Short Stature in Patients with Diamond-Blackfan Anemia: A Cross-Sectional Study

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Objective To systematically describe the short stature of patients with Diamond-Blackfan anemia and to explore factors affecting the height development of patients with Diamond-Blackfan anemia.

Study design This cross-sectional study was conducted at the Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, and the height, weight, and clinical data of 129 patients with Diamond-Blackfan anemia were collected from June 2020 to September 2020.

Results The median height-age-z score (HAZ) of children affected by Diamond-Blackfan anemia was −1.54 (−6.36-1.96). Short stature was found in 37.98% of the patients. Specific Diamond-Blackfan anemia growth curves were developed for weight, height, and body mass index, separately for male and female patients. Multivariable logistic regression models showed that female sex (aOR 4.92; 95% CI 1.29-18.71; P = .0195), underweight (aOR 10.41, 95% CI 1.41-76.98, P = .0217), cardiovascular malformations (aOR 216.65; 95% CI 3.29-14279.79; P = .0118), and *RPL11*(aOR 29.14; 95% CI 1.18-719.10; P = .0392) or *RPS26* (aOR 53.49; 95% CI 1.40-2044.30; P = .0323) mutations were independent risk factors for short stature. In the subgroup of patients who were steroid-dependent, patients with a duration of steroid therapy over 2 years (OR 2.95; 95% CI 1.00-8.66; P = .0494) or maintenance dose of prednisone >0.1 mg/kg per day (OR 3.30; 95% CI 1.02-10.72; P = .0470) had a higher incidence of short stature.

Conclusions Patients with Diamond-Blackfan anemia had a high prevalence of short stature. The risk of short stature increased with age and was associated with sex, underweight, congenital malformations, and *RPL11* or *RPS26* mutations. The duration of steroid therapy and maintenance dose of steroid was significantly associated with the incidence of short stature in steroid-dependent patients with Diamond-Blackfan anemia. *(J Pediatr 2021; ■:1-9)*.

Diamond-Blackfan anemia is classified as an inherited bone marrow failure syndrome that is characterized by red cell aplasia, congenital anomalies, and a predisposition to cancer. In over 90% of the cases reported, the patients present mainly with severe hypoplastic anemia, usually macrocytic, and reticulocytopenia within 1 year of age.[1] It is also known as the first discovered human ribosomopathy.[2] Mutations in or deletions of one of the ribosomal proteins are found in up to 70% of patients with Diamond-Blackfan anemia, with *RPS19* mutations being the most common, accounting for approximately 25% of all cases of Diamond-Blackfan anemia.[3,4]

Chronic glucocorticoid treatment is the mainstay treatment for Diamond-Blackfan anemia. Although approximately 80% of individuals with Diamond-Blackfan anemia respond to corticosteroids, only 40% of the total patients have a sustained response. The other 40% of patients, who do not respond or lose response to glucocorticoids, require frequent blood transfusions. The major problem with transfusion therapy is iron overload, and effective and intense chelation therapy is warranted.[2] The only curative treatment for the hematologic phenotype is hematopoietic stem cell transplantation (HSCT), which is recommended for patients who are transfusion-dependent.[5]

Diamond-Blackfan anemia is frequently linked with a variety of physical anomalies (30%-50%), with the most common malformations being orofacial anomalies, upper limb and hand defects, and genitourinary and cardiac abnormalities.[6] Mutations in *RPL5* and *RPL11* are reported to be associated with orofacial and thumb anomalies, and mutations in *RPS26* are associated with skeletal

BMI	Body mass index
HAZ	Height-age-z score
HSCT	Hematopoietic stem cell transplantation
WAZ	Weight-age-z score

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defects.[7-9] Although growth retardation is also commonly seen in patients with Diamond-Blackfan anemia (in 25%-41% of Diamond-Blackfan anemia),[9,10] limited studies have described the linear growth of these children in detail. The etiology of short stature in patients with Diamond-Blackfan anemia is thought to be multifactorial; chronic steroid therapy, iron overload, and ribosomal protein mutations may play roles in the poor growth of children affected by Diamond-Blackfan anemia.[10-12]

The aims of this cross-sectional study were to describe the incidence of short stature and develop a growth curve for patients affected by Diamond-Blackfan anemia. Based on these findings, factors affecting the height development of patients with Diamond-Blackfan anemia were explored.

Methods

This cross-sectional study included patients who were diagnosed with Diamond-Blackfan anemia and consulted at the Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College from 2003 to 2020. The diagnostic criteria refer to those of the Sixth Annual Daniella Maria Arturi International Consensus Conference and include the following. Diagnostic criteria were age less than 1 year; macrocytic and occasionally normocytic anemia with no other significant cytopenias; reticulocytopenia; and normal marrow cellularity with a paucity of erythroid precursors. Major supporting criteria were gene mutation described in ''classic'' Diamond-Blackfan anemia and a positive family history. Minor supporting criteria were elevated erythrocyte adenosine deaminase activity; congenital anomalies described in ''classic'' Diamond-Blackfan anemia; elevated hemoglobin F; and no evidence of another inherited bone marrow failure syndrome.[13] Patients who had no available height or weight data were excluded from the analyses. This study was approved by the ethics committee of the Institute of Hematology and Blood Diseases Hospital, CAMS and PUMC (DC2020004-EC-1).

