities ^b	8 (50.0)	5 (83.3)	3 (30.0)	0.039 [‡]
Treatment^b				
Pulse methylprednisolone	19 (50.0)	12 (75.0)	7 (31.8)	0.021 [‡]
Methylprednisolone (2 mg/kg/day)	37 (97.4)	16 (100.0)	21 (95.5)	N/A
Methotrexate	36 (94.7)	16 (100.0)	20 (90.9)	N/A
Mycophenolate mofetil	8 (21.1)	6 (37.5)	2 (9.1)	0.049 [‡]
Hydroxychloroquine	16 (42.1)	6 (37.5)	10 (45.0)	0.875 [‡]
Cyclophosphamide	2 (5.3)	2 (12.5)	0 (0.0)	N/A
Prognosis^b				
Remission	30 (78.9)	9 (56.3)	21 (95.5)	0.005 [‡]
Relapse	10 (26.3)	4 (25.0)	6 (27.3)	>0.999 [‡]
Refractory JIIM	19 (50.0)	16 (100.0)	3 (13.6)	N/A

^bMedian (IQR); IQR: interquartile range; [‡]Chi-square test; [‡]Mann-Whitney U test; [‡]n (%); CMAS: Childhood Myositis Assessment Scale; AST: Aspartate Aminotransferase; LDH: Lactate Dehydrogenase; CK: Creatine Kinase; JIIM: Juvenile Idiopathic Inflammatory Myopathies; MSA: Myositis-Specific Antibodies; Anti-NXP2: Anti-nuclear matrix protein 2; Anti-SRP: Anti-signal recognition particle; Anti-TIF1 (TIF1-γ): Anti-transcription intermediary factor 1 gamma; Anti-Mi-2: Anti-Mi-2 nucleosome remodeling deacetylase complex; Anti-MDA5: Anti-melanoma differentiation-associated gene 5; Anti-SAE: Anti-small ubiquitin-like modifier activating enzyme; Anti-PL-7: Anti-threonyl-tRNA synthetase; Anti-OJ: Anti-isoleucyl-tRNA synthetase

Characteristics of patients who received intravenous immunoglobulin treatment

Ten of the 16 patients (62.5%) received IVIG at the time of diagnosis. During the follow-up period, eight patients (50%) required IVIG therapy. Two patients (12.5%) received IVIG therapy in the first three months, and four patients (25%) received it in the second three months. Two patients required a second course of IVIG due to persistent calcinosis and muscle weakness.

The reasons for IVIG administration were severe or unresponsive muscle weakness (56.3%), dysphagia (12.5%), persistent cutaneous involvement (12.5%), arthritis (6.3%), lipodystrophy (6.3%), calcinosis (6.3%), and dysphonia (6.3%). In addition, IVIG was initiated owing to a drug-related adverse effect (6.3%) and as a steroid-sparing strategy (6.3%) due to steroid-induced osteoporosis. The indications for IVIG therapy are shown in Figure 1.

Adverse effects of IVIG therapy were observed in two patients: hemolysis and severe headache.

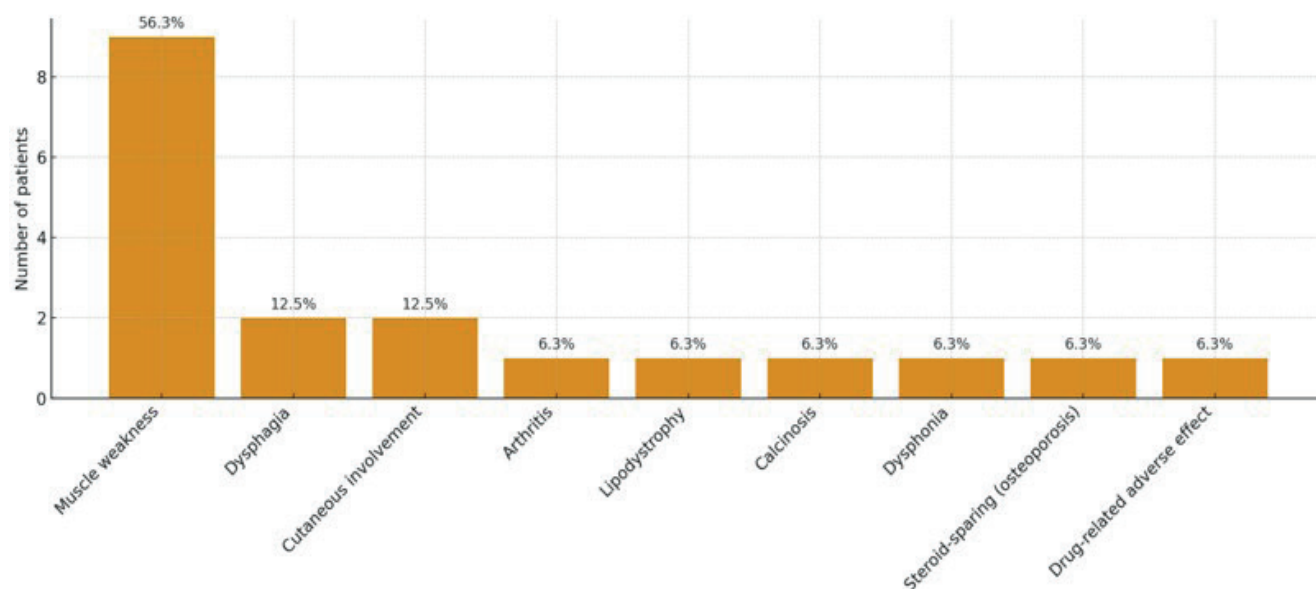


Figure 1. Indications for intravenous immunoglobulin therapy

Comparison of patients with/without intravenous immunoglobulin therapy

Comparison of demographic, laboratory, treatment, and outcome characteristics of JIIM patients according to IVIG treatment is presented in Tables 1 and 2. There were no significant differences between the two groups in demographic and clinical characteristics. AST levels were significantly higher in the IVIG-treated group. In terms of the presence of MSA, there was no significant difference between the two groups.

The CMAS score at onset was significantly lower in IVIG-treated patients ($p=0.021$). By the 3-month follow-up, CMAS remained lower in the IVIG-treated group ($p=0.011$). No significant differences were found at the sixth month ($p=0.109$) and the last visit ($p=0.753$).

Abnormalities in nailfold capillaroscopy (NFC) at onset were significantly more common in IVIG-treated patients ($p = 0.039$).

The remission rate was significantly lower in the IVIG-treated patients ($p=0.005$). All IVIG-treated patients had refractory JIIM, although this finding was not statistically significant.

Predictors of intravenous immunoglobulin therapy

In the bivariate logistic regression analysis, lower CMAS scores were significantly associated with an increased odds of receiving IVIG therapy. At diagnosis, each one-point decrease in the CMAS score was associated with a 1.09-fold increase in the odds of IVIG administration (OR: 0.92; 95% CI: 0.87–0.97; $p=0.007$). Similarly, at the third month, each one-point decrease corresponded to a 1.30-fold increase in IVIG use (OR: 0.77; 95% CI: 0.60–0.98; $p=0.038$). In the multivariate model, the third-month CMAS score remained the only variable significantly associated with IVIG therapy, with each one-point decrease increasing the odds of IVIG administration by approximately 1.30-fold (OR: 0.77; 95% CI: 0.60–0.97; $p=0.038$) (Table 3).

Table 3. Multivariate logistic regression model for predicting IVIG therapy of JIIM

Factor	Bivariate analysis OR (95% CI)	p	Multivariate analysis OR (95% CI)	p
CMAS score at diagnosis	0.92 (0.87-0.97)	0.007		
CMAS score at 3 rd month	0.77 (0.60-0.98)	0.038	0.77 (0.60-0.97)	0.038

Hosmer and Lemeshow $p=0.568$, OR: odds ratio, CI: confidence interval, CMAS: Childhood Myositis Assessment Scale

DISCUSSION

Juvenile idiopathic inflammatory myopathies are a group of diseases with marked clinical heterogeneity that complicates management. Therefore, an individualized treatment approach is required to reduce morbidity and achieve disease control. Our study demonstrated that patients receiving IVIG had lower CMAS scores and more frequent abnormalities in NFC. Furthermore, despite receiving pulse methylprednisolone and mycophenolate mofetil, the remission rate was lower in patients receiving IVIG.

According to the EULAR/ACR and SHARE recommendations, the therapeutic strategy for JIIM is guided by disease severity. For mild disease, the standard first-line therapy is methotrexate in combination with oral corticosteroids. For moderate-to-severe cases, or when the initial treatment is ineffective, high-dose intravenous corticosteroids are administered alongside methotrexate. IVIG is recommended as an adjunctive therapy for patients with refractory disease, frequent relapses, or prominent cutaneous involvement.¹⁷⁻¹⁹ In our study, disease activity, assessed using the CMAS at diagnosis and at the third-month visit, was higher in patients requiring IVIG treatment. Additionally, a higher AST level at onset was defined in the IVIG-treated group. Similarly, Lam et al. reported that patients receiving IVIG had more severe muscle involvement, lower MMT scores, and more frequent dysphagia and photosensitivity than the control group.⁸ In our study, logistic regression analysis demonstrated that lower CMAS scores were associated with IVIG use. Likewise, severe initial muscle weakness and persistent high disease activity despite first-line treatment were more frequently observed in patients who received IVIG. All these findings may suggest both more severe disease activity and clinicians' tendency to prefer IVIG treatment based on this perceived severity.

Recent studies have shown that early aggressive immunosuppressive therapy, such as pulse methylprednisolone and/or IVIG therapy in the first year, provided better clinical response and faster corticosteroid tapering.^{20,21} A pediatric study reported that using IVIG in the early period reduced steroid burden, improved refractory muscle weakness and persistent cutaneous findings.²² Lam et al. found that patients receiving IVIG treatment who had more severe disease at baseline achieved remission slowly. IVIG was shown to significantly reduce disease activity, particularly in steroid-resistant patients.⁸ The CARRA study also demonstrated that most clinicians add IVIG early in the management of patients, particularly

those who are refractory to treatment, steroid-dependent, or experiencing toxicity, and reported significant clinical improvement in severe phenotypes such as refractory muscle weakness, ulceration, and calcinosis.^{16,23} In the present study, we observed that all patients receiving IVIG therapy had a refractory JDM phenotype, and remission rates were lower in this group.

In JIIM, strict sun protection is an indispensable non-pharmacological component of treatment. It is a fundamental preventive measure in disease management. Furthermore, there is a long-term use of corticosteroids, which are the mainstay of treatment. The possibility of adverse effects increases because of the steroid burden.²⁴ Hence, the use of steroid-sparing agents is important to reduce complications. It has previously been reported that IVIG therapy is also used as a steroid-sparing agent in JIIM.^{25,26} In our study, IVIG was added in one patient due to secondary osteoporosis as a steroid-sparing strategy, and in another due to drug-related adverse effects. IVIG may play an important role in individualized treatment approaches, not only for refractory cases but also for steroid sparing.

Nailfold capillaroscopy abnormalities play a crucial role in assessing early changes in vasculopathies. The NFC in JIIM reflects microvascular damage and disease activity. Visualizing early microvascular damage, capillary loss, and neoangiogenic patterns can provide clinicians with insight into disease severity. Schmeling et al. reported that pathogenic NFC is associated with both muscle and skin disease activity.²⁷ Barth et al. demonstrated that a decreased capillary count and neoangiogenesis in NFC are associated with higher disease activity and pulmonary involvement.²⁸ In some studies, a significant reduction in NFC density was found in children with JDM, but no association was observed with CMAS scores.^{29,30} In our study, NFC abnormalities were more frequently observed in the IVIG-treated group. Given these results, NFC appears to be a complementary tool in classifying early disease activity. Furthermore, early-phase NFC findings may indicate that the patients require IVIG.

The limitations of this study are its retrospective design and small sample size. As a tertiary care center, although our patients had high disease severity and serious organ involvement, all patients who met the inclusion criteria were included in the study to prevent bias. One limitation of the study is that MSA tests are not routinely performed at our center, so they could not be evaluated in all patients; this may have limited the analysis of the relationship between autoantibody profiles and clinical findings. IVIG initiation was based on clinician assessment rather

than standardized criteria, and IVIG preparations varied according to hospital availability.

In conclusion, IVIG is an effective treatment option for patients with severe and refractory JIIM. These patients are characterized by lower CMAS scores and marked microvascular involvement. Therefore, NFC can be considered an important.

Author contributions

Conceptualization: E.Ö., Z.E.T.; Methodology: E.Ö., B.Ç.A., E.Ç., Z.E.T., C.K., E.E.T., Ş.E.; Formal analysis and investigation: E.Ö., M.I.E., S.N.Y., D.Ö., Y.U.E., Ş.E.T.; Writing - original draft preparation: E.Ö., Z.E.T.; Writing - review and editing: E.Ö., Z.E.T. 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 Bilkent City Hospital Medical Research Ethics Committee (TABED) (Date: 26.11.2025, Decision/Protocol No: TABED 2-25-1672). 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.

Funding

The authors declare that this study received no funding.

Generative AI statement

The authors declare that no generative AI or AI-assisted technologies were used in the writing or preparation of this study.

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Breaking the allergy chain: Maternal fermented dairy consumption and its impact on children's milk tolerance

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Cite this article as: Tunçerler G, Atar Beşe S, Erge D, Uysal P. Breaking the allergy chain: Maternal fermented dairy consumption and its impact on children's milk tolerance. Trends in Pediatrics 2026;7(3):184-192.

ABSTRACT

Background: A limited number of studies have shown that fermented milk products (FMPs) regularly consumed by mothers during pregnancy may be beneficial for atopic dermatitis and food allergy in their children. In this study, we aimed to investigate the effect of regular maternal consumption of yogurt and cheese during pregnancy on the remission period of cow's milk protein allergy (CMPA) in their children.

Methods: In this retrospective cohort study, 59 consecutive children who presented to the pediatric allergy outpatient clinic between January 2020 and January 2023 and were evaluated for IgE-mediated CMPA were screened; 19 were excluded (incomplete data, non-IgE-mediated findings, or declined participation), leaving 40 children for analysis. The diagnosis of CMPA was made by skin prick test (SPT) and/or cow's milk-specific IgE and oral food challenge (OFC). Demographic characteristics, laboratory data, duration of tolerance development and frequency of regular FMP consumption by mothers during pregnancy were recorded using a structured questionnaire.

Results: Among cases, 42.5% were girls and 57.5% were boys. The median age of cases was 38.5 months (24-47.75). The median duration of breast milk intake was 20 months (14-24). Parental atopy was present in 45% of cases. The median age at symptom onset was 4.5 (2-6) months. Prediagnoses at presentation included atopic dermatitis in 57.5%, allergic proctocolitis in 5%, urticaria and angioedema in 32.5%, and anaphylaxis in 5%. The median cow's milk SPT wheal diameter was 8 (5-13) mm, yogurt SPT wheal diameter was 5 (0-10) mm, and cow's milk-specific IgE value was 0.66 (0.13-5.68) kU/L. Baked cow's milk products were tolerated by 67.5% of subjects. Prevalence of daily FMP consumption during pregnancy was 55%, yogurt consumption was 62.5%, and cheese consumption was 70%. Although there was no significant difference between subgroups in age at onset of clinical symptoms, the age at first tolerance to cow's milk, duration of tolerance development and yogurt SPT wheal diameter were significantly lower in children of mothers who consumed FMPs daily during pregnancy compared with those who did not ($p<0.05$); this lower yogurt wheal diameter may in part reflect a milder baseline sensitization profile in this subgroup. Age at symptom onset was correlated with serum total IgE and cow's milk-specific IgE.

Conclusion: The duration of remission of CMPA in offspring was found to be shorter if the mother used FMPs daily during pregnancy.

