Association of *ZBTB38* gene polymorphism (rs724016) with height and fetal hemoglobin in individuals with sickle cell anemia

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A B S T R A C T

Objectives: Our study evaluated the association of the polymorphism rs724016 in the *ZBTB38* gene, previously associated with height in other populations, with predictors of height, clinical outcomes, and laboratory parameters in sickle cell anemia (SCA).
Methods: Cross-sectional study with individuals with SCA and aged between 3 and 20 years. Clinical, laboratory, molecular, and bone age (BA) data were evaluated. Levels of IGF-1 and IGFBP-3 were adjusted for BA, target height (TH) was calculated as the mean parental height standard deviation score (SDS), and predicted adult height (PAH) SDS was calculated using BA.
Results: We evaluated 80 individuals with SCA. The homozygous genotype of the G allele of rs724016 was associated with a lower height SDS ($p < 0.001$) and, in a additive genetic model, was negatively associated with HbF levels ($p = 0.016$). Lower adjusted IGF-1 levels were associated with co-inheritance of alpha-thalassemia and with the absence of HU therapy. Elevated HbF levels were associated with a lower deficit in adjusted growth potential (TH minus PAH).
Conclusion: Our analysis shows that SNP rs724016 in the *ZBTB38* is associated with shorter height and lower HbF levels, an important modifier of SCA.

1. Introduction

Sickle cell anemia (SCA), defined by homozygosity for the hemoglobin S (HbS) allele, is characterized by episodes of hemolysis and vaso-occlusion triggered by deoxygenation of HbS [1]. Patients with SCA exhibit variable degrees of organic dysfunction, which can affect multiple organs and compromise statural growth. Although SCA is more prevalent in sub-Saharan Africa, the Mediterranean basin, the Middle East, and India, successive migration waves have made the disease a global health problem [2]. The height of children with SCA living in resource-poor regions is lower due to a combination of factors, including worse health conditions and a higher prevalence of nutritional deficiencies [3]. However, an elevated level of fetal hemoglobin (HbF) represents the most important modifier of the severity of SCA, as it can reduce morbidity and mortality, and protect against complications related to growth [3]. HbF is the predominant hemoglobin at birth, then HbF synthesis declines and adult hemoglobin (HbA) becomes the most common hemoglobin (Hb) in children without hemoglobinopathies, while HbS becomes predominant in children with SCA [4]. Because of the progressive decline in HbF levels in children with SCA, the first manifestations of the disease begin after 6 months of age, which coincides with the onset of risk for growth impairment [1,3,4]. Growth

impairment in children with SCA is progressive during childhood and may worsen during puberty, as delayed onset of puberty is common in patients with SCA [3]. Hydroxyurea (HU) therapy can attenuate the tendency for HbF levels to decline after birth and prevent disease complications. In some locations, HU therapy is restricted to children with SCA who are older than 2 years and have complications of the disease, although many authors recommend treatment for all children with SCA [5]. Co-inheritance of alpha-thalassemia, in combination with HbF levels and HU therapy, is another important modifying factor of sickle cell anemia. Previous studies have suggested the possibility that co-inheritance of alpha-thalassemia may impair the growth of children with SCA by affecting the growth hormone/insulin-like growth factor 1 (GH/IGF-1) axis [6].

Genetic factors represent >90% of the variability in human height, according to heritability estimates using monozygotic and dizygotic twins [7]. Genome-wide association studies (GWAS) confirm the polygenic nature of the trait by showing hundreds of common variants associated with height. The loci in the *ZBTB38* (zinc finger and BTB domain-containing 38) gene are considered the most frequently associated with height in GWAS [8,9]. The effect on the height of some single-nucleotide polymorphisms (SNPs) can vary in different populations [10], however, the SNP rs724016 in the *ZBTB38* was associated with growth when several aspects were evaluated, such as length at birth (LB) [11], population of Chinese with short stature [8], individuals of European ancestry [12] and individuals with African ancestry [10].

Despite the diversity of height GWAS, common allelic variants have not been studied in individuals with SCA. In turn, HbF levels are a heritable trait whose modulating elements are poorly understood, and less than half of its genetic variance is explained [1]. Additionally, most genetic variants associated with stature or HbF levels in GWAS act on transcriptional regulation [1,13]. Variants associated with transcription factors, such as the ZBTB38, often act pleiotropically by modifying the expression of multiple genes. While there is increasing effort to conduct studies to assess pleiotropically associated variants with complex traits, such as stature [13], studies involving smaller populations, such as individuals with SCA, or evaluating HbF levels have not yet been carried out. Therefore, it is important to attempt to replicate the results of GWAS of stature in individuals with SCA, as well as to evaluate the influence of HbF levels on the obtained outcomes.