Data Collection

Newly measured (within 1 week) height and weight of patients with Diamond-Blackfan anemia and parents were collected by structured questionnaire from June 2020 to September 2020. To characterize the population, sex, birth date, and clinical information were collected from medical records. To reveal the respective association of sex and genetic height, the mean of the parental heights (midparental height) and sex were chosen as 2 separate variables, instead of corrected midparental height. Calculated z score values were used to quantify height for age, weight for age, and body mass index (BMI) for age. Short stature is defined as patients with height-age-z score (HAZ) <-2 SD based on age- and sex-specific measures under the Chinese criteria (2009).[14] Preshortness is defined as children with HAZ between <-1 SD and ≥-2 SD. Underweight was defined if the weight-age-z score (WAZ) is less than -2 SD. Overweight was defined as BMI >85th percentile, and obesity was defined as BMI >95th percentile. Remission was defined as hemoglobin level maintained at >80 g/L for at least 6 months without corticosteroids or transfusion. Steroid dependence was identified when corticosteroids were required to maintain hemoglobin levels of >80 g/L. Transfusion dependence was defined when the patient needed transfusions to maintain hemoglobin levels of >80 g/L.

Mutation Analysis Method

Genomic DNA was isolated from the peripheral blood samples of patients. Each exon with flanking regions of the following ribosomal protein genes and *GATA1* was tested by target sequencing with method previously described.[15] Common mutated ribosomal protein genes, including *RPS19, RPL5, RPL11, RPS24, RPS17, RPS26, RPL35a,* and other Diamond-Blackfan anemia related ribosomal protein genes (including *RPL29, RPL26, RPL15, RPL28, RPL14, RPL19, RPS7, RPS15, RPS10, RPL15*, and *RPS29*) were tested.

Statistical Analyses

Continuous variables are presented as the "median (min-max)," and categorical data are presented as frequencies (percentages). Normally distributed continuous variables were compared by using 2-sided Student t tests. Continuous variables, which were not normally distributed, were compared by using the Wilcoxon rank test. Two-sided likelihood ratio χ^2 test or Fisher exact test were used to compare categorical variables.

Univariate and multivariable logistic regression models were used to detect the relationship between short stature and conventional risk factors, including the age of measurement, sex, therapeutic status, midparental height, WAZ, BAZ, ribosomal protein mutation, and congenital anomalies. The age of measurement, WAZ, and BAZ were transformed into categorical variables according to the recognized clinical cutoff points. For patients who were steroid-dependent, ORs and aORs with corresponding 95% CIs were estimated to detect the relationship between short stature and the age of steroid therapy start age (months), duration of steroid therapy (months), maintenance dose of steroids (prednisone, mg/kg per day), and hemoglobin level (g/L) at ordinary time.

All statistical analyses were performed with the software packages R (http://www.R-project.org, The R Foundation) and Free Statistics software v 1.3 (www.freestatistics.tk). A 2-tailed P value of $<.05$ was considered statistically significant.

Method to Establish Development of Growth Curves

Centile curves of patients with Diamond-Blackfan anemia (height, weight, and BMI) were drawn by the Cole's Lambda, Mu, Sigma method using the gamlss package in R. The model assumes that the response variable has a flexible distribution (ie, Box-Cox Cole-Green original, Box-Cox Power Exponential original, and Box-Cox t original were chosen as appropriate according to the Akaike Information

Criterion and Schwartz Bayesian Criterion). A power transformation of age was performed and P-splines were used as a smoother.[16] The growth curves of patients with Diamond-Blackfan anemia were merged with the growth curves of healthy Chinese children and World Health Organization Growth Standards reported by Xin-Nan Zong et al.[14]

Results

Participants and Basic Characteristics

A total of 189 patients were diagnosed with Diamond-Blackfan anemia from 2003 to 2020 in our center. In this study, the height and weight data of 129 patients were collected. The basic characteristics of patients and results of all observations are shown in **Table I**. In total, 80 male and 49 female patients were included in the analyses. The median age of onset was 2 months (ranging from 0 to 75 months). Approximately 86.29% (107 of 129) of patients developed anemia by 1 year of age. The median age of the measurement of height and weight was 49 months (ranging from 3 to 189 months). Congenital malformations (not including short stature) were observed in 32.81% (41 of 128) of the patients. Digit abnormalities were the most common and accounted for 8.59% (11 of 128), followed by cardiovascular malformations (5.47%, 7 of 128) and facial deformities (3.12%, 4 of 128). Other kinds of malformations, such as hypospadias and hip dislocation, accounted for 10.16% (13 of 128) of the total malformations. Another 6 (4.69%) patients had 2 or more kinds of congenital malformations. Next-generation sequencing data were available for 112 patients. Mutations involving ribosomal protein genes were identified in 68.75% (77 of 112) of patients. The most prevalent mutated genes were as follows: RPS19 (28.57%), RPL5 (8.04%), RPS26 (7.14%), RPL11 (5.36%), RPS17 (3.57%), RPL35a (3.57%), and RPS24 (2.68%). Mutations in other rare ribosomal protein genes were found in 9.82% of patients. The distribution of ribosomal protein mutations in our population is consistent with previous studies.[2] At the time of height and weight measurement, 17.05% (22 of 129) patients had not yet received steroid therapy, and 55.04% (71 of 129) patients were steroid-dependent. Only 12.40% (16 of 129) patients achieved remission. Patients who were transfusion-dependent accounted for 10.08% (13 of 129) of the total patients evaluated, and 5.43% (7 of 129) patients underwent hematopoietic stem cell transplantation.

