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ORIGINAL PAPER

Haemoglobinopathies

BMP6 and *VDR* gene polymorphisms are associated with osteonecrosis in a sickle cell anaemia cohort

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Summary

The occurrence and severity of osteonecrosis in sickle cell anaemia (SCA) vary due to risk factors, including genetic modifiers. Bone morphogenetic proteins (BMPs), particularly BMP6, and the vitamin D receptor (VDR) play key roles in cartilage and bone metabolism, making them potential contributors to orthopaedic outcomes in SCA. Here, we evaluated the association of polymorphisms in *BMP6* (rs3812163, rs270393 and rs449853) and *VDR* (*FokI* rs2228570 and Cdx2 rs11568820) genes with osteonecrosis risk in a Brazilian SCA cohort. A total of 177 unrelated SCA patients were selected. The AA genotype of *BMP6* rs3812163 was independently associated with a lower osteonecrosis risk ($p = 0.015$; odds ratio (OR): 0.38; 95% confidence interval (CI): 0.18–0.83) and with the long-term cumulative incidence of osteonecrosis ($p = 0.029$; hazard ratio: 0.56, 95% CI: 0.34–0.94). The *VDR* rs2228570 TT genotype was independently associated with a lower osteonecrosis risk ($p = 0.039$; OR: 0.14; 95% CI: 0.02–0.90). In summary, our results provide evidence that *BMP6* rs3812163 and the *VDR* rs2228570 might be implicated in osteonecrosis pathophysiology in SCA and might help identify individuals at high risk.

KEYWORDS

avascular necrosis, bone morphogenic proteins, rs3812163, sickle cell disease, vitamin D receptor

INTRODUCTION

Osteonecrosis, also referred to as avascular necrosis (AVN), stands as a prevalent skeletal complication observed in sickle cell anaemia (SCA). This condition arises due to infarction within joint surfaces, ensuing from vaso-occlusive episodes within the intricate network of bone microcirculation. Over time, these occurrences culminate in the deterioration and eventual disintegration of articular cartilage.[1,2] The femoral head is the most common site affected, followed by the humeral head, the knee, the shoulders and the ankles.[3,4] The prevalence is higher among middle-aged individuals, and

bilateral involvement is a frequent finding.[5,6] Despite its high prevalence in SCA, the molecular mechanism behind osteonecrosis pathophysiology is complex and some studies suggest that its occurrence is influenced by genetic modifiers.[7–10]

Bone morphogenic proteins (BMPs), members of the TGF-beta superfamily, are multifunctional secreted proteins that play a pivotal role in bone formation, tissue development and morphogenesis.[11] Among the 20 different human BMPs described so far, BMP6 stands out due to its involvement with inflammatory processes, osteoblast differentiation, skeletal development and bone induction.[12–14] BMP6 is mainly expressed in cartilaginous tissues, and previous studies demonstrated that it plays a crucial role in cartilage cell proliferation and differentiation.[15,16] Moreover, BMP6 appears to be involved in osteogenic differentiation associated with parathyroid hormone (PTH) and vitamin D_3.[17]

Vitamin D_3 is a steroidal hormone involved in many metabolic pathways and classically acts by regulating intestinal calcium absorption, bone mineralization and bone mass turnover.[18,19] The vitamin D active metabolite, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), regulates genomic pathways by binding to its specific receptor, vitamin D receptor (VDR), which is expressed in almost all tissues and cells such as epithelial cells, osteoblasts, chondrocytes, vascular smooth muscle and immune system, among others.[20] VDR is a nuclear receptor, and after activation, through direct interaction with $1,25(OH)_2D$, binds to regulatory regions of target genes, so-called vitamin D responsive elements (VDREs), resulting in a specific transcriptional response.[21]