We hypothesized that the *ZBTB38*, which is highly expressed in hematopoietic tissue, may have pleiotropic effects on the growth and HbF levels of individuals with SCA. The aim of the study was to assess the association of the rs724016 of the *ZBTB38* with anthropometric data and HbF levels in individuals with SCA aged 3 to 20 years. The data were also associated with other factors capable of influencing the growth of children with SCA. Therefore, we evaluated the association of the polymorphism with levels of IGF-1 and IGFBP-3, as an estimate of the function of the GH/IGF-1 axis. Additionally, we adjusted the data for the main modifiers of SCA, which are HbF levels, HU therapy, and co-inheritance of alpha-thalassemia.

2. Materials and methods

2.1. Participants

Cross-sectional study with individuals who were diagnosed with SCA and followed up between August 2018 and July 2019 at the outpatient clinic of the Regional Blood Center of Governador Valadares, which provides public care to the population in the eastern region of Minas Gerais state, Brazil.

The study was approved by the Ethics Committee of the Hemominas Foundation (number 2,666,921 in May 2018) and the assent form or informed consent was signed by the participants and their parents or guardians, as applicable.

The inclusion criteria were previous diagnosis of SCA, absence of acute disease complications at the time of enrollment, and age between 3 and 20 years. The lower age limit was based on current guidelines at the time of the project's inception, which recommended the use of HU therapy only for children older than 3 years. Our aim was to assess height throughout the individual's growth phase; therefore, we extended the age range to 20 years due to the delayed puberty that occurs in SCA.

The exclusion criteria included the existence of any other systemic disease, intellectual disability, or body dysmorphia that could compromise growth. All eligible patients were invited for clinical, laboratory, and radiological evaluation during routine medical consultations at the blood center. The radiological evaluation was carried out in a clinic that established an agreement with the research project and was located close to the place of care. In patients under chronic transfusion therapy, blood sample for laboratory testing was collected before the start of the transfusion.

2.2. Clinical evaluation

The clinical evaluation included a structured medical interview, physical examination, and review of the participant's medical records. To obtain the best accuracy about the information on the parents' height, the moment of measuring the participant's height was also used to measure or inquire about the parents' height. Data provided about LB were expressed as a SDS using gestational age and sex according to the INTERGROWTH-21st Project [14]. Small for gestational age was defined as LB less than -2 SD. Low birth weight (LBW) was defined as weight at birth of <2500 g. According to the World Health Organization (WHO) criteria, anthropometric data were expressed in SDS. The height SDS of the parents was calculated considering the age of 19 years, and we defined the target height (TH) as the average of the SDS of the parents' height. We classified puberty according to the Tanner stages, and stage I of the breast (female) or genital (male) was defined as prepubertal. Short stature was defined as a height less than -2SD.

2.3. Complementary evaluation

The most recent records from the past 12 months of complete blood count, serum ferritin, and HbF levels were obtained from medical records. The evaluation of the GH/IGF-1 axis was represented by the IGF-1 and IGFBP-3 plasma concentration, which was analyzed by a chemiluminescent assay (IMMULITE® 2000 SYSTEM, Siemens Healthcare Diagnostics Products Ltd.). Levels of IGF-1 and IGFBP-3 were expressed in SDS according to data from a reference population [6] and replacing chronological age with bone age (BA), to adjust the results for the delay in bone maturation that is commonly associated with delayed puberty. The method by Greulich and Pyle [15] was used to determine BA, and then we used BA to calculate the predicted adult height (PAH). Because it is more applicable to patients with delayed bone age [16], we calculated the PAH in SD with the BoneXpert method (http://www.bonexpert.com/adult-height-predictor). We extracted genomic DNA from peripheral white blood cells and identified alpha-globulin deletion genotypes (3.7 and 4.2 kb deletion) using the Multiplex Gap-PCR technique [17]. Additionally, we genotyped rs724016 using the TaqMan real-time PCR assay (Applied Biosystems, Foster City, CA, USA).