The median HAZ score of children affected by Diamond-Blackfan anemia was −1.54 (−6.36 to 1.96). Nearly 90% of the patients were under the 50th percentile for height for children of the same age and sex. Short stature (HAZ <-2 SD) was found in 37.98% of the patients, one-half of whom

Table I. Basic characteristics of the study population and the results of all observations

Characteristics	Median (minimum-maximum)/ number(percent)
Total number	129
Sex	
Male	80 (62.02%)
Female	49 (37.98%)
Age of onset*	2.00 (0.00-75.00)
<12 mo	107 (86.29%)
≥12 mo	17 (13.71%)
Age of measurement	49.00 (3.00-189.00)
<24 mo	35 (27.13%)
24-120 mo	75 (58.14%)
≥120 mo	19 (14.73%)
Midparental height†	167.50 (157.00-179.00)
<165 cm	21 (21.88%)
≥165 cm	75 (78.12%)
Congenital anomalies‡	
Yes	42 (32.81%)
No	86 (67.19%)
Type of congenital anomalies	
None	87 (67.19%)
Cardiovascular malformations	7 (5.47%)
Digit abnormalities	11 (8.59%)
Facial deformities	4 (3.12%)
Other kinds of malformations	13 (10.16%)
≥Two kinds of malformations	6 (4.69%)
Ribosomal protein mutation	
Yes	77 (68.75%)
No	35 (31.25%)
Mutated ribosomal protein gene	
No mutation	35 (31.25%)
RPL5	9 (8.04%)
RPL11	6 (5.36%)
RPS17	4 (3.57%)
RPS19	32 (28.57%)
RPS24	3 (2.68%)
RPS26	8 (7.14%)
RPL35a	4 (3.57%)
Others	11 (9.82%)
Therapeutic status	
No steroid therapy yet	22 (17.05%)
Steroid dependence	71 (55.04%)
Remission	16 (12.40%)
Transfusion dependence	13 (10.08%)
Post HSCT	7 (5.43%)
HAZ	−1.54 (−6.36 to 1.96)
<-3 SD	24 (18.60%)
−3 to <-2 SD	25 (19.38%)
−2 to <-1 SD	32 (24.81%)
−1 to <0 SD	33 (25.58%)
≥0 SD	15 (11.63%)
WAZ	−0.98 (−2.83 to 5.84)
<-2 SD	16 (12.40%)
−2 to 0 SD	79 (61.24%)
≥0 SD	34 (26.36%)
BMI	15.94 (8.40-37.33)
BMI-age-z score	−0.20 (−3.65 to 16.11)
BMI percentile	
≤15th	31 (24.03%)
15-85th	71 (55.04%)
85-95th	8 (6.20%)
>95th	19 (14.73%)

*The age of onset was verified in 124 patients.
†Midparental heights were available in 96 patients.
‡Physical examination and imaging results were available for verifying congenital anomalies in 128 patients.

were severely short (HAZ < −3 SD). Another 24.81% children were preshortness (−2 SD ≤HAZ <-1 SD). The median WAZ of patients with Diamond-Blackfan anemia was −0.98 (−2.83 to 5.84). More than 70% of patients weighed less than the 50th percentile for weight for children of the same age and sex. Underweight (WAZ <-2 SD) patients accounted for 14% of the total patients evaluated. The median BMI of patients with Diamond-Blackfan anemia was 15.94 (8.40-37.33), with the median BMI-age-z score of −0.20 (−3.65 to 16.11). The BMI of approximately 24% of children with Diamond-Blackfan anemia was under the 15th percentile. However, the BMIs of 21% of patients were over the 85th percentile, among whom over two-thirds were obese (Table I).

Using the LMS method, specific Diamond-Blackfan anemia growth curves from 0 to 15 years of age were developed for weight, height, and BMI, separately for male and female patients. Figure shows growth curves for the height (A and B), weight (C and D), and BMI (E and F) of male and female patients.

Factors Associated with Short Stature of Patients with Diamond-Blackfan Anemia

To identify factors related to short stature, univariate and multivariable logistic regression analyses were performed (Table II). Sex, the age of measurement, WAZ, midparental height, congenital malformations, therapeutic status, and ribosomal protein gene mutations were adjusted in multivariable logistic regression analysis.