Keywords: cow's milk allergy, fermented food, skin prick test, cow's milk-specific IgE, milk, yogurt, tolerance

INTRODUCTION

Cow's milk protein allergy (CMPA) represents one of the most common food allergies in early childhood, affecting 2-3% of infants worldwide.¹ While this condition typically

resolves naturally over time, the journey to tolerance can be lengthy and challenging for both children and their families. Recent scientific attention has turned to preventive strategies, particularly the role of maternal



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Received: 27.12.2025 Accepted: 15.09.2026

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diet during pregnancy in modulating allergic outcomes in offspring.²⁻⁴

Dysbiosis is a condition in which the microbiota balance is disturbed by changing the content and quantity of bacteria.⁵ Dysbiosis is thought to affect the function of the intestinal barrier and basophil and type 2 immune responses and has been proposed as a contributing mechanism to allergic responses.⁶

Fermentation is a natural food chemical processing method that occurs through the action of microorganisms in the organic structure of foodstuffs.⁷⁻⁹ Fermented dairy products, with their rich probiotic content and modified protein structures, have emerged as potential modulators of immune response.⁸⁻¹⁰ These products, particularly yogurt and cheese, have been traditionally consumed across various cultures for their nutritional benefits. Fermented foods contain bioactive compounds, antioxidants, antimicrobial enzymes, essential amino acids, vitamins, probiotics, and prebiotics.^{8,9,11} They play an important role in the formation of the intestinal microbiota.^{8,9} However, their specific impact on allergic conditions, especially when consumed during pregnancy, remains an understudied area of considerable interest.

Previous research has suggested associations between maternal consumption of fermented foods during pregnancy and reduced risk of atopic conditions in children.¹²⁻¹⁵ However, limited data exist specifically examining the relationship between maternal fermented dairy intake during pregnancy and the natural history of CMPA in offspring. Our study addresses this critical knowledge gap by investigating whether regular consumption of fermented dairy products during pregnancy influences the duration of IgE-mediated CMPA in children.

METHODS

Participant characteristics

This was a retrospective cohort study; the primary outcomes (age at tolerance and time to tolerance) were assessed longitudinally. All consecutive children aged 0-18 years with IgE-mediated CMPA admitted between January 2020 and January 2023 were screened for eligibility.

Detailed information concerning the patients included in the study is shown in the flowchart in Figure 1.

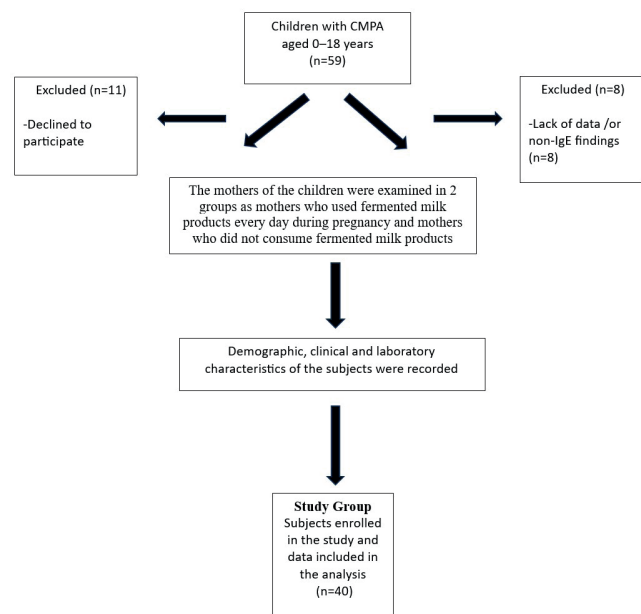


Figure 1. Study flowchart.

Case report form and data collection

The variety and frequency of fermented milk products (FMPs) used by the mothers during pregnancy were assessed by telephone interview using a structured questionnaire. Consumption of yogurt and cheese as FMP was recorded and categorized as “every day” versus “not every day” for each product, following the classification used by Koksall et al. in a comparable cohort of mothers and their children with CMPA.¹²

The definition of fermented milk product was based on the consumption of cheese and yogurt.

Cow's milk oral food challenge (OFC) and skin prick test procedures: Oral food challenges were performed in hospital under physician supervision using a graded, incremental-dose protocol for cow's milk, adapted from the cow's milk product ladder and OFC dosing schedule of the Turkish National Food Challenge Guideline (2019)¹⁶; the challenge was terminated at the first objective sign of an IgE-mediated reaction and considered negative if no reaction occurred after the cumulative target volume was tolerated. Baked cow's milk products were defined, per the same national guideline, as products baked at 180–200 °C for approximately 30 minutes (e.g., biscuits, cakes, muffins, or pastries) containing less than 1 g of cow's milk protein per serving (Step 1 of the cow's milk product ladder); baked-milk tolerance was confirmed by an open OFC with

the baked product without objective reaction. Skin prick tests were performed with commercial allergen extracts together with saline (negative) and histamine (positive) controls; wheals were read at 15–20 minutes, and a wheal diameter ≥ 3 mm greater than the negative control was considered positive, in keeping with standard national practice.

The mothers were divided into 2 subgroups according to whether they consumed FMPs every day or not every day during pregnancy and were compared. Demographic, clinical and laboratory characteristics of the subjects were recorded.

Exclusion criteria

Of 59 consecutively screened patients, 19 were excluded due to incomplete data, non-IgE-mediated laboratory findings, or declined participation. Patients presenting with a clinical phenotype of allergic proctocolitis were retained in the analysis where biochemical evidence of IgE-mediated sensitization (a positive skin prick test and/or cow's milk-specific IgE) was documented; proctocolitis is more commonly a non-IgE-mediated entity, and we clarify this point here because it was not stated explicitly in the original submission.

CMPA diagnosis

The diagnosis of CMPA was made by cow's milk-specific IgE measurement, skin prick test (SPT), diagnostic elimination diet and oral food challenge, together with a thorough clinical history.¹⁷

Sample size

A total of 40 children diagnosed with CMPA who underwent oral food challenge were included in the study retrospectively. A formal a priori sample size or power calculation was not performed, as this was a retrospective analysis of consecutively available patients over the study period; this is acknowledged as a limitation.

Ethics

This study was approved by the local institutional ethics committee (protocol no. 2023/63). The research was conducted in conformity with the principles of the Declaration of Helsinki. Informed consent was obtained from the children's parents or legal guardians.

Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics version 27.0 software (IBM Corp., Armonk, NY, USA). The data obtained were expressed as percentage (%), mean $\pm$ SD and median (25th–75th percentile, IQR). Within-group values were analyzed with parametric and nonparametric tests according to the nature of the data. Chi-square or Fisher's exact test was used to analyze categorical variables, and odds ratios (OR) with 95% confidence intervals (CI) are reported where appropriate. Differences between the two groups were evaluated with Student t test or Mann-Whitney U test; for the latter, rank-biserial correlation is reported as a measure of effect size. A post hoc exploratory multivariable logistic regression analysis was performed to assess whether egg allergy and other food allergy independently predicted baked cow's milk tolerance, with discrimination summarized by the area under the receiver operating characteristic curve (AUC). A p-value < 0.05 was considered statistically significant.

RESULTS

Patient characteristics

Fifty-nine children were initially screened for the study, of whom 19 were subsequently excluded for the reasons detailed in the Methods, leaving 40 children for final analysis. Detailed participation information is shown in the flowchart (Figure 1).

There was no significant difference between the subgroups defined by maternal daily FMP consumption in terms of the demographic characteristics of sex, age, weight, height or BMI SDS ($p > 0.05$).

The median duration of breastfeeding was 20 months. Parental atopy was present in 45% (18) of the patients. The most common presenting complaint was rash, itching, and swelling in 90% (36) of the patients. The most common clinical diagnosis at presentation was atopic dermatitis in 57.5% (23). This diagnosis was followed by urticaria and angioedema in 32.5% (13), proctocolitis in 5% (2), and anaphylaxis in 5% (2), in order of frequency (Table 1).

Baked cow's milk protein was tolerated by 67.5% (27) of patients. The frequency of consumption of fermented milk products (yogurt/cheese) during pregnancy was 55% (22) for mothers who consumed them every day and 45% (18) for mothers who did not consume them every day (Table 1).

Table 1. Demographic characteristics of patients with cow's milk allergy.	
Demographic measures	
Sex, n=40 (%)	
Girl	17 (42.5)
Boy	23 (57.5)
Age (months); median (IQR)	38.5 (24-47.75)
Weight SDS, median (IQR)	0.07 (-0.94 to 0.72)
Height SDS, median (IQR)	-0.09 (-0.092 to 0.65)
BMI SDS, median (IQR)	0.02 (-0.69 to 0.76)
Parental atopy, n (%)	
Yes	18 (45)
No	22 (55)
Age at symptom onset (months); median (IQR)	4.5 (2-6)
Clinical diagnosis, n (%)	
Atopic dermatitis	23 (57.5)
Allergic proctocolitis	2 (5)
Urticaria and angioedema	13 (32.5)
Anaphylaxis	2 (5)
Presenting complaint, n (%)	
Rash, itching, and swelling	36 (90)
Anaphylaxis	4 (10)
Number of patients tolerating baked cow's milk, n (%)	27 (67.5)
Number of patients tolerating any dairy product other than baked cow's milk, n (%)	19 (47.5)
Frequency of consuming FMPs (yogurt and cheese) during pregnancy, n (%)	
Every day:	22 (55)
Not every day:	18 (45)
Frequency of yogurt consumption during pregnancy, n (%)	
Every day:	25 (62.5)
Not every day:	15 (37.5)
Frequency of cheese consumption during pregnancy, n (%)	
Every day:	28 (70)
Not every day:	12 (30)
Duration of cow's milk tolerance (months), median (IQR)	17.5 (14.25-24.25)
Age at cow's milk tolerance (months), median (IQR)	21.5 (7.75-31.25)
Laboratory:	
Skin prick test (SPT) wheal diameters (mm), median (IQR)	
Milk	8 (5-13)
Yogurt	5 (0-10)
Alpha-lactalbumin, n=11	0 (0-0)
Beta-lactoglobulin	6 (0-11)
Casein, n=24	0 (0-5)
FX5 (kU/L), median (IQR)	1.74 (0.29-8.15)
Cow's milk-specific IgE (kU/L), median (IQR)	0.66 (0.13-5.68)
Total IgE (IU/mL), median (IQR), n=16	63.25 (29.5-318.5)

BMI: body mass index, FMP: fermented milk product, FX5: specific IgE screening panel for a mixture of common food allergens (egg white, milk, fish, wheat, peanut, and soybean), IgE: immunoglobulin E, IQR: interquartile range, SDS: standard deviation score, SPT: skin prick test.

Table 2. Comparison of the frequency of fermented milk products consumed by the mother during pregnancy with clinical and laboratory data.

	Mother consumed yogurt and cheese every day	Mother did not consume yogurt and cheese every day	p value
Age at first tolerance to cow's milk (months)	18 (16-24)	40 (31-48)	<0.001
Time to development of cow's milk tolerance (months)	15 (11-18)	34 (24-47)	<0.001
Age at clinical onset (months)	3 (2-6)	6 (1.75-6)	0.840
Number of patients tolerating baked cow's milk, n (%)	21 (77.8%)	6 (22.2%)	<0.001
Presence of parental atopy, n (%)	10 (45.5%)	8 (44.4%)	0.949
Egg allergy, n (%)	15 (68.2%)	12 (66.7%)	0.919
Other food allergy, n (%)	6 (27.3%)	7 (38.9%)	0.435
Number of patients tolerating non-baked cow's milk products, n (%)	14 (77.8%)	5 (27.8%)	0.003
Duration of breastfeeding (months)	17.5 (13.25-24)	21 (16-27)	0.314
Cow's milk-specific IgE (kU/L)	0.65 (0.06-1.98)	2.95 (0.25-19.65)	0.129
Total IgE (IU/mL)	87 (24.5-219.5)	41.5 (30.5-317.8)	0.875
SPT wheal diameter (mm)			
Milk	7 (5-14.25)	9 (5-11.25)	0.882
Yogurt	0 (0-8)	7 (5-11.5)	0.027
Casein	0 (0-1.5)	0 (0-5.25)	0.625
Alpha-lactalbumin	0	0 (0-4.25)	0.429
Beta-lactoglobulin	5.5 (0-9.75)	8 (0-12)	0.347

IgE: immunoglobulin E, SPT: skin prick test. Laboratory comparisons were performed only in patients with available measurements for each biomarker; subset sizes for casein-specific IgE and alpha-lactalbumin/beta-lactoglobulin are reported in Table 1.

In patients who tolerated cow's milk, the median duration of tolerance was 17.5 months, and the median age of tolerance was 21.5 months (Table 1).

When patients with CMPA were compared according to the mother's daily consumption of cheese and yogurt during pregnancy, the age at first tolerance to cow's milk protein was younger, the time to develop tolerance was shorter, and the number of patients who tolerated cow's milk protein in total (baked or unbaked) was higher ($p<0.05$) (Table 2).

Laboratory

The median FX5 (a screening panel of specific IgE to a mixture of common food allergens: egg white, milk, fish, wheat, peanut, and soybean) value was 1.74 kU/L, cow's milk-specific IgE was 0.66 kU/L, and total IgE level was 63.25 IU/mL in children with CMPA (Table 1).

When patients with CMPA were compared according to the mother's daily consumption of cheese and yogurt during pregnancy, no difference was observed in total IgE or cow's milk-specific IgE values between children of mothers who consumed cheese and yogurt daily and children of mothers

who did not, but yogurt SPT wheal diameter was found to be lower in children of mothers who consumed yogurt daily ($p=0.027$) (Table 2). As baseline sensitization severity was not adjusted for, this finding may partly reflect a milder underlying allergic phenotype in this subgroup rather than a direct effect of maternal FMP intake and should be interpreted with caution.

Correlation analysis

According to Pearson correlation analysis, there was a moderate positive correlation between age at symptom onset and serum total IgE ($r=0.647$, $p=0.007$).

There was a moderate positive correlation between age at symptom onset and serum cow's milk-specific IgE ($r=0.523$, $p=0.002$).

We assessed whether egg allergy or other food allergy (positive SPT to soy, wheat, peanut, hazelnut, walnut, fish, beef, or lentil) independently predicted tolerance to baked cow's milk products using multivariable logistic regression. Neither egg allergy (OR 1.72, 95% CI 0.40-7.44, $p=0.47$) nor other food allergy (OR 0.58, 95% CI 0.13-2.51, $p=0.47$) was

an independent predictor, and the model showed poor discrimination (AUC 0.56), likely reflecting the limited sample size; this should be regarded as hypothesis-generating rather than confirmatory. We also examined whether symptom severity at presentation (presence of anaphylaxis) was associated with age at tolerance acquisition, but only 2 of 40 patients presented with anaphylaxis, precluding a statistically meaningful comparison. For the main between-subgroup comparisons in Table 2, effect sizes are as follows: age at first cow's milk tolerance (rank-biserial $r=0.95$) and tolerance development time ($r=0.91$) were both very large, and the odds of tolerating baked cow's milk products were substantially higher in children of mothers with daily FMP consumption (OR 42.0, 95% CI 4.50-391.65), although the wide confidence interval reflects the small cell counts involved.

DISCUSSION

Our study demonstrated that regular maternal consumption of FMPs during pregnancy was associated with shorter remission periods of CMPA in offspring. This finding aligns with and extends previous research in the field of maternal diet and childhood allergies.

Previous case-control studies have shown that lower consumption of yogurt, tarhana, and kefir during pregnancy is associated with increased risk of infant secondary protein allergy (CMPA).^{7,9,11-13,18} Our study extends these findings by showing that daily maternal consumption of FMPs during pregnancy was associated with differences in cow's milk allergy progression. Children of mothers who consumed FMPs daily showed lower ages of milk tolerance, shorter tolerance development periods, and smaller yogurt SPT wheal diameters. Given the retrospective, observational design, these associations may reflect an immunomodulatory effect of maternal FMP intake, but they may equally reflect unmeasured confounding (e.g., baseline sensitization severity, maternal atopy, or other dietary factors), and causality cannot be inferred.

Previous retrospective case-control research has illuminated the potential protective role of maternal fermented dairy product consumption during pregnancy against allergic conditions.¹⁹ Specifically, studies investigating the impact of FMPs on allergic proctocolitis revealed that lower daily consumption of these products was associated with increased incidence of the condition.^{19,20} Additionally, daily consumption of a variety of FMPs, including yogurt, cheese, and tarhana, demonstrated a protective effect against allergic proctocolitis in infants.¹⁹ Our findings are broadly

consistent with this body of research, providing additional observations on the relationship between maternal diet and infant immune responses. While previous studies primarily focused on the presence or absence of allergic proctocolitis, our study extends this by examining cow's milk tolerance timing specifically. These results support the importance of maternal dietary choices during pregnancy as one of several factors potentially relevant to allergic outcomes in offspring.