2.4. Statistical analysis

We performed statistical analyzes with SPSS version 20.0 and stratified descriptive data according to HU therapy. Participants who had been using HU for <1 year, considered to be undergoing a dose adjustment, were allocated to the group not using the drug. Fisher's exact test was used to compare nominal variables between groups when any expected or observed value in the contingency table was <5. For higher values in the table, we used the chi-square test to compare nominal variables between groups. The chi-square test was used to test for Hardy-Weinberg equilibrium of rs724016 genotypes. The association

of genotypes with other variables was tested using dominant, additive, and recessive genetic models. The Shapiro-Wilk test verified the normality of the distribution, and we describe data with normal distribution in mean (SD) and those without normal distribution in median (minimum; maximum). The *t*-test compared continuous variables with normal distribution between two groups, and the Mann-Whitney test compared variables without normal distribution. In the case of the additive genetic model, ANOVA followed by Turkey's test compared variables with normal distribution between groups (GG vs AG vs AA), while the Kruskal-Wallis test followed by Dunn's test compared variables without normal distribution. The generalized linear model (GLM) evaluated the association of more than one predictor variable with different outcomes of interest, such as anthropometric data, adjusted IGF-1 and IGFBP-3 levels, and HbF levels. We considered the homozygous genotype for the A allele (ancestral allele) as the reference variable when the rs724016 additive model genotypes were considered predictors in the GLM. Participants were excluded from analyzes that required missing data (complete-case analyses), with the aim of not harming the statistical power of the height assessment, which was defined as the main objective. We considered *p*-value <0.05 to be significant (two-tailed).

3. Results

Descriptive data were grouped in Table 1 according to HU therapy, which is an important modifying factor for SCA. In the group of patients not using HU, 5 participants who had used the drug for <1 year were included. The level of missing BA data was 26.2% (21/80). The remaining missing data were described in Table 1.

The univariate association of rs724016 genotypes with participants' height SDS was tested in different genetic models. The univariate analysis of the height SDS between the genotypes of the dominant genetic model (GG + AG vs AA) did not show statistical significance ($p = 0.068$), although in the recessive model (GG vs GA + AA) the height SDS of the homozygous GG genotype was significantly lower ($p = 0.002$) than the sum of the genotypes of the AA homozygote with the GA heterozygote. In the additive model (GG vs AG vs AA) the G allele was also associated with shorter height (Fig. 1). The genotype frequencies AA = 23.1% (18/78), AG = 42.3% (33/78), and GG = 34.6% (27/78) showed Hardy-Weinberg equilibrium and the G allele frequency of rs724016 in *ZBTB38* in the evaluated population was 0.45.

The association of rs724016 genotypes with anthropometric outcomes (height SDS and TH minus PAH SDS) was also performed under adjustment for other independent variables in a GLM. As independent variables capable of influencing the growth in SCA, we considered important age, sex, alpha-thalassemia co-inheritance, HU therapy, HbF levels, Hb levels and serum ferritin. In the dominant model, the rs724016 genotypes adjusted for the other predictors were not associated with height ($p = 0.343$). The additive model significantly associated the G homozygous genotype with shorter height ($p = 0.039$). Table 2 shows that in the recessive model, the G homozygous genotype was significantly associated with shorter height ($p < 0.001$). Additionally, the HbF levels were the only significant predictor ($p = 0.042$) for the smaller adjusted deficit in predicted adult height (TH minus PAH).

Although the LB SDS was obtained from only 20 participants, the data were used in the GLM with adjustment for gestational age and sex. The genotype with the G allele was significantly associated with shorter LB in the dominant model. Additionally, in the additive model, only the heterozygous genotype was significantly associated with shorter height, and in the recessive model, there was no significant association between genotypes and LB.

The association of rs724016 genotypes with the GH/IGF-1 axis, represented by BA-adjusted IGF-1 and IGFBP-3 levels, was also tested in the GLM that incorporated the same independent variables used to evaluate the anthropometric data as an outcome. The rs724016 genotypes showed no significant association with adjusted IGF-1 levels in any

Table 1

Characteristics of participants according to hydroxyurea therapy.