Girls had a more than 2 times higher risk of short stature (OR 2.43; 95% CI 1.16-5.08; $P = .0181$) and the result was stable after adjusting for other factors (aOR 4.92; 95% CI 1.29-18.71; $P = .0195$).

The proportion of short stature increased in patients over 2 years old. Compared with children under 2 years old, the ORs of short stature were 7.55 (95% CI 2.42-23.49; $P = .0005$) in children age 2-10 years and 5.64 (95% CI 1.41-22.48; $P = .0143$) in children over 10 years of age. Although in multivariable analyses, the association between age and short stature was not significant.

The incidence of short stature significantly decreased with increasing WAZ. The risk of short stature in patients with WAZ <−2 SD was more than 3-fold higher than that in patients with WAZ ≥ −2 SD (OR 3.16, 95% CI 1.07-9.35, $P = .0373$). The association was more significant in multivariable logistic regression analysis (aOR 10.41, 95% CI 1.41-76.98, $P = .0217$).

Short stature was more frequent in patients with congenital malformations (OR 2.54; 95% CI 1.19-5.43; $P = .0164$). Among the different types of malformations, patients with facial deformities had the highest short stature rate (75%) followed by those with cardiovascular malformations

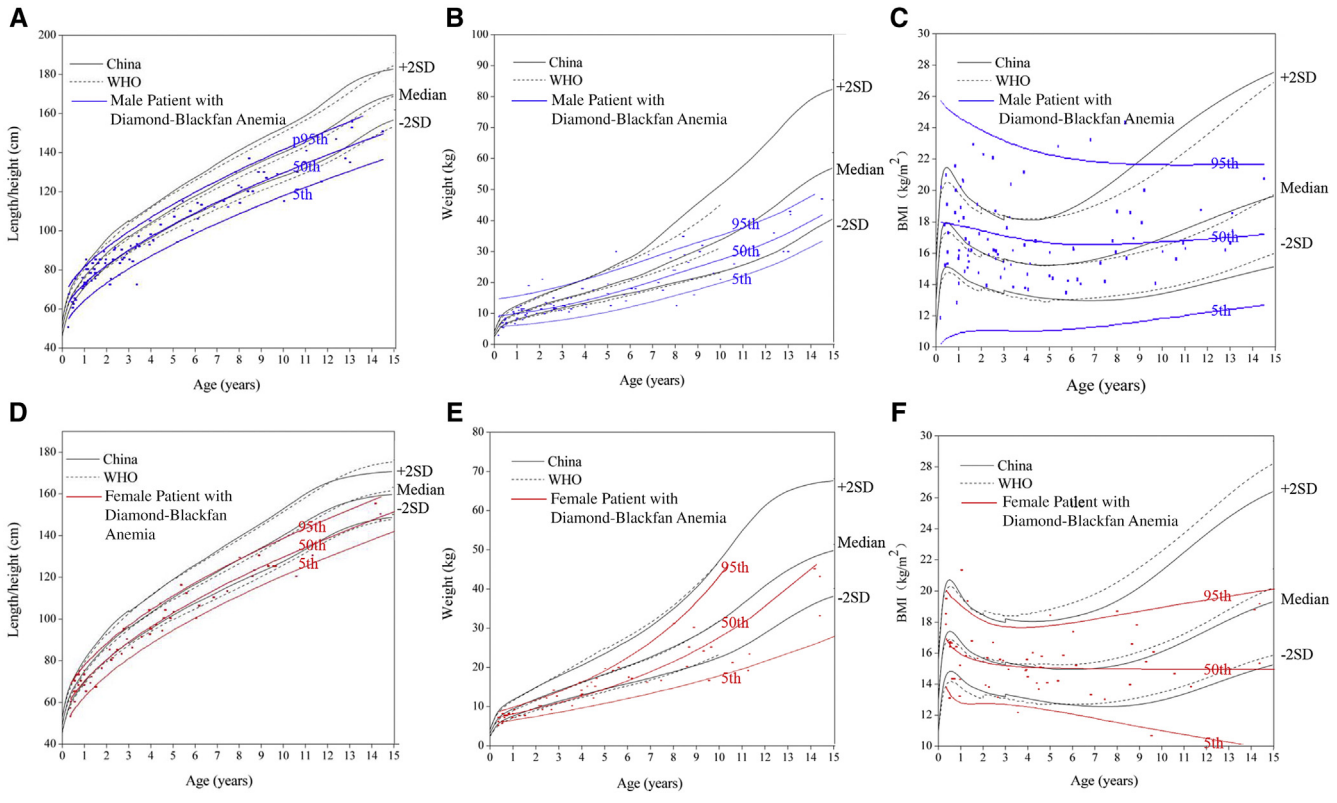

Figure. A-F, Growth curves for height, weight, and BMI for male and female patients with Diamond-Blackfan anemia compared with Chinese and WHO standards. Curves for the 5th, 50th, and 95th in patients with Diamond-Blackfan anemia and -2 SD, median, and +SD in Chinese and WHO standards. *WHO*, World Health Organization.