Considering the crucial role of BMP6 and VDR in bone homeostasis, cartilage differentiation and intestinal calcium absorption, genetic variations in their respective genes might be related to AVN pathophysiology in SCA. Single nucleotide polymorphisms (SNPs) located in intronic and 3′ untranslated regions of the *BMP6* gene were previously associated with osteonecrosis in three independent SCA cohorts.[7–9] Despite the lack of previous investigations with SCA, functional *VDR* SNPs *FokI* (rs2228570) and Cdx2 (rs11568820) were associated with bone-related diseases such as glucocorticoid-induced osteonecrosis,[22] bone mineral density,[23] lumbar spine pathologies[24] and others. *VDR FokI* is a start codon variant that affects VDR efficiency as a transcriptional activator, while Cdx2 is a variant situated in the promoter region at the 5′ end of the *VDR* gene, impacting *VDR* expression in the small intestine.[25,26] Therefore, here we evaluated the association of candidate genetic polymorphisms in *BMP6* and *VDR* genes with osteonecrosis susceptibility in SCA patients.

METHODS

Patients

Between January 2018 and March 2020, peripheral blood samples were collected of 177 unrelated SCA patients, regularly followed at a single reference centre in northeast Brazil. To confirm haemoglobin S homozygosity, all patients' samples were submitted to restriction fragment length polymorphism analysis of a PCR-amplified fragment of the beta gene (*HBB*) using *DdeI* enzyme and haemoglobin quantification by cation-exchange high-performance liquid chromatography (Variant IITM, Bio-Rad Laboratories, Hercules, CA, USA). Clinical and laboratory data were retrospectively obtained from the patients' medical records. All baseline laboratory characteristics were obtained from treatment-free periods for those who received clinical intervention, such as hydroxycarbamide (hydroxyurea) therapy or chronic blood transfusions.

Regarding the osteonecrosis development, patients were classified into case and control groups. The case group consisted of SCA patients with magnetic resonance imaging and/or x-ray with evident manifestations and clinical complaints resulting from osteonecrosis. The control group consisted of SCA patients over 18 years who did not present symptomatic evidence of osteonecrosis or other SCA clinical complications. The local research ethics board approved this study (#1.792.544) and following the Declaration of Helsinki, informed consent was obtained from all participants before the commencement of the study.

Molecular data

Peripheral blood samples were collected at a one-time point, at the time of patients' recruitment in vacuum tubes with EDTA as an anticoagulant, to extract DNA from leukocytes by a standard phenol–chloroform extraction method.[27] Three SNPs located in the 3′ untranslated region of *BMP6* gene rs3812163 (A>T), rs270393 (C>T), rs449853 (C>T), and two functional SNPs located in the *VDR* gene rs2228570 (*FokI*) and rs11568820 (Cdx2) with a minor allele frequency (MAF) higher than 10% for the African (YRI) and Caucasian (CEU) population were selected. Genotyping was performed by real-time PCR using TaqMan® probes, following the manufacturer's instructions. Gap-PCR technique was used to determine the coinheritance with $^{-3.7Kb}$alpha-thalassaemia deletion.[28] The β^S-globin gene cluster haplotypes were also determined as previously described.[29]

Statistical analyses

Patient baseline characteristics were reported descriptively. A chi-squared test was used to evaluate the Hardy–Weinberg equilibrium. Fisher's exact and chi-square tests were employed for genotype and allele frequency comparisons. The non-parametric Mann–Whitney and Kruskal-Wallis tests, followed by Dunn's multiple comparisons post-test, were used to analyse continuous variables. The risk was defined in terms of odds ratio (OR) with confidence interval (CI), with significance levels up to 95%. Cumulative incidence curves were constructed reflecting time to complication development, using the Kaplan–Meier method and the log-rank test to compare the curves.