Parameters	n	Total	n	HU group	n	Non-HU group	p
Age, years	80	1226 (3.73; 20.42)	40	11.8 (4.08; 20.13)	40	13.6 (3.73; 20.42)	0.851
Male, n (%)	80	43 (53.8)	40	20 (50.0)	40	23 (57.5)	0.501
Prepubertal, n (%)	79*	40 (50.6)	40	21 (52.5)	39	19 (48.7)	0.737
Alpha-thal, n (%)	78*	11 (14.1)	38	5 (13.2)	40	6 (15.0)	0.815
VOC <12 months, n (%)	80	45 (56.2)	40	22 (55.0)	40	23 (57.5)	0.822
CTT, n (%)	80	10 (12.5)	40	9 (22.5)	40	1(2.5)	**0.014**
SS, n (%)	80	12 (15.0)	40	7 (17.5)	40	5 (12.5)	0.531
BMI (SD)	80	−0.80 (1.07)	40	−0.70 (1.00)	39	−0.90 (1.00)	0.398
Height (SD)	80	−0.77 (1.15)	40	−0.77 (1.03)	40	−0.77 (1.27)	0.986
TH (SD)	73*	−0.87 (0.77)	36	−0.74 (0.78)	37	−0.99 (0.74)	0.167
PAH (SD)	59*	−0.13 (−3.09; 4.70)	27	−0.19 (−2.81; 4.70)	32	−0.07 (−3.09; 4.42)	0.558
TH − PAH (SD)	54*	−0.55 (−6.48; 2.23)	30	−0.46 (−6.48; 1.67)	24	−0.79 (−5.83; 2.23)	0.462
CA − BA, years	59*	1.39 (0.34)	32	1.42 (1.32)	27	1.35 (1.61)	0.847
LB (SD)	20*	−0.38 (−4.89; 3.65)	11	−0.32 (−4.50; 1.37)	9	−0.42 (−4.89; 3.65)	0.552
SGA, n (%)	20*	2 (10.0)	9	1 (11.1)	11	1(9.1)	1.000
LBW, n (%)	63*	7 (11.1)	36	3(8.3)	27	4(14.8)	0.449
Hb, g/dl	79*	8.22 (1.20)	40	8.52 (1.06)	39	7.92 (1.27)	**0.026**
WBC, × 10^9/L	79*	11.32 (3.17)	40	9.73 (2.73)	39	12.95 (2.75)	**0.002**
HbF, %	71*	10.5 (1.8; 34.7)	38	13.6 (3.1; 31.8)	33	8.9 (1.8; 34.7)	**0.004**
Albumin (g/dL)	77*	4.49 (0.34)	39	4.46 (0.32)	38	4.52 (0.36)	0.431
Ferritin, ng/ml	77*	141.0 (17.0; 1950)	39	200 (23.1; 1242)	38	101.50 (17.0; 1950)	**0.008**
IGF-1-BA (SD)	56*	−1.19 (1.09)	32	−0.97 (1.15)	24	−1.48 (0.94)	0.084
IGFBP-3-BA (SD)	56*	−0.76 (−3.13; 0.79)	33	−0.36 (−3.13; 0.79)	23	−0.99 (−2.63; 0.08)	**0.005**

Data are presented as mean (SD), median (minimum; maximum), or n (%). VOC, vaso-occlusive crisis; VOC <12 months, at least 1 episode in the past 12 months; CTT, chronic transfusion therapy; SS, short stature; BMI, body mass index; TH, target height; PAH, predicted adult height; TH − PAH, TH minus PAH; CA, chronological age; BA, bone age; CA − BA, CA minus BA; LB, length at birth; SGA, small for gestational age; LBW, low birth weight (weight at birth of <2500 g); WBC, white blood cells; HbF, fetal hemoglobin; IGF-1-BA, IGF-1 adjusted for BA; IGFBP-3-BA, IGFBP-3 adjusted for BA.

* Variables with missing values.

genetic model. However, the homozygous genotype of the G allele was significantly associated ($p = 0.044$) with higher IGFBP-3 levels in an additive model. Regarding the other independent variables, HU therapy was significantly associated with adjusted IGF-1 and IGFBP-3 levels, and co-inheritance of alpha-thalassemia was significantly associated only with adjusted IGF-1 levels (Table 2).

We evaluated rs724016 genotypes as predictors of HbF levels, adjusting genotypes in the GLM to other independent variables capable of modifying HbF levels, such as age, sex, HU therapy, co-inheritance of alpha-thalassemia, and IGF-1 levels adjusted for BA. In the recessive
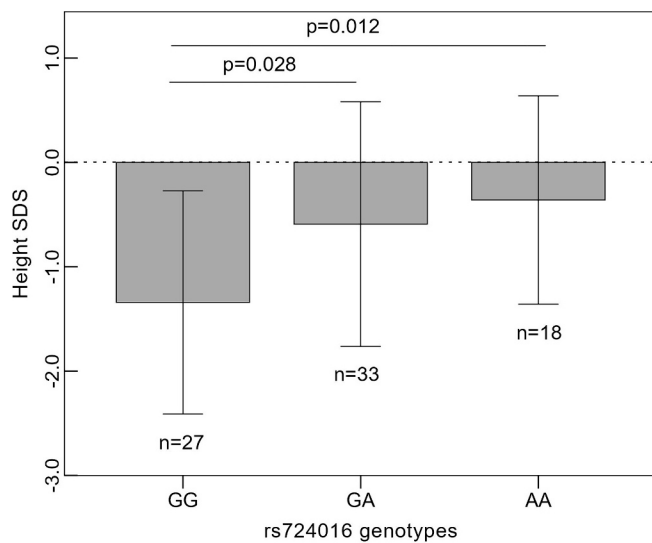
Fig. 1. Height of participants according to rs724016 (*ZBTB38*) genotypes.

model, predictive variables were not associated with HbF levels (*p* = 0.935). In the dominant model, the group with the genotypes with the G allele (GG and GA) was significantly associated with lower HbF levels (*p* = 0.016), and HU therapy was significantly associated with higher HbF levels (*p* = 0.030). Additionally, in the additive model, there were associations between rs724016 genotypes and HU therapy with HbF levels (Table 3).