Prior research has reported potential protective effects of maternal dietary interventions on atopic dermatitis (AD) development in children. Retrospective case-control studies have reported that daily yogurt consumption during pregnancy is associated with reduced AD risk.¹³ A comprehensive systematic review and meta-analysis involving 28 studies and 6,907 participants further substantiated these findings, revealing that maternal probiotic supplementation during pregnancy and early postpartum period substantially decreased AD incidence in children.¹⁵ A notable cohort study tracking infants from birth to six years confirmed these observations, showing markedly reduced AD risk in children whose mothers received probiotic support during pregnancy and early infancy.¹⁴ In our study, 57.5% of cases presented with atopic dermatitis, and milk allergy progression varied with maternal FMP consumption; children of mothers who consumed FMPs daily showed lower ages of milk tolerance and smaller yogurt SPT wheal diameters. These observations are directionally consistent with prior research on probiotic supplementation and AD prevention, though the mechanisms cannot be confirmed by our study design.

Research involving diverse populations, including Western and Japanese cohorts, demonstrated that high maternal consumption of dairy products and calcium during pregnancy was associated with reduced risks of infantile wheezing, asthma, and atopic dermatitis in children aged 16-29 months.²¹ Similarly, our study examines the specific impact of FMPs on CMPA progression, adding to this literature on maternal diet and infant allergic outcomes.

Children of mothers who consumed FMPs daily showed significantly lower ages of milk tolerance and shorter tolerance-development periods, consistent with prior evidence that maternal dietary interventions may modulate offspring immune responses.^{2,22} This finding—that the duration of CMPA remission was shorter in children of mothers consuming daily FMPs—adds a further, more specific data point to the literature on maternal nutrition

and allergic disease progression, while the associative, non-causal nature of this observation should be kept in mind.

The variability in allergic outcomes across different studies, including our own, underscores the need for comprehensive, longitudinal research to fully understand the immunomodulatory effects of maternal diet. While previous studies focused on broad allergic conditions, our research offers detailed insights into milk allergy tolerance mechanisms, suggesting that the impact of maternal nutrition extends beyond simple risk reduction to potentially influencing the fundamental immunological responses in offspring.

The strengths of our study include that all patients were evaluated in a single pediatric allergy and immunology center, that only patients with IgE-mediated CMPA (confirmed by SPT and/or cow's milk-specific IgE) were included, that an OFC was performed in all patients to confirm the diagnosis, that mothers were likely to recall their pregnancy diet accurately given the young age of the patient group, and that mothers were asked about consumption over the entire pregnancy rather than a single time point.

Limitations of our study include its retrospective, single-center design and its relatively small sample size, which limits statistical power and precludes robust multivariable or ROC-based predictive modeling; the exploratory multivariable analysis reported above should be interpreted accordingly. Casein-specific IgE was not measured in all patients, reflecting the retrospective nature of data collection, although an OFC confirming the diagnosis was performed in every patient. Baseline sensitization severity was not formally adjusted for in the subgroup comparisons, so differences such as the lower yogurt SPT wheal diameter in the daily-FMP subgroup cannot be fully separated from a milder baseline allergic phenotype. We did not collect data on maternal or infant antibiotic or probiotic exposure, so the potential role of dysbiosis discussed in the Introduction remains speculative in the context of our findings and was not directly tested. The questionnaire-based assessment of maternal FMP consumption may be subject to recall bias, and as an observational, retrospective study, our design cannot establish causality between maternal FMP consumption and the timing of CMPA remission. Future prospective studies with larger cohorts, a priori sample size calculations, and more detailed dietary and microbiome assessments would be valuable to confirm and extend these findings.

CONCLUSION

Although no significant differences were observed in terms of the amount or variety of FMP consumption by mothers during pregnancy, the duration until tolerance in their children was shorter when mothers consumed yogurt and cheese every day. If confirmed in larger, prospective studies, these findings could have implications for prenatal dietary counseling. While further prospective studies with larger cohorts are needed to validate these findings, our research suggests that regular consumption of fermented dairy products during pregnancy could plausibly be associated with a shorter duration of CMPA in children. Such an association, if causal, could meaningfully affect quality of life for affected families and reduce the associated healthcare burden. However, it is essential to interpret these findings within the context of the study's retrospective, observational nature and sample size limitations, which preclude any causal inference. Future research should focus on elucidating the specific mechanisms through which maternal FMP consumption may relate to allergic outcomes in offspring, as well as determining optimal consumption patterns for maximum benefit.

Key message

CMPA remains a considerable challenge in early childhood. Our exploratory, retrospective study raises the possibility of a preventive role for maternal diet modification, suggesting that regular consumption of fermented dairy products during pregnancy may be associated with a shorter duration of CMPA in offspring. Building on existing knowledge of the potential immunomodulatory properties of fermented dairy, our findings show that children born to mothers who regularly consumed these products during pregnancy developed tolerance earlier and showed smaller yogurt skin prick test wheal diameters. These observations point to a potentially simple dietary strategy worth testing prospectively in expecting mothers. For healthcare providers, this research offers preliminary support for considering fermented dairy products as part of prenatal dietary counseling, though confirmatory research is needed before any practice recommendation can be made. Our findings highlight the potential role of maternal nutrition during pregnancy in shaping children's immune responses and suggest a testable pathway for potentially reducing the burden of milk protein allergy in early childhood.

Author contributions

Data acquisition: S.A.B., D.E.; Data analysis: D.E.; Critical revision of the manuscript: S.A.B., D.E. All authors reviewed the results, approved the final version of this study, and agreed to be accountable for all aspects of this study.

Ethical approval

This study was approved by the Aydın Adnan Menderes University Medical Faculty Local Institutional Ethics Committee (Decision/Protocol No: 2023/63). 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.

Funding

This study was supported by Adnan Menderes University Scientific Research Projects.

Generative AI statement

The authors declare that no generative AI or AI-assisted technologies were used in the writing or preparation of this study.

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Influencing factors on selection of initial treatment dose in children diagnosed with isolated growth hormone deficiency

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Cite this article as: Erbaş İM, Uyar N, Nalbantoğlu Ö, Özkan B. Influencing factors on selection of initial treatment dose in children diagnosed with isolated growth hormone deficiency. Trends in Pediatrics 2026;7(3):193-200.

This study has been previously presented at ESPE 2023 as a e-poster.

ABSTRACT

Objective: In this study, we aimed to reveal the factors that affect the clinician's preferences in the selection of the starting dose of growth hormone (GH) treatment.

Methods: Patients under the age of 18 years who presented to our department between 2013 and 2022, were diagnosed with isolated GH deficiency, and started treatment were included in the study. Relevant data were collected retrospectively from the medical records of the patients. Data are presented as median (interquartile range).

Results: Eighty-eight patients (56.8% male; 65.9% prepubertal) with a chronological age (CA) of 10.4 (7.4-12.8) years were included in the study. The median bone age (BA) of the patients was 8 (5-10) years and height standard deviation score (SDS) was -2.7 [(-3.3) – (-2.3)]. Somatropin treatment was started at a median dose of 35 (30-35) µg/kg/day. Patients were divided into two groups according to the treatment dose as <35 µg/kg/day (n=36) and ≥35 µg/kg/day (n=52). In the univariate analyses, the target height SDS was lower (p=0.033), the annual growth rate was lower (p=0.010), and the CA-BA difference was greater (p=0.018) in the high-dose group, whereas the BA/CA ratio was similar between groups (p=0.730). However, in the multivariable analysis, only lower pre-treatment annual growth rate remained independently associated with a higher prescribed initial GH dose (p=0.028). Other anthropometric measurements, peak GH response in stimulation tests, and serum insulin-like growth factor 1 (IGF-1) and IGF binding protein-3 (IGFBP-3) levels did not differ between the groups (p>0.05). The initial dose of somatropin correlated negatively with annual growth rate (p=0.006) and positively with the CA-BA difference (p=0.009).

Conclusions: Lower pre-treatment annual growth rate was the only factor independently associated with a higher prescribed initial GH dose after adjustment for potential confounders. Although lower target height SDS and greater BA delay were associated with higher prescribed doses in the univariate analyses, these associations were not independently maintained. These findings provide real-world evidence that clinicians primarily rely on auxological severity, particularly impaired growth velocity, when individualizing the initial GH dose in children with isolated GH deficiency.

Keywords: growth hormone therapy, growth hormone dosage, hypopituitarism, height velocity



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Received: 30.03.2026 Accepted: 15.09.2026

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INTRODUCTION

Growth hormone (GH) deficiency in childhood, resulting from congenital or acquired causes, is characterized by impaired linear growth and may adversely affect metabolic health, skeletal development, and quality of life. Recombinant human GH therapy constitutes the cornerstone of treatment and has consistently been shown to improve growth velocity and height outcomes, while also exerting beneficial effects on body composition, bone mineralization, and cardiovascular risk factors.¹ Accordingly, optimizing GH treatment strategies, including appropriate dose selection at therapy initiation, is essential for achieving favorable short- and long-term clinical outcomes in children with GH deficiency.

Different GH doses are recommended according to the underlying indication. Current international guidelines recommend initiating treatment for childhood GH deficiency within a dose range of approximately 22–35 µg/kg/day while emphasizing individualized treatment rather than a fixed-dose strategy.² However, these recommendations provide little guidance regarding which clinical or biochemical parameters should determine the initial dose within this recommended range. Although dose escalation was suggested for selected patients with severe growth failure or during puberty, and individualized dosing was associated with improved growth outcomes in several studies, evidence-based criteria for selecting the initial GH dose at treatment initiation remain limited. Consequently, in routine clinical practice, clinicians often integrate multiple parameters into their decision-making process, including annual growth rate, baseline height deficit, target height, the difference between chronological age (CA) and bone age (BA), despite the absence of standardized recommendations. Previous studies mainly focused on predictors of treatment response rather than the factors influencing clinicians' initial dose selection, leaving an important gap between guideline recommendations and real-world practice.³⁻⁵

In this study, we aimed to identify the clinical and biochemical factors associated with clinicians' preferences for the initial somatropin dose in children with isolated GH deficiency and to characterize real-world prescribing patterns in routine pediatric endocrinology practice.

MATERIALS AND METHODS

Study design

This retrospective observational study included pediatric patients (<18 years of age) who were diagnosed with isolated GH deficiency and initiated on recombinant GH therapy at our tertiary pediatric endocrinology clinic between 2013 and 2022. Patients receiving GH treatment for indications other than isolated GH deficiency (e.g., multiple pituitary hormone deficiencies, syndromic short stature, chronic systemic diseases, or genetic growth disorders) were excluded from the study. All relevant clinical, biochemical, and radiological data were collected from the medical records of the patients.

During the study period, 157 children who initiated GH therapy at our center were screened for eligibility. Patients receiving GH therapy for indications other than isolated GH deficiency, including short stature associated with small for gestational age (n=7), Turner syndrome (n=9), Noonan syndrome (n=5), Prader–Willi syndrome (n=3), and chronic kidney disease (n=6), were excluded. Patients with multiple pituitary hormone deficiency (n=8) and those with incomplete baseline clinical, anthropometric, biochemical, or radiological records (n=31) were also excluded. Consequently, 88 children with isolated GH deficiency were included in the final analysis.

Anthropometric measurements

Routinely performed anthropometric measurements during outpatient follow-ups were used for analysis. Standing height was measured to the nearest 0.1 cm using a Harpenden stadiometer, and body weight was measured using a calibrated digital scale with 0.1 kg precision, with patients wearing only underwear. All anthropometric measurements were performed according to standardized institutional procedures throughout the study period. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m²). Height, weight, and BMI values were converted to standard deviation scores (SDS) according to age- and sex-specific reference data for the Turkish pediatric population.⁶

Pubertal status was assessed by pediatric endocrinologists according to Tanner staging. Patients were classified as prepubertal (Tanner stage I; testicular volume <4 mL in boys) or pubertal (Tanner stage II or higher; testicular volume ≥4 mL in boys) according to the criteria of Marshall and Tanner.^{7,8}

At the initiation of GH therapy, baseline evaluations included anthropometric measurements, BA assessment, serum insulin-like growth factor 1 (IGF-1) and IGF binding protein-3 (IGFBP-3) levels, and target height. Target height was calculated according to the Tanner (mid-parental height) method using parental heights and expressed as SDS.⁹ BA was assessed according to the Greulich and Pyle atlas by a single experienced pediatric endocrinologist, thereby avoiding inter-observer variability.¹⁰ Serum IGF-1 and IGFBP-3 concentrations were measured using assays with validated reference ranges, and age- and sex-specific SDS values were calculated according to published reference data.¹¹

Growth hormone deficiency diagnosis

Growth hormone stimulation tests were performed in patients with a clinical suspicion of GH deficiency, based on growth velocity deceleration, short stature relative to target height, or markedly reduced IGF-1 levels. All stimulation tests were conducted in the fasting state. Before GH stimulation testing, adrenal insufficiency, hypothyroidism, and other pituitary hormone deficiencies were excluded by appropriate clinical, biochemical, and hormonal evaluation. Pharmacological stimulation was performed using insulin, clonidine, or L-dopa according to standardized institutional protocols. Peak GH concentration obtained during the tests was used for diagnosis. The diagnosis of isolated GH deficiency was established according to the international consensus recommendations applicable during the study period.¹² An insufficient peak GH response (<10 ng/mL) in two different pharmacological stimulation tests, together with compatible auxological findings, was considered diagnostic of GH deficiency.

Growth hormone treatment

Recombinant human GH (somatropin) was initiated according to routine clinical practice at our institution. The initial dose was prescribed by the treating pediatric endocrinologist on an individual basis, taking into account the overall clinical assessment of each patient, including auxological findings, growth velocity, BA delay, target height, and biochemical evaluation. No predefined institutional algorithm or standardized protocol was used for dose selection. The prescribed dose was expressed as $\mu\text{g/kg/day}$, consistent with routine pediatric endocrinology practice and international guideline recommendations.

Statistical analyses

Analyses were performed using SPSS version 24.0 software. The normality of the distribution of the data was assessed using the Kolmogorov–Smirnov test. Continuous variables were summarized as mean $\pm$ standard deviation or median (interquartile range), as appropriate according to their distribution. For comparisons between groups, the Mann–Whitney U test and the independent-samples t-test were used for continuous variables, and the chi-square test was used for categorical variables. Correlations between parameters were analyzed using the Spearman correlation analysis. To identify variables independently associated with the initial GH dose, a multiple linear regression analysis was performed. Variables considered clinically relevant or associated with the initial GH dose in the univariate analyses were entered into the model, including age, pubertal status, target height SDS, pre-treatment annual growth rate, BA delay (CA–BA difference), and peak GH concentration. Regression coefficients (β), 95% confidence intervals (95% CI), and p-values were reported. A two-sided p-value <0.05 was considered statistically significant.

Ethics

The Local Ethics Committee approved the study (Approval number: 2025/06-02), and we performed it according to the Declaration of Helsinki.

RESULTS

Eighty-eight patients (56.8% male; 65.9% prepubertal) with a CA of 10.4 (7.4 – 12.8) years were included in the study. The median BA of the patients was 8 (5 – 10) years. Height and target height SDS were -2.7 [$(-3.3) - (-2.3)$] and -1.3 [$(-1.9) - (-0.8)$], respectively. Weight SDS was -2.0 ± 1.1 and BMI SDS was -0.6 ± 1.2 . Annual growth rate was calculated as 3.4 ± 1.2 cm/year. In laboratory evaluations, IGF-1 and IGFBP-3 SDS levels were -1.5 ± 1.1 and -0.2 [$(-0.9) - 0.6$], respectively. The peak GH level in stimulation tests was 5.5 (3.8 – 7.1) ng/mL.

Somatropin treatment was started at a median dose of 35 (30 – 35) $\mu\text{g/kg/day}$. Patients were divided into two groups according to the treatment dose as <35 $\mu\text{g/kg/day}$ ($n=36$) and ≥ 35 $\mu\text{g/kg/day}$ ($n=52$).

Compared with the lower-dose group, children receiving an initial dose ≥ 35 $\mu\text{g/kg/day}$ had a lower target height SDS [-1.5 (-1.9 to -1.2) vs. -1.1 (-1.6 to -0.5), $p=0.033$], and a lower pre-treatment annual growth rate (3.1 ± 1.2 vs. 3.8 ± 1.1 cm/year, $p=0.010$). Pubertal status was

comparable between the two dose groups (63.9% vs. 67.3% prepubertal, $p=0.739$), indicating that puberty did not appear to influence clinicians' initial dose selection in our cohort. While the ratio of BA/CA was similar in both groups ($p=0.730$), the difference between CA and BA was

significantly greater in the high-dose group [2.9 (2.0–3.2) vs. 2.0 (1.6–2.6) years, $p=0.018$]. Other anthropometric measurements, peak responses in GH stimulation tests, and serum IGF-1 and IGFBP-3 levels did not differ between the two groups ($p>0.05$). Table 1 summarizes these results.