Table II. Factors associated with short stature (HAZ <-2SD)

Variables	Percentage of short stature	Univariate logistic regression analysis		Multivariate logistic regression analysis	
		OR (95% CI)/β (95% CI)	*P* Value	aOR (95% CI)/β (95% CI)	*P* Value
Sex					
Male	30.00% (24/80)	1		1	
Female	51.02% (25/49)	2.43 (1.16, 5.08)	.0181	4.92 (1.29, 18.71)	.0195
Age of measurement					
<24 mo	11.43% (4/35)	1		1	
24-120 mo	49.33% (37/75)	7.55 (2.42, 23.49)	.0005	31.49 (0.51, 1953.37)	.1014
≥120 mo	42.11% (8/19)	5.64 (1.41, 22.48)	.0143	16.55 (0.22, 1225.45)	.2013
WAZ		0.41 (0.27, 0.62)	<.0001	0.41 (0.21, 0.81)	.0097
≥2SD	34.51% (39/113)	1		1	
<-2SD	62.50% (10/16)	3.16 (1.07, 9.35)	.0373	10.41 (1.41, 76.98)	.0217
Midparental height		0.91 (0.86, 0.97)	.0021	0.86 (0.73, 1.02)	.0923
≥165 cm	37.33% (28/75)	1		1	
˂165 cm	61.90% (13/21)	2.73 (1.01, 7.39)	.0486	1.66 (0.39, 7.02)	.4941
Congenital anomalies					
No	30.23% (26/86)	1		1	
Yes	52.38% (22/42)	2.54 (1.19, 5.43)	.0164	8.78 (1.34, 57.40)	.0234
Type of congenital anomalies					
None	29.89% (26/87)	1		1	
Cardiovascular malformations	71.43% (5/7)	5.87 (1.07, 32.20)	.0417	216.65 (3.29, 14279.79)	.0118
Digit abnormalities	45.45% (5/11)	1.96 (0.55, 6.98)	.3018	15.29 (0.98, 238.51)	.0517
Facial deformities	75.00% (3/4)	7.04 (0.70, 70.86)	.0977	inf. (0.00, Inf)	.9918
Other kinds of malformations	46.15% (6/13)	2.01 (0.62, 6.57)	.2471	15.87 (0.82, 306.38)	.0672
≥Two kinds of malformations	50.00% (3/6)	2.35 (0.44, 12.40)	.3154	1.97 (0.00, 17944.60)	.8837
Ribosomal protein mutation					
No	14.29% (5/35)	1		1	
Yes	51.95% (40/77)	6.49 (2.28, 18.48)	.0005	4.52 (0.93, 21.93)	.0614
Mutated ribosomal protein gene					
No mutation	14.29% (5/35)	1		1	
RPL5	88.89% (8/9)	48.00 (4.89, 471.32)	.0009	62.11 (0.53, 7252.25)	.0891
RPL11	66.67% (4/6)	12.00 (1.72, 83.81)	.0122	29.14 (1.18, 719.10)	.0392
RPS17	50.00% (2/4)	6.00 (0.68, 52.90)	.1067	4.68 (0.35, 62.53)	.2438
RPS19	37.50% (12/32)	3.60 (1.10, 11.80)	.0344	3.09 (0.48, 19.86)	.2346
RPS24	33.33% (1/3)	3.00 (0.23, 39.61)	.4040	0.35 (0.00, 27.15)	.6394
RPS26	87.50% (7/8)	42.00 (4.21, 418.62)	.0014	53.49 (1.40, 2044.30)	.0323
RPL35a	0.00% (0/4)	0.00 (0.00, Inf)	.9902	0.00 (0.00, Inf)	.9931
Other	54.55% (6/11)	7.20 (1.58, 32.86)	.0108	11.51 (0.80, 165.89)	.0727
Therapeutic status					
No steroid therapy yet	13.64% (3/22)	1		1	
Steroid dependence	49.30% (35/71)	6.16 (1.67, 22.67)	.0063	5.80 (0.54, 62.14)	.1459
Remission	12.50% (2/16)	0.90 (0.13, 6.16)	.9185	0.82 (0.04, 15.14)	.8940
Transfusion dependence	38.46% (5/13)	3.96 (0.76, 20.67)	.1027	0.68 (0.04, 11.92)	.7889
Post HSCT	57.14% (4/7)	8.44 (1.23, 58.16)	.0302	3.95 (0.14, 111.82)	.4202

Adjusted model:
Sex: adjusted for therapeutic status, the age of measurement (months), ribosomal protein gene mutations, congenital anomalies, midparental height, and WAZ.
Age of measurement (months): adjusted for sex, therapeutic status, ribosomal protein gene mutations, congenital anomalies, midparental height, and WAZ.
WAZ: adjusted for sex, therapeutic status, the age of measurement (months), ribosomal protein gene mutations, congenital anomalies, and midparental height.
Midparental height: adjusted for sex, therapeutic status, the age of measurement (months), ribosomal protein gene mutations, congenital anomalies, and WAZ.
Mutated ribosomal protein gene: adjusted for sex, therapeutic status, the age of measurements (months), congenital anomalies, midparental height, and WAZ.
Therapeutic status: adjusted for sex, the age of measurements (months), ribosomal protein gene mutations, congenital anomalies, midparental height, and WAZ.