4. Discussion

The prevalence of short stature was 15% in this cross-sectional study with participants aged 3 to 20 years with SCA. Our study showed an association of SNP rs724016 in the *ZBTB38* gene with shorter height in patients with SCA. Although HbF levels are recognized as a principal factor in reducing complications in SCA, they were not associated with height SDS or with IGF-1 and IGFBP-3 levels in our study. However, HbF levels showed a positive association with the growth potential of individuals with SCA, estimated by the deficit in predicting adult height adjusted for parental height (TH minus PAH). Additionally, the G variant of rs724016 showed a significant negative association with HbF levels, suggesting that this variant may negatively affect the height of individuals with SCA by being associated with lower HbF levels.

Height is a complex and highly polygenic genetic trait. GWAS primarily assess common polymorphisms, which, when considered collectively, can account for up to 45% of the variation in human height.

This estimation was derived from a study involving 5.4 million individuals from diverse ancestries and an evaluation of 12,111 SNPs [18]. These genetic variants can impact human height by influencing various tissues, including endocrine glands, bone structure, and growth plates. On an individual basis, each allele can have an impact on height of <0.5 cm [19]. Several GWAS studies have associated the G allele of rs724016 in the *ZBTB38* with greater height [10–12,20]. Although the direction of effect of our data was different from the GWAS, the association we found between the G allele of rs724016 and shorter LB has the same direction of effect as that of a recent study with a cohort of 261 children with short stature of undefined etiology [9]. GWAS involve large populations, but often the effect of variants found in a study does not apply to other populations. The lack of transferability between populations is due to failures in sample stratification, small samples with low statistical power, epigenetic factors, and different linkage disequilibrium pattern, which affects the interaction between genes [10,21]. Using epigenetic factors, the variants can interact with the environment and be heterogeneously associated with the phenotypes, which may explain the difference in the impact of some variants on height according to age, pubertal stage, and sex [9,10].

The SNP rs724016 is in the intron of the *ZBTB38* at 3q23, which encodes a zinc finger transcription factor that binds to methylated CpG dinucleotides at various sites in the human genome involved in the control of gene expression. Although the role of the ZBTB38 in height is not defined, its importance in the regulation of apoptosis leads to the assumption that its participation in the chondrocytes of the epiphyseal plate may impact on height [22]. Additionally, binding of ZBTB38 to the differently methylated *H19/IGF2* locus at 11p15 [23,24] associates its

Table 3
Association of rs724016 (*ZBTB38)* with HbF levels in a crude analysis or adjusted for other predictors, including age and sex.

	HbF (n = 48*)	
Predictors	Coefficient (CI 95%)	p
Crude analysis		
GG vs. AA	−0.26 (−0.70; 0.18)	0.251
GA vs AA	−0.45(−0.88; −0.02)	**0.038**
Adjusted analysis		
GG vs. AA	−0.43 (−0.85; −0.00)	**0.047**
GA vs AA	−0.58 (−1.01; −0.14)	**0.009**
HU group	0.34 (0.04; 0.64)	**0.024**
Alpha-thal	0.20 (−0.18; 0.59)	0.304
EZ IGF1-BA	0.12 (−0.03; 0.27)	0.116

HbF, fetal hemoglobin; HU group, hydroxyurea therapy group; BA, bone age; IGF-1-BA, IGF-1 adjusted for BA.
* Variables with missing values.

Table 2
Association of rs724016 (*ZBTB38*) with anthropometric data and with the GH/IGF-1 axis in a crude analysis or adjusted for other predictors, including age and sex.