Table 1. Comparison of clinical and laboratory findings of patients with initial somatropin doses <35 and ≥ 35 $\mu\text{g/kg/day}$.

	Somatropin dosage <35 $\mu\text{g/kg/day}$ (n=36)	Somatropin dosage ≥ 35 $\mu\text{g/kg/day}$ (n=52)	p-value
Age (years)	9.5 (6.0 – 12.5)	10.8 (8.3 – 12.9)	0.107
Weight SDS	-2.0 ± 1.0	-2.0 ± 1.2	0.739
Height SDS	$-2.7 [(-3.3) - (-2.3)]$	$-2.7 [(-3.3) - (-2.3)]$	0.410
BMI SDS	-0.5 ± 1.1	-0.7 ± 1.3	0.380
Male sex (n, %)	20 (55.6%)	30 (57.7%)	0.842
Prepubertal (n, %)	23 (63.9%)	35 (67.3%)	0.739
Difference of CA – BA (years)	2.0 (1.6 – 2.6)	2.9 (2.0 – 3.2)	0.018
BA/CA ratio	0.78 (0.64 – 0.84)	0.74 (0.67 – 0.83)	0.730
Target height SDS	$-1.1 [(-1.6) - (-0.5)]$	$-1.5 [(-1.9) - (-1.2)]$	0.033
Height – Target height SDS	$-1.8 [(-2.6) - (-0.9)]$	$-1.1 [(-1.9) - (-0.7)]$	0.085
IGF-1 SDS	-1.3 ± 1.4	-1.6 ± 0.9	0.252
IGFBP-3 SDS	$-0.2 [(-0.7) - 0.7]$	$-0.2 [(-1.0) - 0.5]$	0.756
Peak GH response (ng/mL)	5.9 (3.7 – 7.6)	5.2 (3.8 – 6.8)	0.415
Annual growth rate (cm/year)	3.8 ± 1.1	3.1 ± 1.2	0.010

BA: bone age, BMI: body mass index, CA: chronological age, GH: growth hormone, IGF-1: insulin-like growth factor-1, IGFBP-3: IGF binding protein-3, SDS: standard deviation score. Weight SDS, BMI SDS, IGF-1 SDS, and annual growth rate are presented as mean $\pm$ SD. Age, height SDS, difference of CA – BA (years), BA/CA ratio, target height SDS, height-target height SDS, IGFBP-3 SDS, peak GH response (ng/mL) are presented as median (interquartile range).

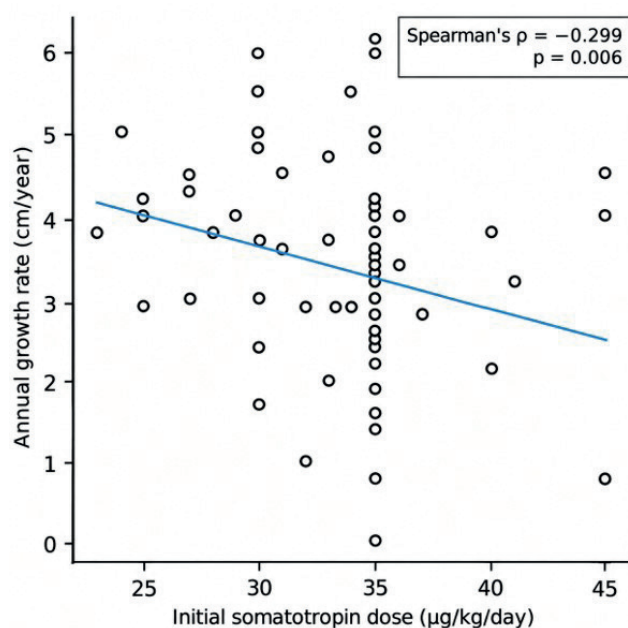


Figure 1. The correlation between initial somatropin dose and pre-treatment annual growth rate.

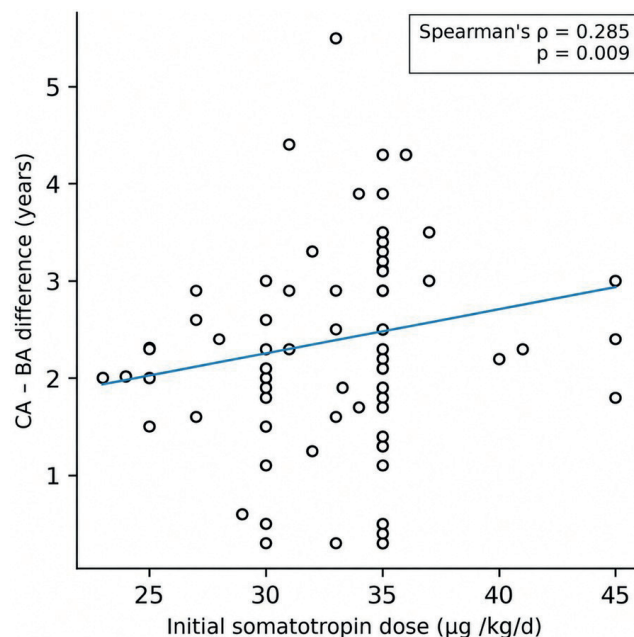


Figure 2. The correlation between initial somatropin dose and chronological age (CA) – bone age (BA) difference.

Table 2. Multiple linear regression analysis of factors independently associated with the initial growth hormone dose.

Variable	B (95% CI)	p-value
Age (years)	0.36 (-0.04 to 0.76)	0.080
Pubertal status	-2.48 (-5.01 to 0.05)	0.055
Target height SDS	-0.25 (-1.31 to 0.82)	0.645
Annual growth rate (cm/year)	-0.86 (-1.63 to -0.10)	0.028
Bone age delay (CA-BA, years)	0.55 (-0.36 to 1.46)	0.234
Peak GH level (ng/mL)	0.04 (-0.26 to 0.34)	0.809

Model statistics: $R^2 = 0.166$; adjusted $R^2 = 0.087$. The multivariable analysis included patients with complete data for all variables entered into the regression model (complete-case analysis, $n = 70$). BA: bone age, CA: chronological age, CI: confidence interval, GH: growth hormone, SDS: standard deviation score.

In correlation analyses, the initial somatropin dose showed a negative correlation with pre-treatment annual growth rate (Spearman's $\rho = -0.299$, $p=0.006$) and a positive correlation with the difference between CA and BA (Spearman's $\rho = 0.285$, $p=0.009$) (Figure 1 and Figure 2).

To identify variables independently associated with the initial GH dose, a multiple linear regression analysis was performed including age, pubertal status, annual growth rate, target height SDS, BA delay (CA-BA difference), and peak GH concentration. Lower pre-treatment annual growth rate remained independently associated with a higher initial GH dose ($B = -0.86$, 95% CI: -1.63 to -0.10, $p=0.028$), whereas target height SDS, BA delay, pubertal status, age, and peak GH concentration were not independently associated with the initial dose (Table 2).

DISCUSSION

The principal finding of this study is that lower pre-treatment annual growth rate was the only variable independently associated with the prescribed initial GH dose after adjustment for potential confounders. Although lower target height SDS and greater BA delay were associated with higher prescribed doses in the univariate analyses, these associations were no longer statistically significant in the multivariable model.

Several studies have investigated predictors of response to GH therapy, focusing primarily on treatment outcomes rather than treatment initiation strategies. Large registry-based analyses and prediction models have consistently identified auxological parameters as key determinants of growth response during the first year of therapy and beyond. However, such studies typically compared predefined dosing regimens or assessed responsiveness to GH rather than examining how these variables influence initial dose selection.¹³ The guidelines provide a dose

range for GH treatment according to the indication, but do not clearly emphasize which factors should be taken into consideration in dose selection.^{14,15} Current recommendations are based on weight-based dosing within a relatively narrow range for GH deficiency and emphasize individualization based on growth response.¹⁶ In addition, previous guidelines acknowledged the importance of familial height expectations in the evaluation of short stature; however, they provided limited guidance on how target height influenced GH dosing decisions.^{2,15} In a cohort study, higher somatropin doses were independently associated with better final height, together with mid-parental height, underscoring the importance of both genetic potential and adequate dosing in height prognosis.¹⁷ In the univariate analyses of our study, children with lower target height SDS, lower pre-treatment growth velocity, and greater BA delay were more likely to receive higher initial GH doses. However, after adjustment for other clinical variables, only pre-treatment annual growth rate remained independently associated with the prescribed dose. This practice is consistent with the literature data demonstrating a dose-response relationship between GH dose and both short-term growth velocity and final height in GH deficiency.¹⁸ However, because of the retrospective observational design, the present study cannot determine the actual decision-making process or establish causality. In addition, the inverse association between the initial GH dose and pre-treatment growth velocity should be interpreted with caution. This finding is likely influenced by confounding by indication, a common limitation of observational treatment studies, whereby children with more severe growth impairment are preferentially prescribed higher GH doses. Although higher GH doses may be considered during puberty because of increased physiological GH requirements and accelerated growth, pubertal status was not associated with clinicians' initial dose selection in our cohort.

Recent guidelines have stated that the daily GH dose in children should be calculated on the basis of weight or body surface area and not titrated primarily on IGF-1 levels.¹⁶ Although evidence regarding factors influencing initial GH dose selection is limited in children, an adult study similarly reported that baseline IGF-1 levels had little influence on initial dose selection.¹⁴ While long-acting GH development studies use IGF-1 dynamics to define dose bands, routine clinical practice for daily somatropin appears to rely more on auxologic parameters and BA than on baseline biochemical severity.¹⁹ We showed that basal IGF-1 and IGFBP-3 levels did not influence initial dose in our cohort. In addition, despite being central to the diagnosis of GH deficiency, peak GH responses were not associated with the prescribed starting dose. Our findings therefore highlight a gap between the large amount of effort invested in biochemical characterization and its limited role in initial dose decisions for daily GH treatment in clinical practice. The lack of association between peak GH and starting dose also reflects the poor correlation between pharmacological test results and subsequent growth response, which was reported in prior studies and acknowledged in guidelines.¹⁶ Moreover, GH stimulation tests are known to have considerable intra-individual variability and limited reproducibility, which may reduce their value in guiding dose selection beyond establishing the diagnosis of GH deficiency.¹² These findings suggest that, in routine clinical practice, clinicians may place greater emphasis on the overall clinical phenotype and auxological severity than on the biochemical severity of GH deficiency when selecting the initial dose within the recommended therapeutic range.

A greater CA–BA difference usually reflects greater growth potential and a larger window for catch-up growth.²⁰ The association between greater BA delay and higher prescribed doses observed in the univariate analyses is clinically plausible, as clinicians may perceive delayed skeletal maturation as a marker of greater auxological severity. However, this association was no longer significant after adjustment for other clinical variables, suggesting that BA delay alone was not an independent determinant of dose selection. Although the absolute difference between chronological age and BA was significantly greater in the higher-dose group, the BA/CA ratio was comparable between groups. These findings are not contradictory because the two indices reflect different aspects of skeletal maturation. The CA–BA difference represents the absolute delay in skeletal maturation expressed in years, whereas the BA/CA ratio reflects the relative delay after accounting for chronological age. Consequently, children of different

chronological ages may have similar BA/CA ratios despite different absolute BA delays. Together, our findings suggest that clinicians primarily rely on auxological assessment, particularly impaired pre-treatment growth velocity, when selecting the initial GH dose within the guideline-recommended range, rather than biochemical indices. This approach is consistent with the principle emphasized in international consensus statements that GH treatment should be individualized to optimize growth outcomes while avoiding unnecessary overtreatment.^{12,15} However, future prospective studies should examine whether formalized dosing algorithms based on baseline height deficit, target height, growth velocity, and the CA–BA difference can optimize both growth outcomes and safety, preferably integrating long-term data from cohorts treated with both daily and long-acting GH formulations.^{21–23} Although target height SDS and BA delay were associated with the initial GH dose in the univariate analyses, these associations were no longer statistically significant after adjustment for other clinical variables. In contrast, lower pre-treatment annual growth rate remained independently associated with higher prescribed doses, suggesting that clinicians may place particular emphasis on impaired growth velocity when selecting the initial GH dose.

This study has several limitations. The retrospective design limits causal inference and reflects clinician-driven decision-making rather than standardized dosing protocols. However, this also represents a strength, as it provides insight into real-world clinical practice, which may differ from controlled trial settings. In addition, our cohort consisted exclusively of children with isolated GH deficiency, improving internal consistency but limiting generalizability to patients with syndromic short stature or multiple pituitary hormone deficiencies. In addition, we did not assess early growth response or long-term outcomes such as near-adult height in relation to the initial dose, so it remains unknown whether the observed dose selection patterns translate into clinically meaningful differences in growth or metabolic endpoints. Despite these limitations, the study offers novel insight into how pediatric endocrinologists manage “individualized dosing” when initiating somatropin in children with isolated GH deficiency.

In conclusion, the principal finding of this study is that lower pre-treatment annual growth rate was the only factor independently associated with the initial GH dose after adjustment for potential confounders. Although lower target height SDS and greater BA delay were associated with higher prescribed doses in the univariate analyses, these

associations were not independently maintained. Our findings suggest that, in routine clinical practice, clinicians place greater emphasis on impaired pre-treatment growth velocity than on biochemical indices when individualizing the initial GH dose in children with isolated GH deficiency. These results provide novel real-world evidence regarding prescribing patterns and may contribute to the optimization of individualized GH treatment strategies.

Author contributions

Conception and design: İ.M.E., Ö.N., B.Ö.; Data acquisition: N.U.; Data analysis: İ.M.E.; Data interpretation: İ.M.E.; Drafting of the manuscript: N.U., İ.M.E.; Critical revision of the manuscript: İ.M.E., N.U., Ö.N., B.Ö. 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 İzmir Dr Behcet Uz Children's Hospital Ethics Committee (Date: 20.03.2025, Decision/Protocol No: 2025/06-02). 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.

Funding

The authors declare that this study received no funding.

Generative AI statement

The authors declare that no generative AI or AI-assisted technologies were used in the writing or preparation of this study.

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Suspected local anesthetic allergy in children referred from dental clinics: A 10-year experience

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Cite this article as: Çelebi Çelik F, Günder S, Soyöz Ö, Karkiner CS, Sancaklı Ö, Can D. Suspected local anesthetic allergy in children referred from dental clinics: A 10-year experience. Trends in Pediatrics 2026;7(3):201-209.

Preliminary findings of this study were presented at the 20th International Congress of Pediatric Allergy, Immunology and Asthma, in Antalya.

ABSTRACT

Objective: Suspected local anesthetic (LA) allergy is a frequent reason for referral to pediatric allergy clinics, particularly from dental settings. However, true drug hypersensitivity is considered rare. This study aimed to determine the frequency of confirmed LA allergy and to identify clinical risk factors in children referred from dentistry.

Methods: This retrospective study included children aged 2–18 years referred to a tertiary pediatric allergy unit with suspected LA hypersensitivity between 2015 and 2025. A stepwise diagnostic protocol was used: skin prick test (SPT) at full strength (1:1), intradermal test (IDT) at 1:100 dilution if SPT negative, and subcutaneous drug provocation test (DPT) in two graded steps if both skin tests were negative. True LA allergy was defined as objective positivity on SPT, IDT, and/or DPT, interpreted in the context of the clinical history when an index LA reaction was present.

Results: Of 94 referred children, 61 with completed diagnostic evaluation were included in the final analysis. The median age was 9 years (IQR 6.1–12.9), and 45.9% were female. Most referrals were related to allergic comorbidities rather than a prior reaction suggestive of LA hypersensitivity. LA allergy was confirmed in 5 patients (8.2%). Confirmation was significantly more frequent in children with a history suggestive of a prior hypersensitivity reaction to an LA ($p=0.024$; OR 12, 95% CI 1.2–116) and in those with a history of anaphylaxis ($p=0.005$; OR 26.5, 95% CI 3.1–223.9). Articaine or prilocaine was also associated with confirmed LA allergy ($p=0.012$). No positivity was observed in children referred solely because of asthma, food allergy, or chronic urticaria. Among children with negative SPT results, two had positive IDT findings; no positive reactions occurred during subcutaneous provocation in those with negative skin tests. The negative predictive value of undiluted SPT was 96.4%.