(71%). After adjusting for other factors, cardiovascular malformations were independent risk factors for short stature (aOR 216.65; 95% CI 3.29-14279.79; $P = .0118$).

Mutations in ribosomal protein genes were also risk factors for short stature (OR 6.49; 95% CI 2.28-18.48; $P = .0005$). The percentage of short stature was 52% in patients with ribosomal protein gene mutations and only 14% in patients without ribosomal protein gene mutations. The highest percentage of short stature was seen in patients with *RPL5* mutations (89%), followed by those with *RPS26* mutations (87.5%) and *RPL11* mutations (67%). Mutations of *RPL5, RPL11, RPS19, RPS26,* and rare ribosomal protein gene mutations were associated with a high risk of short stature. Further multivariable logistic regression analysis indicated that *RPL11* (aOR 29.14; 95% CI 1.18-719.10; $P = .0392$) and *RPS26* (aOR 53.49; 95% CI 1.40-2044.30; $P = .0323$) mutations were independent risk factors for short stature.

In different therapeutic statuses, compared with patients who had not yet received steroid therapy, steroid-dependent (OR 6.16, 95% CI 1.67-22.67, $P = .0063$) and post-HSCT (OR 8.44, 95% CI 1.23-58.16, $P = .0302$) patients had higher incidences of short stature. Patients in remission had a short stature rate that was similar to that of patients before steroid therapy (12.5% vs 13.6%). The OR of short

stature in patients who were transfusion-dependent was not significant (OR 3.96, 95% CI 0.76-20.67, $P = .1027$).

In summary, short stature in patients with Diamond-Blackfan anemia was associated with sex, age, WAZ, midparental height, congenital malformations, ribosomal protein gene mutation, and therapeutic status. Female sex, underweight (WAZ <-2 SD), cardiovascular malformations, and *RPL11* or *RPS26* mutations are independent risk factors for short stature.

Factors Associated with Short Stature in Patients who were Steroid-dependent with Diamond-Blackfan Anemia

Patients who were steroid-dependent comprised the majority of children in our study. We collected information on the steroid therapy start age, duration of steroid therapy, maintenance dose of steroids (mg/kg per day of prednisone), and hemoglobin level (g/L) of patients who were steroid-dependent. Univariate and multivariable logistic regression analyses were then performed to find associations between short stature and the factors above (Table III).

The median steroid therapy start age was 12 months, ranging from 0 to 55 months. The rate of short stature was similar in patients who started steroid therapy before or after 12 months (51.5% vs 48.6%). No significant association between short stature and steroid therapy start age (OR 0.89; 95% CI 0.35-2.28; $P = .8108$) was observed.

The duration of steroid therapy in patients involved in our study ranged from 0.89 to 174.63 months. A higher rate of short stature was seen in patients under steroid therapy for more than 2 years compared with patients with less than 2 years of steroid therapy (59.6% vs 33.3%). The duration of steroid therapy was significantly associated with short stature (OR 2.95; 95% CI 1.00-8.66; $P = .0494$). Although the P value was not significant, the aOR of duration of steroid therapy remained above 1 (aOR 2.06; 95% CI 0.22-19.61; $P = .5313$).

The maintenance dose of prednisone in these patients ranged from 0.003 to 1.220 mg/kg per day. Patients with maintenance doses of prednisone >0.1 mg/kg per day had a higher incidence of short stature than those with maintenance doses of prednisone under 0.1 mg/kg per day (64.3% vs 35.3%). Univariate logistic regression analysis suggested that maintenance dose of steroids tended to be a risk factor for short stature (OR 3.30; 95% CI 1.02-10.72; $P = .0470$). After adjusting for sex, the age of measurement, ribosomal protein gene mutations, congenital anomalies, midparental height, WAZ, and the duration of steroid therapy, short stature remained associated with an increasing maintenance dose of steroids (aOR 9.52; 95% CI 0.83-109.57; $P = .0706$).

More than two-thirds of children could maintain hemoglobin levels over 100 g/L. The incidence of short stature was higher in patients with hemoglobin levels of 80-100 g/L than in patients with hemoglobin levels ≥100 g/L (65% vs 49%). By regression analysis, hemoglobin level did not appear to be associated with short stature.

In patients who were steroid-dependent, short stature was associated with an increasing duration of steroid therapy and the maintenance dose of steroids, but not associated with steroid therapy start age.