	Height (SD) (n = 68*)		TH − PAH (SD) (n = 45*)		IGF-1-BA (SD) (n = 48*)		IGFBP-3-BA (SD) (n = 49*)	
Predictors	Coefficient (CI 95%)	p	Coefficient (CI 95%)	p	Coefficient (CI 95%)	p	Coefficient (CI 95%)	p
Crude analysis								
GG vs. GA + AA	−0.87 (−1.38; −0.25)	**0.001**	0.49 (−0.61; 1.60)	0.384	0.16 (−0.43; 0.77)	0.587	0.17 (−0.28; 0.63)	0.446
Adjusted analysis								
GG vs. GA + AA	−0.91 (−1.40; −0.41)	**<0.001**	0.65 (−0.33; 1.64)	0.195	0.070 (−0.42; 0.56)	0.782	0.35 (−0.04; 0.74)	0.082
Hb, g/dl	0.24 (0.01; 0.49)	0.066	0.26 (−0.32; 0.86)	0.376	0.01 (−0.28; 0.30)	0.935	−0.10 (−0.34; 0.12)	0.375
Ferritin, ng/ml	0.00 (−0.00; 0.00)	0.185	−0.00 (−0.00; 0.00)	0.310	0.00 (−0.00; 0.00)	0.406	0.00 (−0.00; 0.00)	0.258
HbF, %	−0.02 (−0.07; 0.01)	0.242	−0.09 (−0.19; −0.00)	**0.042**	0.02 (−0.02; 0.07)	0.346	0.00 (−0.03; 0.04)	0.886
Alpha-thal	−0.57 (−1.24; 0.08)	0.087	0.24 (−1.15; 1.64)	0.735	−0.65 (−1.28; 0.02)	**0.041**	−0.16 (−0.66; 0.34)	0.524
HU group	−0.11 (−0.61; 0.38)	0.652	0.762 (−0.20; 1.73)	0.124	0.55 (0.06; 1.05)	**0.027**	0.70 (0.31; 1.09)	**<0.001**

TH, target height; PAH, predicted adult height; TH − PAH, TH minus PAH; BA, bone age; IGF-1-BA, IGF-1 adjusted for BA; IGFBP-3-BA, IGFBP-3 adjusted for BA; HbF, fetal hemoglobin; HU group, hydroxyurea therapy group.
* Variables with missing values.

role in growth with increased IGF-2 synthesis, as occurs in some genetic syndromes. Like IGF-1, IGF-2 binds to receptors on growth plate chondrocytes involved in hypertrophic differentiation, which results in embryonic and postnatal growth [25]. Beckwith-Widermann hypergrowth syndrome is characterized by changes in the methylation pattern at the *H19/IGF2* locus, leading to an increase in *IGF2* expression in fetal life [26]. Silver-Russel syndrome is associated with a decrease in *IGF2* expression, which compromises intrauterine and postnatal growth [27]. Variants associated with complex traits such as height often act in gene regulation through DNA methylation [13], which may alter the expression of many genes and have a pleiotropic effect, as our result suggests when associating the SNP rs724016 in the *ZBTB38* with lower HbF levels in individuals with SCA. High IGF-2 levels can compromise the homeostasis and regeneration of hematopoietic stem cells, generating senescence of young hematopoietic cells [28,29]. Additionally, as primitive erythrocytes differentiate into a definitive lineage, there is also a decrease in the expression of the γ-globin gene, important for the synthesis of HbF [30]. HbF (α2γ2) is formed by 2 α- chains and 2 γ-globin chains that are encoded by two different genes located in the β-globin gene cluster. HbF protects people with SCA from complications of the disease by inhibiting the formation of deoxygenated Hb polymer, preventing cellular damage associated with hemolysis and vaso-occlusion [31]. Therefore, we consider that the effect of the G allele of rs724016 may decrease the synthesis of HbF by increasing *IGF2* expression, leading to permanent differentiation of hematopoietic stem cells into erythrocyte lineages that do not have HbF synthesis. Although elevated IGF-2 levels may be associated with greater height in other populations, we believe that low HbF levels have a greater impact on individuals with SCA, who start to have a greater number of vaso-occlusive crisis (VOC), which in turn secondarily compromise the GH/IGF-1 axis and chondrocytes of the epiphyseal plate (Fig. 2). On the other hand, the excessive production of reactive oxygen species (ROS) by HbS in SCA represents a significant therapeutic target of the disease due to its ability to promote vaso-occlusion, endothelial dysfunction, hemolysis, and inflammation [32]. In this regard, studies in human cells have demonstrated the importance of the ZBTB38 in controlling oxidative stress by limiting intracellular levels of ROS [33]. Therefore, the rs724016 of the *ZBTB38* may be associated with shorter stature in individuals with SCA due to its ability to increase oxidative stress, which leads to organic

dysfunction. For a better understanding of the presumed mechanisms, it is necessary to conduct functional studies with the ZBTB38, validate the results in studies with greater statistical power, evaluate the interaction with epigenetic mechanisms, and assess the existence of linkage disequilibrium variants capable of interfering with the results.