Conclusion: In children referred from dental clinics, confirmed LA allergy was mainly associated with a convincing prior LA-related reaction history, particularly anaphylaxis, rather than with unrelated allergic comorbidities. These findings support a risk-based diagnostic approach in which high-risk children undergo formal evaluation to confirm allergy and identify safe alternatives, whereas unnecessary testing may be avoided in low-risk patients.

Keywords: local anesthetic allergy, children, skin testing, drug provocation test, anaphylaxis, pediatric dentistry



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Received: 18.04.2026 Accepted: 08.07.2026

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INTRODUCTION

Local anesthetics (LAs) are essential for pain control during routine and invasive procedures in contemporary pediatric dentistry. Amide-type LAs are widely preferred in children because of their rapid onset, effective anesthesia, and generally favorable safety profile.¹ However, suspected LA allergy remains one of the most common reasons for referral to pediatric allergy clinics, especially when a child requires further dental treatment after a previous event attributed to LA exposure.

Although concern about LA hypersensitivity is frequent, objectively confirmed immediate-type allergic reactions are rare. Pediatric studies consistently show that only a small proportion of referred children are ultimately diagnosed with true LA allergy following formal allergologic evaluation.²⁻⁴ In most cases, the index event is later attributed to non-allergic causes such as procedural anxiety, vasovagal syncope, toxic effects, or symptoms related to the underlying dental pathology rather than the anesthetic agent itself. This diagnostic uncertainty is particularly relevant in children, as symptom descriptions are often incomplete and dental procedures frequently occur under emotionally stressful conditions.

This situation creates a clinical dilemma. While some children are referred with a history suggestive of immediate hypersensitivity, many others are referred solely because of co-existing conditions such as asthma, food allergy, chronic urticaria, or hypersensitivity to unrelated drugs. Current pediatric evidence indicates that these background allergic conditions do not substantially increase the likelihood of true LA allergy.⁵⁻⁸ Consequently, a considerable number of children undergo extensive diagnostic testing despite a low pre-test probability, resulting in unnecessary healthcare costs, heightened parental anxiety, and potential delays in essential dental care. At the same time, children with a convincing history of immediate reaction must not be overlooked, given the risk of genuine hypersensitivity upon re-exposure.

Despite growing interest in this area, pediatric data remain limited, particularly for children referred specifically from dental settings. Moreover, reliable clinical predictors of confirmed LA allergy have not been clearly defined, and the value of risk-stratified diagnostic approaches in reducing unnecessary testing in low-risk patients requires further clarification. These gaps have important implications for pediatric dentistry, where both over-investigation and

inappropriate avoidance of LAs can compromise timely treatment.

In this context, we conducted a 10-year retrospective study of children referred from dental clinics to our pediatric allergy and immunology unit with suspected LA allergy. The aim of the study was to determine the frequency of confirmed true LA allergy in this population and to identify clinical risk factors predictive of genuine hypersensitivity.

MATERIALS AND METHODS

Study design and setting

This retrospective study evaluated pediatric patients referred from dental clinics to the Pediatric Allergy and Immunology Unit of Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital with suspected LA allergy between 2015 and 2025. The study protocol was approved by the Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital Local Ethics Committee (Approval number: GOA-319). Written informed consent was obtained from all participants and their families. All assessments were performed in a specialized allergy setting under direct physician supervision, with immediate access to emergency equipment and intramuscular epinephrine.

Study population and data collection

Children aged 2–18 years who were referred with suspected immediate-type LA hypersensitivity and underwent diagnostic testing for at least one LA agent were included. Patients were excluded if medical records lacked essential information on the index reaction history, diagnostic steps performed, or final assessment. Demographic and clinical data, including age at referral, sex, referral reason, personal and family history of atopy, details of the index reaction, and diagnostic test results, were extracted from electronic and paper medical records. Anaphylaxis was defined according to internationally accepted World Allergy Organization (WAO)/European Academy of Allergy and Clinical Immunology (EAACI) criteria.⁹

During the study period, some children were referred prior to planned dental treatment due to allergic comorbidities such as asthma, food allergy, or chronic urticaria. These children had no history of reactions related to LA and were considered a low-risk referral subgroup. The tests performed on this subgroup reflected pre-procedural institutional practice to provide reassurance prior to subsequent dental treatment, rather than routine screening based solely on

allergic comorbidity. This patient group was retained in the current retrospective analysis to determine the diagnostic efficacy of this low-risk referral model. All procedures were performed after informed consent and individual risk-benefit assessment in a supervised allergy unit. True LA allergy was defined as an objective positive finding on SPT and/or IDT and/or DPT together with a clinically consistent history.

Allergy work-up and testing protocol

All children were evaluated using a stepwise diagnostic algorithm consisting of clinical history, basic laboratory assessment (complete blood count and total IgE), skin prick test (SPT), intradermal test (IDT) if SPT was negative, and subcutaneous drug provocation test (DPT) if both skin tests were negative. Skin prick tests and intradermal tests were conducted using the recommended concentrations in accordance with international guidelines.¹⁰ Diagnostic testing with local anesthetics was scheduled no earlier than 4 weeks after the suspected hypersensitivity reaction. Antihistamines and medications containing antihistaminic components were discontinued at least 1 week before skin testing. Skin tests were deferred in the presence of acute infection, fever, or active inflammatory conditions unless urgent testing was clinically required.

The SPT was performed on the volar surface of the forearm using undiluted LA agent at full strength (1:1) without epinephrine. Histamine (10 mg/mL) served as the positive control and 0.9% saline as the negative control. Reactions were read at 15–20 minutes. A positive SPT was defined as a wheal diameter at least 3 mm larger than the negative control.

In patients with a negative SPT, IDT was performed using a 1:100 dilution of the relevant LA agent, and 0.02–0.05 mL was injected intradermally into the volar forearm to produce a 5 mm bleb similar to that formed by the saline control. Reactions were read at 15–20 minutes. IDT positivity was defined as a clearly increased wheal size compared with the initial bleb and/or an erythematous response consistent with an immediate hypersensitivity reaction.

When a specific LA was implicated in the index reaction, testing was performed with the suspected culprit agent. In children without a known culprit LA, testing was performed with the LA considered most likely to be used for the planned dental procedure. If a test result was positive,

alternative LAs were subsequently evaluated to identify a safe option for future dental treatment.

Children with negative SPT and IDT proceeded to a provocation test with the respective LA agent administered subcutaneously in a graded manner. The provocation consisted of two steps at full strength (1:1): 0.1 mL followed by 1.0 mL, with a 30-minute interval between doses. Patients were observed for 2 hours after administration of the final dose. The development of objective skin, respiratory, or cardiovascular symptoms was accepted as positive. The absence of these symptoms excluded LA allergy.

Statistical analysis

Statistical analyses were performed using SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). Categorical variables were compared using Pearson's chi-square test or Fisher's exact test, as appropriate. Continuous variables were expressed as median and interquartile range (IQR), and comparisons between groups were performed using the Mann–Whitney U test. Univariate analysis was used to calculate odds ratios (ORs) with corresponding 95% confidence intervals (CIs). A two-sided p-value <0.05 was considered statistically significant.

RESULTS

A total of 94 children were referred to the pediatric allergy clinic at Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital with suspected LA allergy from dental clinics over a 10-year period. In 14 patients, the history was not considered compatible with immediate-type hypersensitivity and was interpreted as a non-allergic adverse reaction, while angioedema was attributed to dental abscess-related infection in 3 additional patients. Seven patients did not complete the diagnostic work-up, and 9 had insufficient medical records. Therefore, 61 children with a completed diagnostic evaluation and adequate documentation were included in the final analysis (28 females [45.9%], 33 males [54.1%]).

The median age at referral was 9 years (IQR 6.1–12.9). Concomitant allergic diseases were common in the overall cohort, most frequently asthma (17, 27.9%) and drug allergy (14, 22.9%), followed by food allergy (6, 9.8%) and urticaria (5, 8.2%); atopic dermatitis and allergic rhinitis were each recorded in 1 patient (1.6%). A family history of atopy was reported in 21 patients (34.4%). Laboratory

findings showed a median absolute eosinophil count (AEC) of 180 cells/ μ L (IQR 107.5–350), eosinophil percentage of 2.4% (IQR 1.4–4.3), and total IgE of 102 IU/mL (IQR 35–207) (Table 1).

Most referrals (70.5%) were due to other allergic conditions rather than a suspected immediate hypersensitivity reaction to LA. A suspected allergy history to LA was observed in 29.5% of patients. Other frequent referral reasons included a history of previous allergy to non-LA drug (26.2%) and a history of asthma (26.2%), followed by food allergy (9.8%) and chronic urticaria (8.2%). In children referred with a history consistent with hypersensitivity reactions to LAs (n=18), urticaria and/or angioedema were

reported in 10 patients (55.6%), anaphylaxis in 6 patients (33.3%), vomiting in 1 patient (5.6%), and tachycardia in 1 patient (5.6%).

Testing profile, positivity, and confirmed LA hypersensitivity

Overall, 56 children were tested with a single LA, 4 children with two different LAs, and 1 child with three LAs. Across the 61 included patients, a total of 67 skin prick tests were performed using full-strength LAs. All tested agents were amide-type LAs. The distribution of tested agents was as follows: lidocaine (n=47; 70.1%), articaine (n=8; 11.9%), mepivacaine (n=5; 7.5%), prilocaine (n=4; 6.0%), and bupivacaine (n=3; 4.5%).

LA allergy was confirmed in 5 of 61 children (8.2%) based on positive skin testing, with positivity observed on skin prick testing in 4 patients and on intradermal testing in 2 patients. Two patients showed hypersensitivity to articaine (P4, P5), one patient to prilocaine (P1), one patient to lidocaine (P3), and one patient to both prilocaine and lidocaine (P2) (Table 2). In all patients with confirmed LA allergy, safe alternative agents were identified using negative results from both skin testing (SPT/IDT) and subcutaneous DPT. SPTs were negative in 57 patients. Among these, two patients had positive IDT results at the 1:100 dilution and therefore did not proceed to provocation. Based on these findings, the negative predictive value of the 1:1 SPT was calculated as 96.4%. In patients with negative SPT and IDT results, no positivity was detected in subcutaneous provocation tests.

An exploratory sensitivity analysis was also performed by restricting the cohort to children with a genuine index reaction compatible with immediate LA hypersensitivity (n=18). In this subgroup, confirmed LA allergy was detected in 4 patients. Among children without an LA reaction history, 1 of 43 patients (2.3%) had confirmed LA allergy; this patient had been referred because of hypersensitivity to a non-LA drug. No confirmed LA allergy was detected among children referred solely for asthma, food allergy, or chronic urticaria (0/27).

Comparative analysis and risk factors

Children with confirmed LA allergy (n=5) were compared with those in whom LA allergy was not confirmed (n=56). Age at referral and sex distribution were comparable between groups, and no meaningful differences were

Table 1. Demographic data and laboratory findings of all patients.

Data	Total (n=61)
Age (year), median (IQR)	9 (6.1-12.9)
Sex, n (%)	
Female	28 (45.9)
Male	33 (54.1)
Parental allergic disease, n (%)	21 (34.4)
Concomitant allergic disease, n (%)	
Asthma	17 (27.9)
Drug allergy	14 (22.9)
Food allergy	6 (9.8)
Urticaria	5 (8.2)
Atopic dermatitis	1 (1.6)
Allergic rhinitis	1 (1.6)
Referral reason, n (%)	
Allergy to LA	18 (29.5)
Allergy to non-LA drug	16 (26.2)
History of Asthma	16 (26.2)
Food allergy	6 (9.8)
Chronic urticaria	5 (8.2)
Allergic reaction to LA, (n=18) (%)	
Urticaria and/or angioedema	10/18 (55.6)
Anaphylaxis	6/18 (33.3)
Vomiting	1/18 (5.6)
Tachycardia	1/18 (5.6)
Confirmed allergic reaction, n (%)	5 (8.2)
AEC (cells/ μ L), median (IQR)	180 (107.5-350)
Eosinophil (%), median (IQR)	2.4 (1.4-4.3)
Total IgE (IU/mL), median (IQR)	102 (35-207)

AEC, absolute eosinophil count; IQR, interquartile range; IgE, immunoglobulin E; LA, local anesthetic.

Table 2. Clinical characteristics and test results of the patients with allergic reactions to LA.

Patient No	Sex	Referral Clinical Finding	Testing LAs	SPT 1:1	IDT 1:100	DPT 1:1	Safe alternative LA
P1	M	Allergic reaction to non-LA drug	Prilocaine	+	N/A	N/A	Mepivacaine
			Mepivacaine	-	-	-	
P2	F	Anaphylaxis with LA (Lidocaine)	Lidocaine	-	+	N/A	Bupivacaine
			Prilocaine	+	N/A	N/A	
			Bupivacaine	-	-	-	
P3	F	Anaphylaxis with LA (Lidocaine)	Lidocaine	+	N/A	N/A	Prilocaine
			Prilocaine	-	-	-	
P4	M	Anaphylaxis with LA (Articaine)	Articaine	+	N/A	N/A	Prilocaine
			Prilocaine	-	-	-	
P5	F	Urticaria with LA (Unknown LA)	Articaine	-	+	N/A	Lidocaine
			Lidocaine	-	-	-	

P= Patient, M= Male, F= Female, LA= Local anesthetic, SPT= Skin prick test, IDT= Intradermal test, DPT= Drug provocation test, N/A= Not applicable.

Table 3. Comparison of clinical characteristics between children with and without confirmed LA allergy.

Data	LA allergy (+) (n=5)	LA allergy (-) (n=56)	p
Age (year), median (IQR)	7.8 (6.8-12.5)	9.5 (5.9-12.9)	0.976
Sex, n (%)			0.421
Female	3 (60)	25 (44.6)	
Male	2 (40)	31 (55.4)	
Parental allergic disease, n (%)	1 (20)	20 (35.7)	0.433
Atopy history of the children, n (%)	2 (40)	42 (75)	0.127
Concomitant allergic disease, n (%)			
Asthma	0 (0)	17 (30.4)	0.309
Drug allergy	2 (40)	12 (21.4)	0.322
Food allergy	0 (0)	6 (10.7)	-
Urticaria	0 (0)	5 (8.9)	-
Atopic dermatitis	0 (0)	1 (1.8)	-
Allergic rhinitis	0 (0)	1 (1.8)	-
Referral reason, n (%)			0.126
Allergy to LA	4 (80)	14 (25)	
Allergy to non-LA drug	1 (20)	15 (26.7)	
Asthma	0 (0)	16 (28.6)	
Food allergy	0 (0)	6 (10.7)	
Chronic urticaria	0 (0)	5 (8.9)	
Previous allergic reaction suspect to LA, n (%)	4 (80)	14 (25)	0.024 OR: 12 (1.2-116)
Previous Anaphylaxis suspect to LA, n (%)	3 (60)	3 (5.4)	0.005 OR: 26.5 (3.1-223.9)
AEC (cells/ μ L), median (IQR)	245 (122.5-1140)	180 (100-350)	0.452
Eosinophil (%), median (IQR)	2.7 (1.6-9.2)	2.4 (1.4-4.3)	0.519
Total IgE (IU/mL), median (IQR)	151 (78-1021)	95.5 (29.7-202.7)	0.337

AEC, absolute eosinophil count; IQR, interquartile range; IgE, immunoglobulin E; LA, local anesthetic; OR, odds ratio.

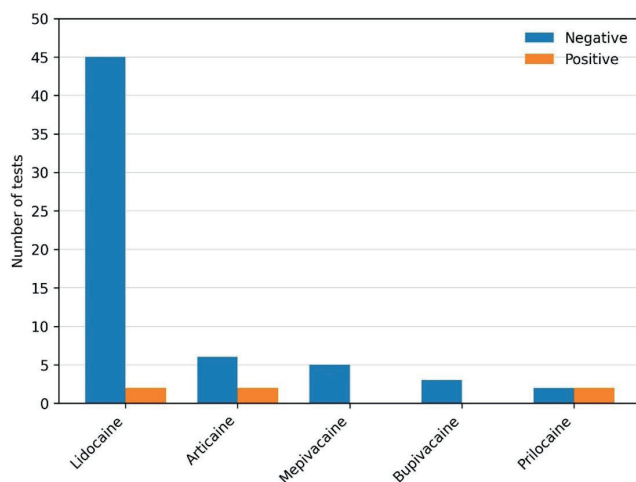


Figure 1. Local anesthetics that have been tested.

observed with respect to accompanying allergic diseases or a family history of atopy.