Binary logistic regression and Cox proportional hazards regression analyses were performed to evaluate prognostic factors for osteonecrosis risk. Univariate logistic regression was performed for each genetic variant as a predictor for osteonecrosis. A multivariate logistic regression analysis was performed to evaluate whether the genetic factors were independently associated with osteonecrosis. Potential prognostic variables considered for model construction were included based on their biological significance for the pathophysiology of osteonecrosis. The covariates included were sex, age, haemoglobin and fetal haemoglobin (HbF) levels, and the number of vaso-occlusive crises *per year* (in the last year prior the end of the follow-up time). Statistical analysis was performed using SPSS Statistics 19.0 (IBM Corporation, Somers, NY, USA), with the level of significance set to 5%.

RESULTS

Patients had a median follow-up of 22 years (range: 4–39 years). The median age was 36 years (20–59 years), with 96 females (54.2%). Baseline clinical and laboratory data are described in detail in Table 1. Based on medical records, 82 patients presented confirmed osteonecrosis and were defined as the case group. The remaining 95 patients were free of evidence of osteonecrosis during the period of the study and were defined as the control group. Patients with osteonecrosis had higher vaso-occlusive crisis (VOC) episodes *per* year that required hospitalization ($p < 0.0001$) and lower HbF levels ($p = 0.019$). No association was observed between alpha thalassaemia −3.7-kb mutation (αα/−α and -α/−α) and β^S-globin haplotypes with osteonecrosis ($p > 0.05$; Table 1). Of note, 51% (90/177) of the patients were under hydroxycarbamide therapy during the study recruitment, not necessarily related to the presence of osteonecrosis.

All patients were fully genotyped for *BMP6* rs3812163, rs270393, rs449853, and *VDR* rs2228570 and rs11568820, with no deviation from Hardy–Weinberg equilibrium detected ($p > 0.05$; Table S1). For *BMP6* rs3812163, the A allele ($p = 0.002$) and the AA genotype ($p < 0.0001$) were associated with osteonecrosis frequencies. Moreover, the TT genotype of *VDR* rs2228570 polymorphism ($p = 0.01$) and the AA genotype of *VDR* rs11568820 polymorphism ($p = 0.03$) were associated with osteonecrosis frequencies (Table 1).

In univariate logistic regression analysis, AA genotype of *BMP6* rs3812163 polymorphism was significantly associated with lower osteonecrosis risk ($p = 0.001$; OR: 0.35; 95% CI: 0.19–0.64). In multivariate logistic regression analysis, *BMP6* rs3812163 AA genotype was independently associated with lower osteonecrosis frequency ($p = 0.015$; OR: 0.38; 95% CI: 0.18–0.83), considering sex, age, haemoglobin levels, HbF levels and number of VOCs *per year* as covariates (Table 2). For *VDR* rs2228570 polymorphism, TT genotype was associated with lower osteonecrosis risk ($p = 0.035$; OR: 0.19; 95% CI: 0.04–0.88) in univariate analysis and was independently associated with osteonecrosis in multivariate analysis ($p = 0.039$; OR: 0.14; 95% CI: 0.02–0.90). Finally, *VDR* rs11568820 AA genotype was associated with higher osteonecrosis risk in univariate analysis ($p = 0.032$; OR: 2.25; 95% CI: 1.07–4.74), although these data were not consistent with multivariate logistic regression analysis ($p = 0.363$; OR: 1.56; 95% CI: 0.59–4.07) (Table 2).

Cumulative incidence analysis showed that patients with AA genotype for *BMP6* rs3812163 had a lower cumulative incidence of osteonecrosis (51%, mean follow-up of 41 years, range: 37–44 years) compared to patients with AT and TT genotypes (84%, mean follow-up of 33 years, range: 30–36 years, $p = 0.002$; Figure 1). In Cox proportional hazards regression analysis, *BMP6* rs3812163 polymorphism was associated with lower risk of osteonecrosis in both univariate ($p = 0.013$; hazard ratio (HR): 0.56; 95% CI: 0.36–0.88) and multivariate analyses ($p = 0.029$; HR: 0.56; 95% CI: 0.34–0.94; Table 2).