Discussion

In this cross-sectional study, we described the basic clinical features and anthropometric characteristics (height, weight, and BMI) of children and adolescents age 0-15 years in our

Table III. Factors associated with short stature (HAZ <-2SD) in steroid-dependent patients with Diamond-Blackfan anemia

		Univariate logistic regression analysis		Multivariate logistic regression analysis	
Variables	Percentage of patients with short stature	OR (95% CI)/β (95% CI)	*P* Value	aOR (95% CI)/β (95% CI)	*P* Value
Steroid therapy start age	12.00 (0.00-55.00)				
<12 mo	51.52% (17/33)	1		1	
≥12 mo	48.65% (18/37)	0.89 (0.35, 2.28)	.8108	0.31 (0.02, 4.13)	.3782
Duration of steroid therapy	37.58 (0.89-174.63)				
<24 mo	33.33% (7/21)	1		1	
≥24 mo	59.57% (28/47)	2.95 (1.00, 8.66)	.0494	2.06 (0.22, 19.61)	.5313
Maintenance dose (prednisone) mg/kg per d	0.179 (0.003-1.220)				
<0.1 mg/kg per d	35.29% (6/17)	1		1	
≥0.1 mg/kg per d	64.29% (27/42)	3.30 (1.02, 10.72)	.0470	9.52 (0.83, 109.57)	.0706
Hemoglobin levels					
≥100 g/L	48.78% (20/41)	1		1	
80-100 g/L	64.71% (11/17)	1.92 (0.60, 6.19)	.2718	0.94 (0.12, 7.54)	.9528

Adjusted model:
Steroid therapy start age (months): adjusted for sex, the age of measurement (months), ribosomal protein gene mutations, congenital anomalies, midparental height, WAZ, the duration of steroid therapy (months), and maintenance dose (prednisone) (mg/kg per day).
Duration of steroid therapy (months): adjusted for sex, the age of measurement (months), ribosomal protein gene mutations, congenital anomalies, midparental height, WAZ, and maintenance dose (prednisone) (mg/kg per day).
Maintenance dose (prednisone): adjusted for sex, the age of measurement (months), ribosomal protein gene mutations, congenital anomalies, midparental height, WAZ, and the duration of steroid therapy (months).
Hemoglobin levels: adjusted for sex, the age of measurement (months), ribosomal protein gene mutations, congenital anomalies, midparental height, WAZ, steroid therapy start age (months), the duration of steroid therapy (months), and maintenance dose (prednisone) (mg/kg per day).

Diamond-Blackfan anemia cohort (2003-2020). We then developed the first specific growth curves of height, weight, and BMI for Chinese patients with Diamond-Blackfan anemia. Although the development of standardized growth curves for evaluating the growth of this special group of children still needs more data from multicenter studies, our results provided an overview of the growth and development of children with Diamond-Blackfan anemia. Through univariate and multivariable logistic regression analysis, we revealed that female sex, age over 2 years, underweight (WAZ <-2 SD), cardiovascular malformations, and *RPL11* or *RPS26* mutations were independent risk factors for short stature. Moreover, in the subgroup of patients who were steroid-dependent, the duration of steroid therapy and maintenance dose of prednisone were significantly associated with the incidence of short stature.

Children with Diamond-Blackfan anemia had poor height development, as the majority of patients had lower heights compared with the standard heights of the same age and sex. Short stature was observed in 38% of the patients. This incidence was compatible with previous findings.[9,10] Besides height development, we described the weight and BMI of children with Diamond-Blackfan anemia. Underweight were observed in 12% patients. However, overweight was also common in children with Diamond-Blackfan anemia. The BMI of >20% patients were above the 85th percentile. The Diamond-Blackfan anemia Registry of North America reported data on overweight and obesity and the relative proportion of overweight and obese individuals was highest in the transfusion-dependent patients. However, owing to the limited number of patients who were transfusion-dependent in our study, further studies are needed to determine whether other reasons, such mutations or steroid therapy are associated with overweight in patients with Diamond-Blackfan anemia.

Although stunting is a common manifestation in patients with Diamond-Blackfan anemia, the main reason for poor height growth in these children is still not clear, as a variety of factors may contribute. One previous study of a French registry, using multiple regression analysis, revealed that the risk of growth retardation increases with age and is associated with malformations and treatment dependence.[10] In our study, the median age at measurement was nearly 4 years, ranging from 0 to 15 years old, which was different from the study of the French registry (the mean age at survey was 13.5 ± 1.6 years). Although the French registry study and our study had different age compositions, our results also suggested an increasing risk of short stature with age. Second, according to our results, congenital malformations were also independently associated with short stature, which is consistent with the French registry study. We further analyzed the incidence of short stature with different types of malformations, and we found significant correlation between cardiovascular malformations and short stature. Moreover, our study reported that mutations in the ribosomal protein gene were strongly associated with short stature. The incidence of short stature in patients with *RPL5, RPL11, RPS19, RPS26,* and rare ribosomal protein gene mutations was markedly increased. The association remained significant between short stature and *RPL11* or *RPS26* mutations after adjusting for other related factors. Correlations between clinical features and mutations in specific ribosomal protein genes have been reported. *RPL5* mutations were associated with physical malformations such abnormal thumbs, cleft palate, and cardiac defects. *RPL11* mutations were associated with thumb abnormalities and *RPS26* mutations were associated with skeletal defects.[7-9] Growth retardation has also been reported in other ribosomopathies.[17,18] These findings support the hypothesis that abnormalities in genes related to ribosomal function may affect the multisystemic development of early embryo, and these abnormalities presents clinically as bone marrow failure and congenital malformations, including short stature.