Four of the children with true LA allergy were referred with a history of immediate hypersensitivity to LA administration. The rate of confirmed LA allergy was significantly higher in patients presenting with a history of suspected local anesthetic allergy compared with other referral reasons ($p=0.024$; OR 12, 95% CI 1.2–116) (Table 3). A suspected history of anaphylaxis with LA was more frequently recorded among children with true LA allergy and was associated with true LA allergy in the comparative analysis ($p=0.005$; OR 26.5, 95% CI 3.1–223.9) (Table 3).

Of the 16 patients referred because of suspected hypersensitivity to non-LA drugs, one was diagnosed with confirmed LA allergy. Concomitant non-LA drug allergy was present in 2 of 5 (40.0%) children with confirmed LA allergy and in 12 of 56 (21.4%) children without confirmed LA allergy; however, this difference was not statistically significant ($p=0.322$). No test positivity or true allergy was detected in patients referred for other allergic conditions such as asthma, food allergy, or urticaria. Atopy did not differ significantly between the groups ($p=0.127$). When the drugs tested were examined, prilocaine or articaine was also associated with true LA allergy ($p=0.012$) (Figure 1).

DISCUSSION

In this 10-year retrospective study of children referred from dental clinics, confirmed immediate-type hypersensitivity

to amide LAs was identified in a small percentage (8.2%) of fully evaluated children. Most referrals were prompted by co-existing allergic conditions rather than a history of LA-related reactions. True LA allergy was confirmed in only 5 of 61 fully evaluated children, and a safe alternative LA was successfully identified in every case using a stepwise diagnostic approach combining skin testing and subcutaneous DPT. Four of the five confirmed cases had a prior history of suspected LA allergy, while one had been referred because of hypersensitivity to a non-LA drug. Notably, true LA allergy was almost never detected in children without a history of an LA-related reaction, identifying this group as low-risk. Comparative analysis showed that a history of anaphylaxis and testing involving articaine or prilocaine were significantly associated with confirmed allergy, although these findings should be interpreted with caution given the small number of positive cases.

Pediatric literature over the past decade consistently demonstrates that true immediate-type LA allergy is rare among referred children.⁵⁻⁸ Recent real-world series report confirmation rates ranging from 1.3% to 4.3%, with most index events attributed to non-allergic mechanisms such as anxiety, vasovagal reactions, or dental pathology rather than the anesthetic itself.⁵⁻⁸ In the largest pediatric cohort from our country ($n=231$), Ocak et al. confirmed true LA allergy in 4.3% of cases, with mucocutaneous symptoms predominating.⁸ Similar diagnostic yields were reported by Aslan et al., Caliskan et al., and Selmanoglu et al.⁵⁻⁷ International data further support this observation; Kvisselgaard et al. found no IgE-mediated cases among 162 Danish patients, while Hascoët et al. estimated the incidence of anaphylaxis to LAs in dental practice at $<0.01\%$.^{11,12} Our confirmation rate of 8.2% is slightly higher than most pediatric series. This difference likely reflects our selected population: we included only children with completed diagnostic work-up and adequate documentation, and all referrals originated from a tertiary dental service managing more complex cases.⁵⁻⁸

The low rate of confirmed LA allergy in the literature is largely explained by frequent misattribution of symptoms to the LA when other causes—such as non-LA drugs, disinfectants, latex, or procedural factors—are responsible.^{13,14} Studies evaluating LA allergy have reported that non-LA causes constitute 1.7–32% of all suspected reactions, depending on the procedure.¹⁵⁻¹⁷ Vasovagal reactions, hyperventilation, anxiety attacks, or toxicity symptoms can mimic allergy,

but skin symptoms are usually absent.^{16,17} Despite the low rate of confirmed allergy, children with a suggestive history of immediate reaction to LA warrant thorough evaluation, as recommended by EAACI/ENDA guidelines, particularly when future exposure is anticipated.¹⁸

Clinical history alone cannot reliably differentiate true hypersensitivity from non-allergic reactions; therefore, objective testing is essential. SPT followed by IDT and DPT remain the cornerstone of diagnosis, whereas specific IgE assays and basophil activation tests are not widely used for LA reactions.¹⁷⁻¹⁹ Current literature recommends performing skin tests with preservative-free solutions and without vasoconstrictors such as epinephrine.¹⁵ Various IDT dilution protocols have been described.^{5-8,20} In our practice, IDT was performed at a 1:100 dilution using preservative- and vasoconstrictor-free preparations to minimize irritant false-positive results, and children with positive IDT were not challenged with the same agent.^{5-8,20} This cautious approach, together with the tertiary nature of our referrals, may partly explain our slightly higher confirmation rate compared with studies using 1:10 IDT dilutions.^{6,9,18-21} Subcutaneous DPT, performed in two graded steps at full strength, enabled identification of safe alternatives in all confirmed cases.²²⁻²⁷

The negative predictive value of undiluted SPT in our high-risk pediatric cohort was 96.4%, and no positive DPT results were observed after negative skin tests, consistent with previous reports.^{6,22,24,28} An important practical outcome of our study was the successful identification of a tolerated alternative LA in every child with confirmed allergy. This finding aligns with previous reports demonstrating that cross-reactivity among amide LAs is neither universal nor predictable.^{20,29} Although articaine and prilocaine showed higher positivity rates in our cohort, individualized testing allowed safe alternatives (most frequently mepivacaine) to be found in all cases, in line with EAACI/ENDA recommendations.¹⁸

Consistent with recent pediatric evidence, our findings indicate that the strongest predictors of true LA allergy are a convincing history of immediate reaction—particularly anaphylaxis—rather than background atopy or unrelated allergic comorbidities.^{6,8,11,30,31} Age, sex, family history of atopy, and co-existing allergic diseases did not differ between allergic and non-allergic groups. In contrast, history of anaphylaxis to LA and testing with articaine or prilocaine emerged as key risk signals. These observations parallel the findings of Ocak et al.⁸ The role of hypersensitivity to other drugs is less clear. Some adult data suggest that multiple

drug allergies or concomitant drug allergies may be more common in patients with positive LA test results.²⁰ Suleyman et al. found no significant difference between patients with LA allergy and the control group in terms of confirmed drug allergy to NSAIDs or β -lactams.³⁰ A study by Kemoklidze et al., involving 450 pediatric and adult patients, found that drug allergy co-occurring with food allergy was associated with an increased risk of LA allergy.³² Importantly, suspected hypersensitivity to a non-LA drug as a referral indication should be distinguished from concomitant non-LA drug allergy as a comorbidity. In our cohort, concomitant drug allergy was numerically more frequent among children with confirmed LA allergy, although this difference was not statistically significant. Previous studies have suggested a possible association between concomitant drug allergies and LA hypersensitivity; however, the available evidence remains inconsistent, and larger studies are needed to clarify whether concomitant drug allergy represents an independent risk factor. Importantly, our data should not be interpreted as supporting routine pre-procedural LA skin testing in children with atopy or allergic comorbidities alone. In our cohort, these low-risk referrals reflected institutional and referring-physician practice during the study period. The absence of confirmed LA allergy in this subgroup supports counseling dentists and families that unrelated allergic comorbidities alone are not an indication for routine LA testing. In summary, this study confirms that true LA allergy is infrequent but not negligible in children referred from dentistry. A history of immediate reaction, especially anaphylaxis, identifies a high-yield subgroup that benefits from formal allergologic evaluation, whereas routine testing appears unnecessary in low-risk children referred for other allergic conditions. These results support the implementation of risk-stratified diagnostic algorithms to optimize resource use while ensuring patient safety.

This study has several limitations. Its retrospective, single-center design and tertiary dental referral setting may limit generalizability and introduce selection bias. The number of confirmed cases was small, resulting in wide confidence intervals and precluding multivariable analysis. Some records lacked detailed information on index symptoms or subsequent dental exposures, and the diagnostic approach evolved within institutional practice over the 10-year period. In addition, some children without a previous LA reaction underwent IDT because of pre-procedural referral requests; although testing was performed with diluted, vasoconstrictor-free preparations under direct supervision, the theoretical risk of iatrogenic sensitization cannot be

completely excluded. These limitations further support cautious interpretation and a risk-stratified approach.

In conclusion, although immediate-type allergy to local anesthetics is rare in children, it can occur and may involve cross-reactivity between agents. Structured diagnostic testing combining skin tests and drug provocation is essential for accurate confirmation or exclusion of hypersensitivity and for identifying safe alternatives. Many evaluations, however, are performed unnecessarily in low-risk patients. A risk-based approach—prioritizing children with a history of immediate reaction to LA, particularly anaphylaxis—should guide clinical decision-making. The development of standardized, risk-stratified diagnostic pathways has the potential to reduce unnecessary testing, decrease parental anxiety, and facilitate timely dental care without compromising safety.

Author contributions

Conception and design: F.Ç.Ç., Ö.S., D.C.; Data acquisition: F.Ç.Ç., S.G., Ö.S.; Data analysis: F.Ç.Ç., S.G., C.Ş.K., Ö.S.; Data interpretation: F.Ç.Ç., S.G., C.Ş.K., Ö.S.; Drafting of the manuscript: F.Ç.Ç., Ö.S., D.C. 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 Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital Institutional Local Ethics Committee (Date: 12 Mar 2026, Decision/Protocol No: GOA-319). 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.

Funding

The authors declare that this study received no funding.

Generative AI statement

The authors declare that during the preparation of this study, the following AI-assisted technology was used: ChatGPT (OpenAI) on Not specified. Extent of Use: English language editing, including improvements in grammar and fluency. 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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Genotype–phenotype correlations and functional outcomes assessed by the North Star Ambulatory Assessment in ambulatory children with dystrophinopathy

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Cite this article as: Yavuz M, Bingöl İ, Kılıç RK, Öztürk N. Genotype–phenotype correlations and functional outcomes assessed by the North Star Ambulatory Assessment in ambulatory children with dystrophinopathy. Trends in Pediatrics 2026;7(3):210-218.

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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Received: 05.06.2026 Accepted: 24.07.2026

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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.

Funding

The authors declare that this study received no funding.

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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First-year efficacy of 3.75 mg versus 11.25 mg depot leuprolide acetate and predictors of biochemical non-suppression in girls with central precocious puberty

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Cite this article as: Helvacioğlu D, Keleştemur E, Kurt İ, Kahveci A, Gürpınar Tosun B, Bereket A. First-year efficacy of 3.75 mg versus 11.25 mg depot leuprolide acetate and predictors of biochemical non-suppression in girls with central precocious puberty. Trends in Pediatrics 2026;7(3):219-228.

ABSTRACT

Objective: To compare first-year biochemical and auxological outcomes of monthly 3.75 mg and 3-monthly 11.25 mg depot leuprolide acetate (dLA) treatment in girls with central precocious puberty (CPP) and to evaluate factors associated with biochemical non-suppression and first-year growth velocity.

Methods: This single-center prospective quasi-randomized comparative study included 87 girls with CPP treated with dLA for 12 months. Patients received either monthly 3.75 mg (n=46) or 3-monthly 11.25 mg dLA treatment (n=41). Auxological parameters, gonadotropin-releasing hormone (GnRH)-stimulated luteinizing hormone (LH) responses, and LH levels measured 40 minutes after dLA injection (40-minute post-dLA LH) were evaluated. Biochemical non-suppression was defined as a year-one 40-minute post-dLA LH level >3.07 IU/L. Logistic regression was used to identify predictors of biochemical non-suppression, and linear regression was performed to determine factors associated with first-year annual growth velocity.

Results: One year after initiation of treatment, there was no difference in annual growth velocity (5.73 ± 1.01 vs 5.81 ± 0.92 cm/year, $p=0.956$), bone age, Δ bone age, and other auxological and pelvic ultrasonographic parameters between treatment groups. Year-one biochemical suppression was achieved in 33/46 patients (71.7%) in the 3.75 mg group and 33/41 patients (80.5%) in the 11.25 mg group, with no significant between-group difference ($p=0.450$). Biochemical non-suppression was associated with higher annual growth velocity and higher pretreatment GnRH-stimulated peak LH; in logistic regression analysis, pretreatment GnRH-stimulated peak LH was the only independent predictor of biochemical non-suppression (adjusted OR 1.04, 95% CI 1.01–1.07, $p=0.012$). First-year annual growth velocity correlated with 6-month GnRH-stimulated peak LH ($r=0.320$, $p=0.003$), 6-month 40-minute post-dLA LH ($r=0.339$, $p=0.001$), year-one basal LH ($r=0.314$, $p=0.003$), and year-one 40-minute post-dLA LH ($r=0.517$, $p<0.001$). In multivariable analysis, year-one 40-minute post-dLA LH remained independently associated with annual growth velocity ($B=0.39$, 95% CI 0.26–0.52, $p<0.001$), whereas other parameters did not.

Conclusions: Monthly 3.75 mg and 3-monthly 11.25 mg depot leuprolide acetate provided comparable first-year auxological outcomes. Higher pretreatment GnRH-stimulated peak LH predicted biochemical non-suppression, whereas year-one 40-minute post-dLA LH level was independently associated with first-year growth velocity, suggesting that it may represent a more robust parameter for monitoring residual pubertal activity during treatment.

Keywords: puberty, precocious, gonadotropin-releasing hormone, luteinizing hormone, treatment outcome, leuprolide



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Received: 24.07.2026 Accepted: 14.08.2026

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INTRODUCTION

Central precocious puberty (CPP) is characterized by premature activation of the hypothalamic–pituitary–gonadal axis, resulting in progressive pubertal development before 8 years of age in girls. Accelerated growth and advanced skeletal maturation may compromise adult height if treatment is not initiated in appropriate patients. The main goals of therapy are to suppress pubertal progression, normalize growth velocity, slow bone age advancement, and preserve height potential.¹ Gonadotropin-releasing hormone (GnRH) agonists (GnRHa) are the standard treatment for CPP. Depot leuprolide acetate (dLA) is available as monthly 3.75 mg and 3-monthly 11.25 mg formulations, and previous studies have reported broadly comparable biochemical and auxological efficacy between these regimens. The 3-monthly formulation may also reduce injection burden during long-term treatment.^{2–5}

Monitoring treatment adequacy during GnRHa therapy remains challenging. Although basal or random luteinizing hormone (LH) measurements are practical, elevated pre-injection or random LH levels may occur despite adequate clinical suppression, limiting their reliability as isolated markers of treatment adequacy.^{6–8} GnRH-stimulated LH testing has traditionally been used to assess biochemical suppression during treatment; however, it requires venous access and repeated sampling, limiting its practicality for routine follow-up. Post-dLA LH sampling has therefore been proposed as a practical dynamic alternative that assesses the acute gonadotropin response to the therapeutic agonist.^{9,10}

Building on our previously identified 40-minute post-dLA LH threshold for monitoring pubertal suppression¹¹, this study compared first-year biochemical and auxological outcomes of monthly 3.75 mg and 3-monthly 11.25 mg dLA treatment in girls with CPP. We also evaluated factors associated with year-one biochemical non-suppression and first-year annual growth velocity, with particular emphasis on the year-one 40-minute post-dLA LH response.

MATERIALS AND METHODS

Study design and patients

This single-center prospective quasi-randomized comparative study was conducted between 2022 and 2025 in a tertiary pediatric endocrinology clinic. Patients were allocated in a 1:1 ratio to receive either monthly dLA 3.75 mg or 3-monthly dLA 11.25 mg according to a

predefined alternating sequence. Treatment allocation was implemented by the principal investigator without allocation concealment.

No a priori sample size calculation was performed. All eligible patients who met the inclusion criteria and provided consent during the predefined study period were enrolled prospectively. A total of 91 patients were allocated to the two treatment groups evaluated in the present study: 46 patients to the monthly 3.75 mg group and 45 patients to the 3-monthly 11.25 mg group. Of the four patients excluded from the 3-monthly 11.25 mg group, one moved to another city, and three did not attend scheduled follow-up visits regularly. We calculated scheduled dates for dLA administration and hormonal testing in advance, and the same experienced pediatric endocrinology nurse performed all injections, blood sampling, and stimulation tests. No procurement or administration problems were documented. Therefore, the final analysis included 87 girls who completed 12 months of follow-up and had complete first-year auxological and hormonal data: 46 patients in the monthly 3.75 mg group and 41 patients in the 3-monthly 11.25 mg group. The analyses were therefore performed according to the per-protocol principle. Patient flow is shown in Figure 1.

CPP was diagnosed according to established criteria: onset of breast development before 8 years of age, accelerated growth velocity and/or advanced bone age, and a pubertal LH response to GnRH stimulation (peak LH ≥ 5 IU/L).¹ Exclusion criteria were peripheral precocious puberty, organic CPP, any chronic systemic disease affecting growth, and incomplete 12-month follow-up data.