For *VDR* rs2228570 polymorphism, patients with variant TT genotype had a lower cumulative incidence of osteonecrosis (38%, mean follow-up of 44 years, range: 40–48 years) compared to patients with CT and CC genotypes (85%, mean follow-up of 36 years, range: 33–38 years, $p = 0.046$; Figure 2). The prognostic value of *VDR* rs2228570 polymorphism was not confirmed in both univariate ($p = 0.094$; HR: 0.30; 95% CI: 0.07–1.22) and multivariate analyses ($p = 0.089$; HR: 0.29; 95% CI: 0.07–1.20) of proportional hazards regression (Table 2). *VDR* rs11568820 was not associated with cumulative incidence of osteonecrosis ($p > 0.05$).

DISCUSSION

Multiple mechanisms related to increased blood viscosity, such as high haematocrit or haemoglobin level, coinheritance with alpha thalassaemia and low HbF levels, have been linked to frequent VOCs and osteonecrosis predisposition.[30–33] Repeated VOCs contribute to impairment of cartilage blood flow, leading to ischaemia and tissue necrosis, especially on the femoral head.[31,34] Moreover, low fetal haemoglobin levels are associated with increased HbS polymerization and intravascular sickling, contributing to high viscosity and osteonecrosis.[7,32] Finally, the presence of genetic variants not linked to haemoglobin gene cluster might be an additional factor of AVN pathophysiology.[7–10] Here we demonstrated that genetic variants in *BMP6* (rs3812163 (A>T)) and *VDR* (rs2228570 (C>T) and rs11568820 (G>A)) genes might be implicated in osteonecrosis risk in a Brazilian cohort of SCA patients.

BMP6 plays a critical role in cartilage proliferation and differentiation[15] and contributes to bone formation and remodelling.[12] Findings with samples of osteonecrotic femoral head show that BMP6 is elevated in the necrotic area of bone tissue, suggesting a role of BMPs in bone repair after AVN.[35] Moreover, the expression of vascular endothelial growth factor and BMP6 induces angiogenesis and bone formation in vitro and in vivo, supporting the relevance of BMPs in AVN therapy.[36] In SCA, components of BMP6 and SMAD signalling partway were upregulated and induced apoptosis

TABLE 1 Baseline characteristics of SCA patients according to the avascular necrosis (AVN) status.