Besides genetic factors, our results demonstrated that short stature was markedly more frequent in steroid-dependent and patients with HSCT. Steroid therapy is the mainstay treatment for Diamond-Blackfan anemia. The proportion of steroid dependence in patients with Diamond-Blackfan anemia was reported to be 21%-45%, and there was a huge difference in steroid maintenance dose.[15,19-21] Because children affected by Diamond-Blackfan anemia may receive potentially life-long steroid therapy starting in infancy, side effects of steroid therapy have long been an important issue of concern. Glucocorticoid strongly downregulates the plasma concentration of FGF23, which regulates the renal phosphate reabsorption and active vitamin D, 1.25(OH) 2D3 formation.[22] And it is well known that chronic steroid use is a dose-related factor affecting growth development in children. Ribeiro et al analyzed the long-term impact of glucocorticoids on growth and bone mineral density in children with idiopathic nephrotic syndrome and demonstrated that increasing doses (cut-off doses >0.2 mg/kg/day) of prednisone were significantly associated with lower height and bone mineral density z scores.[23] According to the recommendations of the 2008 Diamond-Blackfan anemia international clinical consensus conference, steroid therapy is should be started after the age of 1 year, and the target maintenance dose should not be higher than 0.5 mg/kg per day. Our study, analyzed the association of steroid therapy start age, duration, and maintenance dose with the incidence of short stature especially in steroid-dependent patients with Diamond-Blackfan anemia. Hemoglobin level was also considered in these patients as chronic anemia may also affect height development. Our results suggested that the duration of steroid therapy and maintenance dose of steroids, but not the steroid therapy start age and hemoglobin level, were significantly associated with short stature. Close surveillance of growth and other side effects as well as hemoglobin level, is necessary to obtaining the ideal therapeutic level of steroids. Although the steroid therapy start age is not associated with short stature, taking steroid before 6 months of age was not recommended because glucocorticoids not only affect height but also have significant effects on other systems in children, such as the central nervous system,[24] immune system, and endocrine system.

HSCT was recommended for transfusion-dependent patients with Diamond-Blackfan anemia. Poor growth was reported to be prevalent despite reduced-intensity chemotherapy for HSCT in children.[25] Patients who are post-HSCT had the highest incidence of short stature (57%), nearly 20% higher than transfusion-dependent patients (38%). One possible reason for this finding was the effect of transplantation on endocrine glands. The impact of growth needs more attention when HSCT is considered for patients with Diamond-Blackfan anemia.

Our data also revealed that female patients had a higher risk of short stature. As no significant difference in the age of measurement, therapeutic status, ribosomal protein gene mutations, congenital anomalies, midparental height, and WAZ score was found between boys and girls, further study is needed to determine whether other factors, such as the difference in the endocrine system between boys and girls, may lead to this result. In addition, underweight was also found to be a risk factor for short stature, indicating a consistent delay in weight and height development and the importance of nutritional support to promote linear growth. Another supportive piece of evidence was that the occurrence of short stature (HAZ <-2 SD, approximately <3th percentile) in patients who had not yet received steroid therapy and remission patients was also much higher (12%-14%) than healthy children. On the basic of this, nongenetic factors, mainly treatment side effects exacerbated the severity of short stature.

Our work has some limitations. The population included in this study was under 15 years old, which is a relatively small age span. More data from adult patients are needed to verify our results. Second, as the proportions of transfusion-dependent and post-HSCT patients were low in this study, it difficult to confirm the influence of the HSCT and iron overload on the height development of children. Endocrine dysfunction is common in patients with Diamond-Blackfan anemia, and growth hormone therapy could improve short stature in children with Diamond-Blackfan anemia.[11,12] However, as systematic data concerning endocrine function in our patients with Diamond-Blackfan anemia were not available at the time of this research and no patient accepted growth hormone therapy in our cohort, it hard to determine the association between endocrine system function and the incidence of short stature. Finally, the potential recording errors of the height and weight measurement of patient and their parents should be considered.

In conclusion, our cross-sectional research provides a detailed description of the height and weight development and growth curves for patients affected by Diamond-Blackfan anemia age 0-15 years. According to our results, short stature is common in children with Diamond-Blackfan anemia. The risk for short stature increases with age and is associated with sex, underweight, congenital malformations, and *RPL11* or *RPS26* mutations. In steroid-dependent patients, the duration of steroid therapy and maintenance dose of steroids are significantly associated with the incidence of short stature.

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