Changes in the GH/IGF-1 axis are often implicated in changes in the growth of individuals with SCA [6,34]. The pituitary gland is a well-known gland sensitive to iron overload injury, while in SCA the pituitary gland may also be susceptible to organic dysfunction, which can compromise the secretion of GH and the synthesis of IGF-1 and IGFBP-3. However, improvements in healthcare, particularly with the more frequent use of HU, have reduced the impact of the disease on growth [5,34,35]. HU can increase the number of erythroblasts synthesizing HbF in the bone marrow, and the elevation of HbF levels represents the main mechanism of action of the medication in preventing disease complications [31]. Numerous safety studies of HU therapy have contributed to its growing utilization worldwide [36]. However, in countries with limited resources, its usage remains less frequent or at lower doses due to concerns regarding myelotoxicity, which necessitates increased spending on clinical and laboratory monitoring of this complication [37].Therefore, despite many authors recommending HU for all children with SCA, only half of the study population had been using HU for at least 1 year. This is due to local guidelines that restrict its prescription to the most severe cases. This study shows a positive association of HU therapy with higher adjusted IGF-1 and IGFBP-3 levels, and with HbF levels. Although height is not significantly associated with HU therapy in our study, we believe this is justified by the observational characteristics of our study, in which only individuals with the most severe disease received HU therapy. The absence of a difference in reporting VOC in the last year between the groups of patients who underwent HU therapy and those who did not is also justified by the observational aspect of the study. Patients under HU therapy likely received the medication due to having a more severe disease, yet they behaved similarly to untreated patients regarding the frequency of VOC, the main complication of the disease. Thus, in the HU therapy group were also most patients under chronic transfusion therapy, which is known to compromise the GH/IGF-1 axis and height through iron overload without adequate chelation [35]. Although IGF-1 can compromise HbF synthesis by sharing the same intracellular signaling
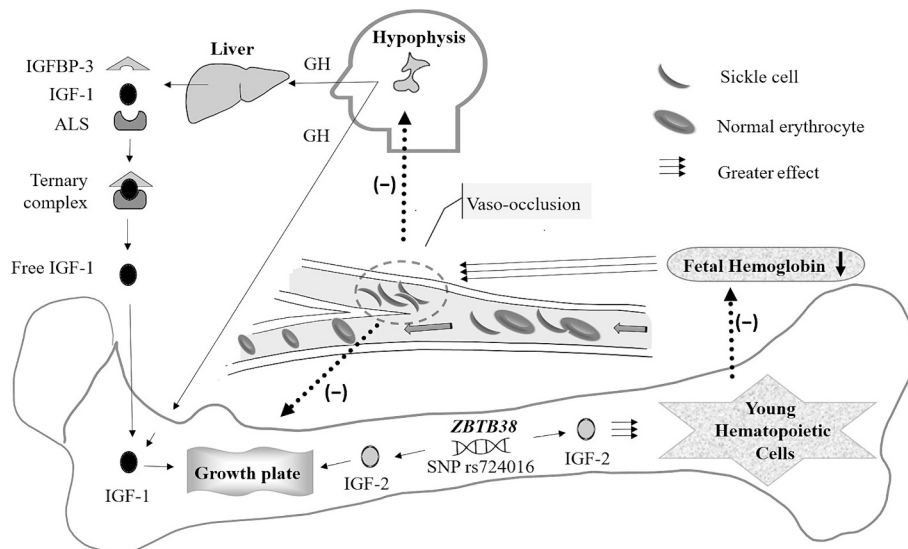

Fig. 2. Model for the association of the SNP rs724016 (*ZBTB38*) with the GH/IGF-1 axis, height, and fetal hemoglobin (HbF) levels in sickle cell anemia (SCA). The figure shows that despite the positive effect of the SNP rs724016 on the growth plate by increasing IGF-2 synthesis, its main effect in SCA depends on the negative impact of IGF-2 on young hematopoietic cells. As a result, lower HbF synthesis aggravates the disease, progressing with the impairment of the growth plate and the GH/IGF-1 axis.
ALS, acid labile subunit.

pattern with IGF-2 [38], our study does not show this association. Although the secretion of IGF-2 is not dependent on the secretion of GH, as is the secretion of IGF-1, IGFBP-3, and acid labile subunit (ALS), it is also secreted in the liver and dependent on the complex formed with the ALS and IGFBP-3. In this way, the performance of IGF-2 and IGF-1 in the tissues will depend on the availability of the free fraction of the hormone, which in turn depends on the degree of affinity that the hormone binding establishes with the complex and the stability that the complex provides by increasing the circulating half-life of IGFs [27].