Auxological and pubertal assessments

Clinical follow-up visits were scheduled at 3-month intervals throughout the first treatment year. At baseline and at each follow-up visit, height, weight, and body mass index (BMI) were measured and expressed as standard deviation scores (SDS) according to national reference data.¹² Pubertal stage was assessed according to Tanner criteria.¹³ Bone age was evaluated from left hand and wrist radiographs using the Greulich and Pyle method¹⁴, and the bone age/chronological age (BA/CA) ratio and bone age advancement (BA–CA) were calculated. Change in bone age (Δ BA) was calculated as year-one bone age minus baseline bone age. Annualized growth velocity during the first 6 months was calculated from baseline and 6-month height measurements, whereas first-year annual growth velocity was calculated from baseline and 12-month

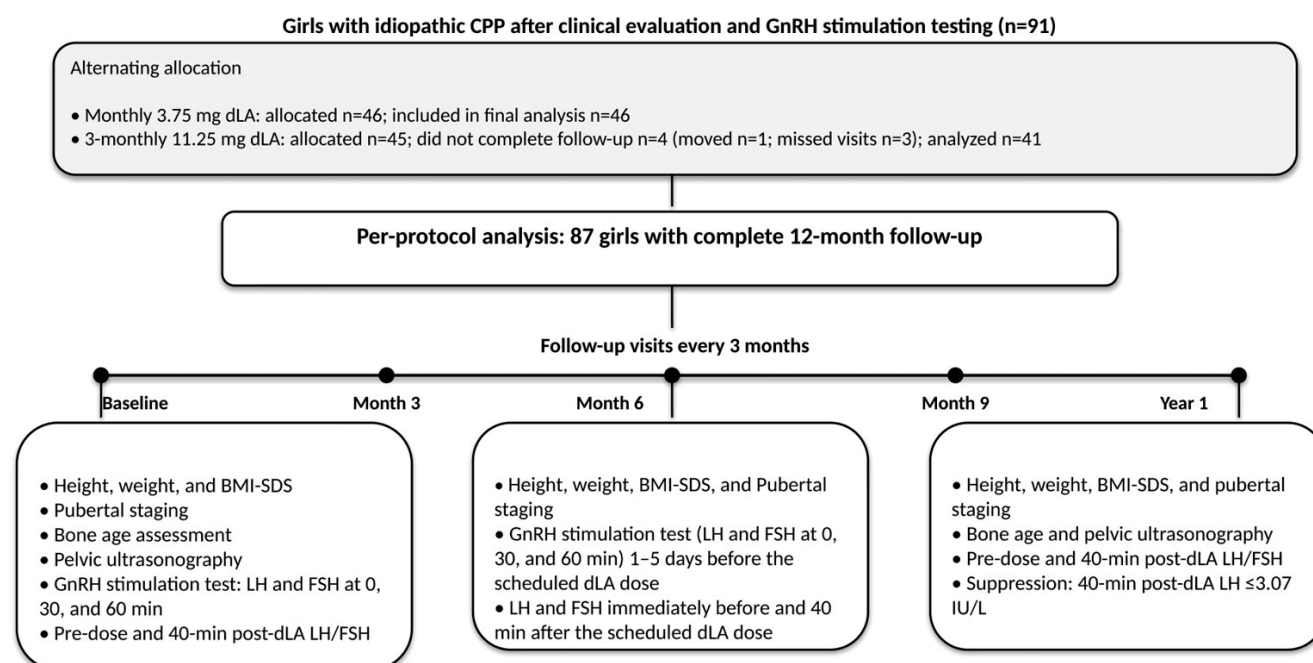


Figure 1. Study flow and follow-up protocol.

CPP, central precocious puberty; dLA, depot leuprolide acetate; LH, luteinizing hormone; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; BMI-SDS, body mass index standard deviation score.

height measurements. Standing height was measured using a calibrated Harpenden stadiometer (Holtain Ltd., Crymych, UK) and recorded to the nearest 0.1 cm. Pelvic ultrasonography was performed at baseline and at year 1 as part of routine clinical assessment.

Hormonal assessments

All blood samples were collected in the morning between 08:00 and 08:30. At baseline, the GnRH stimulation test was performed after insertion of an intravenous cannula. Blood samples were obtained for basal LH and follicle-stimulating hormone (FSH), followed by intravenous administration of gonadorelin acetate at 2.5 µg/kg, up to a maximum dose of 100 µg. Blood samples for LH and FSH were then collected at 30 and 60 minutes, and peak LH was recorded. At treatment initiation, serum LH and FSH were measured immediately before the first dLA injection and 40 minutes after injection. At month 6, a standard GnRH stimulation test was performed 1–5 days before the scheduled dLA dose. On the day of the scheduled dLA injection, LH and FSH were again measured immediately before and 40 minutes after dLA administration. The same pre-injection and 40-minute post-dLA sampling protocol was repeated at the year-one dose visit. All hormonal samples were

analyzed on the day of collection and were not stored for subsequent batch analysis. Serum LH, FSH, and estradiol were measured using a chemiluminescent immunoassay system (Beckman Coulter Diagnostics, CA, USA) according to the manufacturer's protocol. The minimum detectable concentration was 0.07 IU/L for both LH and FSH.

Definition of biochemical suppression

At 6 months, biochemical suppression was assessed using the standard GnRH stimulation test and was defined as a GnRH-stimulated peak LH level <3 IU/L, consistent with previous CPP treatment studies.¹⁵⁻¹⁷ Six-month GnRH-stimulated peak LH and 6-month 40-minute post-dLA LH levels were also evaluated as on-treatment hormonal parameters. As a repeat GnRH stimulation test was not included in the year-one protocol, year-one biochemical suppression was assessed using the previously validated 40-minute post-dLA LH threshold. Year-one biochemical suppression was defined as a year-one 40-minute post-dLA LH level of ≤ 3.07 IU/L, and biochemical non-suppression was defined as a level >3.07 IU/L. This threshold was derived from our group's previously published receiver operating characteristic (ROC) analysis in a prospective cohort of girls with CPP, in which a 40-minute post-dLA LH

level of 3.07 IU/L optimally discriminated between GnRH-stimulated suppressed and non-suppressed patients, with an area under the curve (AUC) of 0.83, sensitivity of 80.8%, and specificity of 75.5%.¹¹

Statistical analysis

The normality of continuous variables was assessed using the Shapiro–Wilk test. Continuous variables are presented as mean $\pm$ standard deviation or median (25th–75th percentiles), as appropriate. Categorical variables are presented as number and percentage. Between-group comparisons were performed using the independent-samples t-test or Mann–Whitney U test for continuous variables and the chi-square or Fisher’s exact test for categorical variables. We calculated the magnitude of decline in growth velocity for each patient by subtracting the first-year annual growth velocity from the 6-month annualized growth velocity and compared it between treatment groups using an independent-samples t-test. We evaluated within-group changes in growth velocity between 6 months and year one using a paired-samples t-test. We used Pearson or Spearman correlation analyses to assess associations between LH parameters and annual growth velocity. A multivariable logistic regression model including treatment regimen and pretreatment GnRH-stimulated peak LH was used to evaluate factors associated with year-one biochemical non-suppression; adjusted odds ratios (OR) with 95% confidence intervals (CI) are reported. A multivariable linear regression model including treatment regimen, age at treatment initiation, pretreatment GnRH-stimulated peak LH, and year-one 40-minute post-dLA LH was used to evaluate factors associated with annual growth velocity. Variables were selected based on clinical relevance and their associations with the outcomes, while correlated on-treatment LH measurements were not included simultaneously. All analyses were performed using GraphPad Prism version 10.0 (GraphPad Software, Boston, MA, USA). A two-sided p-value < 0.05 was considered statistically significant.

Ethical approval

The study was approved by the institutional Ethics Committee, approval number 09.2022.486, and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from parents or legal

guardians of all participants, and patient assent was obtained where appropriate.

RESULTS

At treatment initiation, 87 girls with CPP were included in the baseline comparison: 46 patients received monthly dLA 3.75 mg and 41 patients received 3-monthly dLA 11.25 mg. Chronological age was higher in the monthly 3.75 mg group than in the 3-monthly 11.25 mg group (8.27 ± 0.60 vs 7.86 ± 0.94 years, $p=0.014$), whereas bone age and the BA/CA ratio did not differ significantly between groups ($p=0.065$ and $p=0.961$, respectively).

Baseline anthropometric parameters, basal and GnRH-stimulated gonadotropin responses, LH/FSH ratios, and pelvic ultrasonographic findings were comparable between groups, except for uterine length and pre-stimulation basal FSH during the pretreatment GnRH test, both of which were higher in the monthly 3.75 mg group (both $p=0.046$) (Table 1). At year one, annual growth velocity was comparable between the monthly 3.75 mg and 3-monthly 11.25 mg groups (5.73 ± 1.01 vs 5.81 ± 0.92 cm/year, $p=0.956$). Other first-year auxological and pelvic ultrasonographic outcomes, including height SDS, BMI-SDS, bone age, BA/CA ratio, BA advancement, uterine measurements, and ovarian volumes, were also similar between treatment regimens. The year-one 40-minute post-dLA LH level did not differ significantly between groups, whereas basal LH, basal FSH, basal LH/FSH ratio, 40-minute FSH, and 40-minute LH/FSH ratio differed between groups (Table 2). In the 3-monthly 11.25 mg group, growth velocity decreased from 8.03 ± 2.12 to 5.81 ± 0.92 cm/year, and in the monthly 3.75 mg group, it decreased from 8.09 ± 2.08 to 5.73 ± 1.01 cm/year (both $p<0.001$). The magnitude of decline in growth velocity was 2.36 ± 2.14 cm/year in the monthly 3.75 mg group and 2.22 ± 2.24 cm/year in the 3-monthly 11.25 mg group, with no significant between-group difference (mean difference, 0.14 cm/year; 95% CI, -0.80 to 1.08 ; $p=0.767$). At the year-one dose visit, using the predefined 40-minute post-dLA LH threshold, biochemical suppression was achieved in 33/46 patients (71.7%) in the monthly 3.75 mg group and 33/41 patients (80.5%) in the 3-monthly 11.25 mg group, with no significant between-group difference ($p=0.450$). Patients with year-one biochemical non-suppression and those with suppression were comparable in age at treatment initiation,

Table 1. Pretreatment clinical, auxological, ultrasonographic, and hormonal characteristics by treatment regimen.

	dLA 3.75 mg (n=46)	dLA 11.25 mg (n=41)	p
Age, years	8.27 ± 0.60	7.86 ± 0.94	0.014
Bone age, years	9.80 ± 1.15	9.71 ± 1.32	0.065
BA/CA ratio	1.18 ± 0.12	1.18 ± 0.14	0.961
Height SDS	0.87 ± 1.11	1.06 ± 1.05	0.394
Weight SDS	1.03 ± 1.19	1.05 ± 0.98	0.929
BMI SDS	0.83 ± 1.17	0.69 ± 1.03	0.569
Basal LH, IU/L	0.84 (0.43–2.00)	0.62 (0.47–0.88)	0.225
Basal FSH, IU/L	3.40 (1.65–4.70)	2.73 (1.95–3.94)	0.401
Uterine length, mm	35.00 (33.00–41.00)	32.00 (29.00–37.00)	0.046
Uterine volume, cm ³	2.80 (1.70–4.70)	2.62 (1.88–4.25)	0.397
Left ovarian volume, cm ³	2.30 (1.70–3.60)	1.60 (1.00–3.25)	0.410
Right ovarian volume, cm ³	2.20 (1.40–3.10)	1.65 (0.90–2.60)	0.065
Basal LH during GnRH test, IU/L	0.94 (0.36–1.62)	0.68 (0.49–1.46)	0.781
Peak LH during pretreatment GnRH test, IU/L	10.80 (6.64–15.75)	10.10 (6.81–21.35)	0.879
Basal FSH during GnRH test, IU/L	3.90 (2.43–5.48)	2.94 (2.05–4.10)	0.046
Peak FSH during pretreatment GnRH test, IU/L	12.73 ± 4.86	13.42 ± 3.70	0.212
Peak LH/FSH ratio during GnRH test	0.91 (0.70–1.54)	0.85 (0.50–1.61)	0.745

Values are presented as mean±SD or median (25th–75th percentiles), according to data distribution. BA, bone age; BMI, body mass index; CA, chronological age; dLA, depot leuprolide acetate; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; SD, standard deviation; SDS, standard deviation score.

Table 2. Year-one auxological, ultrasonographic, and hormonal outcomes by treatment regimen.

	dLA 3.75 mg (n=46)	dLA 11.25 mg (n=41)	p
Height SDS	1.06 ± 1.05	1.24 ± 1.04	0.411
Weight SDS	1.18 ± 1.11	1.20 ± 0.96	0.920
BMI-SDS	0.91 ± 1.07	0.80 ± 1.11	0.643
Bone age (BA), years	11.04 ± 0.95	10.66 ± 1.18	0.094
BA/CA ratio	1.21 ± 0.10	1.22 ± 0.14	0.640
BA advancement (BA–CA), years	1.23 ± 0.69	1.38 ± 0.61	0.191
Uterine length, mm	32.54 ± 7.52	28.96 ± 5.53	0.425
Uterine volume, cm ³	2.45 ± 2.74	1.90 ± 1.80	0.240
Left ovarian volume, cm ³	1.89 ± 1.32	1.74 ± 1.11	0.663
Right ovarian volume, cm ³	1.95 ± 1.16	1.63 ± 1.11	0.201
Annual growth velocity, cm/year	5.73 ± 1.01	5.81 ± 0.92	0.956
Hormonal outcomes at year one			
Basal LH, IU/L	0.68 ± 0.34	0.58 ± 0.40	0.020
Basal FSH, IU/L	1.42 ± 0.67	1.90 ± 1.00	0.005
Basal LH/FSH ratio	0.56 ± 0.32	0.36 ± 0.28	<0.001
40-minute post-dLA LH, IU/L	2.52 ± 1.22	2.63 ± 1.51	0.782
40-minute post-dLA FSH, IU/L	3.31 ± 1.59	4.63 ± 2.21	0.001
40-minute post-dLA LH/FSH ratio	0.87 ± 0.41	0.63 ± 0.34	0.003

Values are presented as mean±SD. BA, bone age; BMI-SDS, body mass index standard deviation score; CA, chronological age; dLA, depot leuprolide acetate; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SD, standard deviation; SDS, standard deviation score.

BA/CA ratio, and BMI-SDS (all $p>0.05$). Pretreatment GnRH-stimulated peak LH was significantly higher in the non-suppressed group than in the suppressed group (15.60 [9.27–25.00] vs 8.91 [6.54–14.84] IU/L, $p=0.004$). In addition, both 6-month GnRH-stimulated peak LH and 6-month 40-minute post-dLA LH were higher in patients with year-one biochemical non-suppression than in those with suppression (2.82 [2.09–4.04] vs 1.63 [1.18–2.22] IU/L and 4.04 [3.11–5.27] vs 2.33 [1.85–2.83] IU/L, respectively; both $p<0.001$). Patients with biochemical non-suppression also had higher annual growth velocity than those with suppression (6.45 [6.00–6.95] vs 5.50 [4.94–5.90] cm/year, $p<0.001$), whereas Δ BA was similar between groups ($p=0.510$) (Table 3). A multivariable logistic regression model including treatment regimen and pretreatment

GnRH-stimulated peak LH was constructed. Pretreatment GnRH-stimulated peak LH remained independently associated with biochemical non-suppression (adjusted OR 1.04, 95% CI 1.01–1.07, $p=0.012$), whereas treatment regimen was not independently associated with this outcome (Table 4). First-year annual growth velocity was positively correlated with both 6-month GnRH-stimulated peak LH ($r=0.320$, $p=0.003$) and 6-month 40-minute post-dLA LH ($r=0.339$, $p=0.001$). Year-one basal LH was also positively correlated with annual growth velocity ($r=0.314$, $p=0.003$); however, the strongest correlation was observed with year-one 40-minute post-dLA LH ($r=0.517$, $p<0.001$) (Table 5). In the multivariable linear regression model, year-one 40-minute post-dLA LH remained independently associated with annual growth velocity after adjustment

Table 3. Clinical and hormonal parameters according to year-one biochemical suppression status.