Anemia has a multifactorial etiology and is characterized by a reduction in the ability to transport oxygen to tissues, which can impact the growth of children, especially younger ones [39–41]. Regarding children with hemoglobinopathies, the need for repeated transfusions can lead to iron overload, and if they do not receive adequate chelation, their growth may also be affected [42,43]. In our study, anthropometric outcomes and those related to the GH/IGF-1 axis were adjusted for Hb and ferritin levels; however, there was no significance in the association. Other studies evaluating children with sickle cell anemia confirm the lack of definition, where some authors mention the absence of association between Hb levels and anthropometric data [3], while a study that assessed a population of 33 adolescents with SCA showed a significant positive association between Hb levels and stature [44].

In the present study, we found co-inheritance of alpha-thalassemia in 14.1% of participants, consistent with global data that show the condition affecting around 10 to 30% of people with SCA [45,46]. Alpha-thalassemia is caused by impaired synthesis of the α-globin chain, which is regulated by four genes in two loci on chromosome 16p13.3. The impairment of one or two α-globin genes, the most common molecular defect, leads to mild forms of alpha-thalassemia, which may manifest solely with microcytosis [47]. Some complications of the disease such as stroke, cholelithiasis, and chronic kidney disease are less frequent in individuals with co-inheritance of alpha-thalassemia, while others such as osteonecrosis, splenic sequestration, and VOC are purportedly more frequent [48]. The decrease in the production of α-globin subunits in individuals with alpha-thalassemia co-inheritance favors the reduction of intracellular HbS concentration, resulting in decreased formation of deoxygenated Hb polymers, reduced hemolysis, and VOC, justifying the beneficial effects of the condition [49]. On the other hand, decreased hemolysis increases hematocrit and purportedly also red blood cell adhesiveness, favoring the increased frequency of VOC, explaining cases where co-inheritance exacerbate SCA complications [50]. Although adjusted IGFBP-3 levels and anthropometric data were not associated with alpha-thalassemia co-inheritance in our study, IGF-1 levels were negatively associated with *HBA* gene deletion. This pattern of association between IGF-1 levels and co-inheritance of alpha-thalassemia was previously reported in a study of children with SCA [6], suggesting the modulating effect that the condition establishes by predisposing to a greater number of vaso-occlusive events and disease complications [45].

The study has some limitations, such as the small sample size, which increases the risk of a type I statistical error when analyzing polymorphisms with an allele frequency >0.3 [51], and many missing data, especially those that depended on information regarding LB, BA, and HbF levels. The lack of BA data was primarily due to logistical challenges, as the examination wasn't available at the same facility where patients were seen. This missing BA data also affected other assessments, particularly the measurement of serum levels of IGF-1 and IGFBP-3, which were adjusted based on BA, as well as the calculation of PAH, reducing the accuracy of assessments involving these data. Missing data in other assessments, such as HbF levels, were due to occasional errors in the laboratory records. Birth length measurement is critical for assessing growth, as up to 15% of infants born SGA experience short stature unrelated to SCA. The complexity of BL measurement, requiring assistance and gestational age information, led to a higher rate of missing BL data compared to birth weight. However, positive events like a history of SGA births are more easily recalled, suggesting that missing birth data may

be due to its normalcy. Additionally, the history of low birth weight (LBW), including premature infants, was evaluated in a larger group (*n* = 63). The incidence of LBW in Latin America (9%) closely matches our findings (11%), indicating similar intrauterine growth issues between our study group and the general population. We considered the missing data to be random and chose not to exclude participants to maintain the statistical power for our main assessments, including the association of rs724016 with stature and HbF levels. Our approach contrasts with GWAS by including adjustments for various factors and using different genetic models, which enhances the depth of our analysis compared to the typical additive model used in GWAS studies.

5. Conclusion

In conclusion, our study in a population of individuals with SCA shows a negative association of a common variant in the *ZBTB38* (rs724016) with height, in the opposite direction of effect to GWAS. The study supports the importance of replicating data from large GWAS in other populations with distinctive characteristics that may alter the relationship between genes through epigenetic factors or linkage disequilibrium. Additionally, our data associates the SNP rs724016 with lower HbF levels, the main modifier factor of SCA.

CRediT authorship contribution statement

Domício Antônio Costa-Júnior: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Thaisa N. Souza Valente:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **André Rolim Belisário:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Gisele Queiroz Carvalho:** Writing – review & editing, Writing – original draft, Conceptualization. **Miguel Madeira:** Writing – review & editing, Writing – original draft, Conceptualization. **Cibele Velloso-Rodrigues:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors have no conflicts of interest to declare.

Data availability

Data will be made available on request.

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