	Non-suppressed (n=21)	Suppressed (n=66)	p
Age, years	8.35 (7.62–8.83)	8.09 (7.59–8.63)	0.380
BA/CA ratio	1.19 (1.08–1.32)	1.17 (1.09–1.26)	0.510
BMI-SDS	0.91 (–0.24–1.73)	0.66 (0.23–1.52)	0.580
Pretreatment GnRH-stimulated peak LH, IU/L	15.60 (9.27–25.00)	8.91 (6.54–14.84)	0.004
6-month GnRH-stimulated peak LH, IU/L	2.82 (2.09–4.04)	1.63 (1.18–2.22)	<0.001
6-month 40-minute post-dLA LH, IU/L	4.04 (3.11–5.27)	2.33 (1.85–2.83)	<0.001
Δ BA, years	1.20 (1.00–1.90)	1.15 (0.97–2.00)	0.510
Annual GV, cm/year	6.45 (6.00–6.95)	5.50 (4.94–5.90)	<0.001

Values are presented as median (25th–75th percentiles). BA, bone age; BMI-SDS, body mass index standard deviation score; dLA, depot leuprolide acetate; GnRH, gonadotropin-releasing hormone; GV, growth velocity; LH, luteinizing hormone. Biochemical non-suppression was defined as a year-one 40-minute post-dLA LH level >3.07 IU/L, and suppression as ≤ 3.07 IU/L.

Table 4. Baseline predictors of year-one biochemical non-suppression.

	Adjusted OR (95% CI)	p
Treatment regimen, 11.25 mg vs 3.75 mg	0.57 (0.20–1.65)	0.298
Pretreatment GnRH-stimulated peak LH, IU/L	1.04 (1.01–1.07)	0.012

Year-one biochemical non-suppression was defined as a year-one 40-minute post-dLA LH level >3.07 IU/L. Model fit: omnibus likelihood-ratio $\chi^2(2)=8.32$, $p=0.016$; Nagelkerke $R^2=0.136$; Hosmer–Lemeshow $\chi^2(8)=1.17$, $p=0.997$. The maximum variance inflation factor was 1.00. CI, confidence interval; dLA, depot leuprolide acetate; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; OR, odds ratio.

Table 5. Correlations between LH parameters and annual growth velocity during the first year of treatment.

Variable	r	p
Pretreatment GnRH-stimulated peak LH	0.067	0.530
6-month GnRH-stimulated peak LH	0.320	0.003
6-month 40-minute post-dLA LH	0.339	0.001
Year-one basal LH	0.314	0.003
Year-one 40-minute post-dLA LH	0.517	<0.001

dLA, depot leuprolide acetate; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone.

Table 6. Factors associated with first-year annual growth velocity.

Variable	B coefficient	95% CI	p
Treatment regimen	−0.06	−0.40 to 0.29	0.745
Age at treatment initiation	−0.14	−0.36 to 0.09	0.231
Pretreatment GnRH-stimulated peak LH	−0.01	−0.02 to 0.00	0.213
Year-one 40-minute post-dLA LH	0.39	0.26 to 0.52	<0.001

Model fit: $R^2=0.304$; adjusted $R^2=0.270$; $F(4,82)=8.95$, $p<0.001$. B coefficients are unstandardized regression coefficients. The maximum variance inflation factor was 1.31. B, unstandardized regression coefficient; CI, confidence interval; dLA, depot leuprolide acetate; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone.

for treatment regimen, age at treatment initiation, and pretreatment GnRH-stimulated peak LH ($B=0.39$, 95% CI 0.26–0.52, $p<0.001$) (Table 6).

DISCUSSION

In this prospective quasi-randomized comparative study of girls with CPP treated with depot leuprolide acetate for 12 months, monthly 3.75 mg and 3-monthly 11.25 mg regimens provided comparable first-year auxological outcomes, with a numerically higher biochemical suppression rate in the 3-monthly regimen. Annual growth velocity, height SDS, BMI-SDS, bone age parameters, and pelvic ultrasonographic findings were similar between groups, and growth velocity declined significantly from 6 months to year one in both regimens. These findings support effective first-year auxological control in the majority of patients with both formulations and are consistent with previous studies reporting comparable clinical efficacy of monthly and longer-acting leuprolide acetate regimens in CPP.³⁻⁵

Beyond regimen comparison, pretreatment GnRH-stimulated peak LH independently predicted year-one biochemical non-suppression in our cohort. Previous studies have evaluated basal, pre-injection, and stimulated LH measurements as biochemical monitoring tools during GnRHa treatment; however, isolated LH elevations do not always correspond to clinical pubertal progression or unfavorable auxological outcomes.^{6,18-20} Although the specific relationship between pretreatment GnRH-stimulated peak LH and subsequent biochemical non-suppression has not been extensively investigated, our finding suggests that greater hypothalamic–pituitary–gonadal axis activation before treatment may be associated with a higher likelihood of incomplete biochemical suppression during follow-up. This finding requires confirmation in independent cohorts. Previous studies have also demonstrated that a single post-leuprolide LH measurement may provide a practical assessment of biochemical suppression, primarily based on

its agreement with stimulated LH criteria.^{9,10} Nevertheless, biochemical LH measurements have not consistently correlated with clinical or growth-related outcomes and should therefore be interpreted together with clinical and auxological parameters.²¹ In the present study, year-one 40-minute post-dLA LH remained independently associated with concurrent annual growth velocity. To our knowledge, this specific association has not previously been reported.

Previous randomized and comparative studies have shown effective pubertal suppression with both monthly and 3-monthly leuprolide acetate formulations, although stimulated gonadotropin responses may differ according to regimen, sampling time, and suppression threshold.^{3-5,22} In a recent comparative study, 3-monthly leuprolide acetate was associated with greater hormonal suppression and lower growth velocity and bone age advancement; however, predicted adult height did not differ significantly between monthly and 3-monthly regimens.²² In our cohort, biochemical suppression rates and first-year auxological outcomes were comparable between regimens, despite slightly different hormonal responses. This supports the interpretation that hormonal differences should be evaluated together with growth velocity and skeletal maturation rather than as isolated endpoints.

Biochemical monitoring during GnRH agonist therapy remains challenging, because basal or random LH measurements are practical but may not reliably reflect clinically relevant residual gonadotropic activity during treatment.^{7,8,18} Although GnRH-stimulated LH levels are considered the gold standard for monitoring hormonal suppression during treatment, they require intravenous cannula insertion and repeated sampling, and are therefore cumbersome and invasive. We have recently demonstrated that 40-minute post-dLA LH levels show close agreement with GnRH-stimulated LH levels and can be used for both diagnostic assessment and treatment monitoring in girls with CPP.¹¹ The physiological rationale for post-dose LH monitoring is supported by pharmacodynamic studies

showing that LH rises shortly after depot leuprolide administration, creating a brief stimulatory window for post-injection assessment.²³ Previous studies also suggested that single post-leuprolide LH measurements may be useful for evaluating gonadotropin suppression during GnRHa therapy.^{9,10} Building on this concept, our previous prospective study identified a 40-minute post-dLA LH threshold of 3.07 IU/L for treatment monitoring using GnRH-stimulated suppression as the reference standard.¹¹ In the present study, year-one basal LH correlated with annual growth velocity, but this association was weaker than that observed for year-one 40-minute post-dLA LH and was no longer independent in multivariable analysis. By contrast, year-one 40-minute post-dLA LH remained independently associated with annual growth velocity. These findings suggest that the 40-minute post-dLA LH response is a better marker than basal LH for evaluating gonadotropic activity during dLA treatment.

Year-one biochemical non-suppression was not associated with treatment regimen, age at treatment initiation, BA/CA ratio, or baseline BMI-SDS. Patients with biochemical non-suppression had higher annual growth velocity than those with suppression, whereas bone-age advancement was comparable between groups. This dissociation may reflect the limited timeframe of first-year follow-up, as changes in growth velocity may precede detectable differences in skeletal maturation. Biochemical suppression is generally accompanied by regression or stabilization of clinical pubertal signs and reduced growth velocity; however, the relationship between the degree of LH suppression and the magnitude of the auxological response is not uniform across studies. In a cohort of 64 girls and seven boys treated with depot leuprolide, peak-stimulated and basal LH levels during treatment were not significantly associated with growth velocity, rate of skeletal maturation, or predicted adult stature²⁴, whereas a separate series of 30 girls reported a significant correlation between lower pre-injection LH levels and greater improvement in predicted adult height.²⁰ This discordance may reflect heterogeneity in cohort composition, assay methodology, and the timing of hormonal sampling relative to the injection interval. Regarding final adult height, long-term studies have primarily focused on clinical and auxological determinants rather than biochemical suppression status during the first treatment year.^{25,26} Consequently, direct evidence that the degree of first-year biochemical suppression predicts final adult height remains to be established. Thus, biochemical

non-suppression defined by a laboratory threshold should be interpreted together with growth velocity, skeletal maturation, and clinical pubertal progression rather than as an isolated indicator of treatment failure. In the present study, year-one 40-minute post-dLA LH was independently associated with concurrent annual growth velocity after multivariable adjustment, suggesting potential auxological relevance. Whether this association translates into differences in predicted or final adult height requires prospective long-term follow-up.

This study has some limitations. No a priori sample size calculation was performed, the sample size was modest (particularly for subgroup analyses), and follow-up was limited to the first treatment year. Therefore, the long-term implications of year-one biochemical non-suppression for predicted adult height and final adult height could not be determined. In addition, the 3.07 IU/L threshold was derived from our previous cohort and requires external validation across different populations, assays, and GnRHa protocols. Furthermore, pelvic ultrasonography was performed by multiple radiologists, and inter-observer reliability was not assessed; therefore, measurement variability between observers cannot be excluded. Finally, despite quasi-randomized allocation, chronological age differed significantly between the treatment groups at baseline, whereas bone age was comparable. This baseline imbalance may have introduced residual confounding when interpreting between-group outcomes. Nevertheless, the prospective design, predefined follow-up protocol, and combined assessment of biochemical and auxological outcomes strengthen the clinical relevance of the findings.

In conclusion, monthly 3.75 mg and 3-monthly 11.25 mg depot leuprolide acetate provided comparable first-year auxological outcomes in girls with CPP. Pretreatment GnRH-stimulated peak LH independently predicted year-one biochemical non-suppression, whereas year-one 40-minute post-dLA LH was independently associated with first-year annual growth velocity. These findings suggest that the 40-minute post-dLA LH response may serve as an adjunctive marker of residual gonadotropic activity when interpreted together with growth velocity and other clinical indicators.

Author contributions

Conception and design: D.H., A.B.; Data acquisition: D.H., E.K., I.K., A.K., B.G.T.; Data analysis: D.H., A.K., A.B.; Data

interpretation: D.H., A.K., A.B.; Drafting of the manuscript: D.H.; Critical revision of the manuscript: E.K., I.K., A.K., B.G.T., A.B. 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 Marmara University Ethics Committee (Date: 29.04.2022, Decision/Protocol No: 09.2022.486). 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.

Funding

The authors declare that this study received no funding.

Generative AI statement

The authors declare that no generative AI or AI-assisted technologies were used in the writing or preparation of this study.

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Methodological considerations in interpreting a synbiotic trial for pediatric functional abdominal pain

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Cite this article as: Prasath C H, Kumar PA. Methodological considerations in interpreting a synbiotic trial for pediatric functional abdominal pain. Trends in Pediatrics 2026;7(3):229-230.

We read with interest the randomized, double-blind, placebo-controlled trial by Arsoy et al. investigating the clinical and immunological effects of a synbiotic in children with functional abdominal pain not otherwise specified (FAPNOS).¹ Randomized controlled trials examining synbiotics in pediatric functional gastrointestinal disorders remain scarce, and the inclusion of a cytokine panel alongside symptom outcomes adds an immunological dimension largely absent from prior work in this age group. We wish to raise several methodological points that bear on the interpretation of the results.

The reporting of interleukin-13 (IL-13) requires closer examination. The authors identify the post-treatment rise in IL-13 within the synbiotic group ($p < 0.001$) as the most notable finding and use it as the primary basis for their conclusions. However, the between-group comparison of post-treatment IL-13 levels did not reach statistical significance ($p = 0.519$). In a randomized controlled trial, the between-group test is the correct inferential comparison. Within-group pre-post changes in a single arm do not establish a treatment-specific effect, particularly when the placebo group showed a directionally similar rise from 0.61 to 0.70 pg/mL. Conclusions built on within-group findings that lack between-group confirmation do not accurately reflect the trial's immunological evidence.²

The question of statistical power also deserves consideration. The rate of symptom reduction was numerically greater in the synbiotic group than in the placebo group (median 100% versus 80%), with a p-value of 0.054. In a trial of 80 participants with a condition characterized by considerable biological heterogeneity, a borderline result of this magnitude may reflect an underpowered sample rather than true equivalence between treatments. No post hoc power calculation was reported, and underpowering is not considered among the study's limitations. Declaring a negative outcome on the basis of a borderline p value, without addressing the possibility of a type II error, limits the conclusions that can be drawn from the symptom data.³

The placebo response rate warrants further discussion. Complete symptom resolution occurred in 43.9% of the placebo group, consistent with the high placebo response rates reported across functional gastrointestinal disorder trials.⁴ Moreover, patients in both groups received weekly telephone contact throughout the 8-week period. In functional pain disorders, structured clinical attention can independently reduce symptom burden through modulation of the gut-brain axis. The placebo condition in this trial therefore incorporated an active psychosocial element, which may have attenuated the detectable



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Received: 24.06.2026 Accepted: 29.07.2026

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between-group difference and warrants acknowledgment when interpreting the null symptom outcome.

Psychosocial factors represent a further dimension that was not addressed. The authors' introduction explicitly identifies psychosocial distress as a key driver of FAPNOS. Despite this, no psychological assessment was performed at baseline or follow-up: no anxiety screening, no quality-of-life measure, and no family functioning assessment. In a disorder where gut-brain dysregulation is a primary pathophysiological mechanism, the absence of psychological characterization at enrollment means that the two groups may have differed in an important and unmeasured dimension, limiting the ability to attribute observed symptom changes to the biological properties of the synbiotic.⁵

Finally, the mechanistic basis of the cytokine findings cannot be assessed without gut microbiome data. The rationale for synbiotic therapy in FAPNOS rests on the assumption that modifying microbial composition leads to downstream immune modulation. No stool microbiome profiling was performed, and the observed shifts in IL-13 and interferon- γ within the synbiotic group therefore cannot be connected to any microbiome-level change. The statistically significant rise in transforming growth factor- β in both the synbiotic and placebo groups ($p < 0.001$ in each arm) adds to this uncertainty. A shared immunological trajectory across both study arms points toward a nonspecific temporal effect, the nature of which microbiome data might have helped clarify.

This trial makes a useful contribution to a field where well-designed pediatric studies are few. The randomized design, double-blinding, and inclusion of a multicytokine panel reflect careful planning. Future studies in this area would be strengthened by prospective power calculations, validated symptom assessment instruments, standardized psychosocial evaluation at baseline, and stool microbiome profiling to support mechanistic interpretation of immunological findings. The authors' work provides a constructive reference point from which such studies can be designed.

Author contributions

Conception: P.A.K.; Design: P.A.K.; Data acquisition: H.P.C., P.A.K.; Data analysis: H.P.C., P.A.K.; Data interpretation: H.P.C., P.A.K.; Drafting of the manuscript: H.P.C. All authors reviewed the results, approved the final version of the manuscript, and agreed to be accountable for all aspects of this study.

Data availability statement

Data sharing is not applicable to this article as no new datasets were generated or analyzed during this study.

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.

Funding

The authors declare that this study received no funding.

Generative AI statement

The authors declare that no generative AI or AI-assisted technologies were used in the writing or preparation of this study.

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Erratum to: Research on soft neurological signs in obese children

In the article titled "Research on soft neurological signs obese children" by Tuğba Doğanç, Elif Çelik, Ahmet Anık, and Ayşe Tosun, published in Trends in Pediatrics. 2026;7(2):94-101 (<https://doi.org/10.59213/TP.2026.247>), the article title contained a typesetting error.

The title was published as:

Research on soft neurological signs obese children

The correct title is:

Research on soft neurological signs in obese children

This error occurred during the production process and was not the responsibility of the authors. The online version of the article has been corrected. The publisher apologizes for any inconvenience this may have caused.

Correct citation: Doğanç T, Çelik E, Anık A, Tosun A. Research on soft neurological signs in obese children. Trends in Pediatrics 2026;7(2):94-101. <https://doi.org/10.59213/TP.2026.247>