	All patients		AVN		Non-AVN		
	(*n* = 177)		(*n* = 82)		(*n* = 95)		
Characteristics of patients	**No.**	**%**	**No.**	**%**	**No.**	**%**	***p*-Value[a]**
Sex							
Female	96	54.2	38	46.3	58	61.1	0.069
Male	81	45.8	44	53.7	37	38.9	
Age (years), median	36		40		33		0.001[b]
Range	20–59		21–59		20–52		
VOCs/per year							
<3	79	44.6	19	23.2	60	63.2	<0.0001[b]
3–6	72	40.7	42	51.2	30	31.6	
>6	26	14.7	21	25.6	5	5.3	
RBC ($\times 10^{12}$/μL)	2.58 ± 0.49		2.61 ± 0.52		2.57 ± 0.46		0.852
Hb (g/dL)	8.0 ± 1.1		8.1 ± 1.1		8.0 ± 1.1		0.710
Haematocrit (%)	24.4 ± 3.4		24.7 ± 3.5		24.2 ± 3.4		0.250
HbF (%)	7.0 ± 4.9		6.5 ± 4.0		8.0 ± 5.3		0.019[b]
Reticulocyte (%)	9.5 ± 4.7		8.8 ± 4.3		9.6 ± 4.9		0.154
WBC ($\times 10^9$/L)	11.2 ± 3.6		10.9 ± 3.4		11.5 ± 3.7		0.051
Platelets ($\times 10^9$/L)	406 ± 136		390 ± 151		434 ± 123		0.169
TB (mg/dL)	3.1 ± 2.0		3.4 ± 2.4		3.0 ± 1.7		0.471
IB (mg/dL)	2.4 ± 1.9		2.7 ± 2.2		2.3 ± 1.6		0.494
LDH (U/L)	705 ± 522		770 ± 602		704 ± 469		0.711
Hydroxycarbamide therapy							
Yes	90	51	47	57.3	43	45.3	0.132
No	87	49	35	42.7	52	54.7	
β^S haplotype							
CAR/CAR	101	57.1	47	57.3	54	56.8	1.000
Non-CAR/CAR	76	42.9	35	42.7	41	43.2	
α-thalassaemia ($\alpha^{-3.7kb}$)							
Mutated	45	25.4	23	28.0	22	23.2	0.492
Non-mutated	132	74.6	59	72.0	73	76.8	
BMP6 rs3812163 (A>T)							
AA	89	50.3	30	37.6	59	62.1	
AT	77	43.5	45	54.9	32	33.7	<0.0001[b]
TT	11	6.2	07	8.5	04	4.2	
BMP6 rs270393 (C>T)							
CC	58	32.8	27	32.9	31	32.6	
CT	95	53.7	41	50.0	54	56.8	0.41
TT	24	13.6	14	17.1	10	10.5	
BMP6 rs449853 (T>C)							
TT	47	26.6	22	26.8	25	26.3	
TC	87	49.2	39	47.6	48	50.5	0.91
CC	43	24.3	21	25.6	22	23.2	
VDR rs2228570 (C>T)							
CC	88	49.7	38	46.3	50	52.6	
CT	76	42.9	42	51.2	34	35.8	0.01[b]
TT	13	7.3	02	2.4	11	11.6	

TABLE 1 (Continued)

Characteristics of patients	All patients (*n* = 177)		AVN (*n* = 82)		Non-AVN (*n* = 95)		*p*-Value[a]
	No.	%	No.	%	No.	%	
VDR rs11568820 (G>A)							
GG	38	21.5	20	24.4	18	18.9	
GA	102	57.6	39	47.6	63	66.3	0.03[b]
AA	37	20.9	23	28.1	14	14.7	

Note: Laboratorial parameters are described as median ± standard deviation. Mutated alpha thalassaemia is defined by one or two deletional α genes.

Abbreviations: CAR, Central African Republic; Hb, haemoglobin; HbF, fetal haemoglobin; IB, indirect bilirubin; LDH, lactate dehydrogenase; RBC, red blood cell; VOC, vaso-occlusive crisis; WBC, white blood cell.

[a]Fisher's exact or chi-squared tests for categorical variables. Mann–Whitney or Kruskal-Wallis tests for continuous variables.

[b]Statistically significant difference ($p < 0.05$).

TABLE 2 Binary logistic regression and Cox proportional hazards regression for osteonecrosis development.

	Risk of AVN				Cumulative incidence of AVN			
	OR	95% CI		*p*-Value	HR	95% CI		*p*-Value
Univariate analysis								
BMP6 rs3812163								
AA vs. AT/TT	0.35	0.19	0.64	0.001[a]	0.56	0.36	0.88	0.013[a]
VDR rs2228570								
TT vs. CC/CT	0.19	0.04	0.88	0.035[a]	0.30	0.07	1.22	0.094
VDR rs11568820								
AA vs. GG/GA	2.25	1.07	4.74	0.032[a]	1.29	0.79	2.11	0.304
Multivariate analysis								
BMP6 rs3812163								
AA vs. AT/TT	0.38	0.18	0.83	0.015[a]	0.56	0.34	0.94	0.029[a]
VDR rs2228570								
TT vs. CC/CT	0.14	0.02	0.90	0.039[a]	0.29	0.07	1.20	0.089
VDR rs11568820								
AA vs. GG/GA	1.56	0.59	4.07	0.363	1.01	0.57	1.77	0.968

Note: OR or HR >1or <1 indicate an increased or decreased risk, respectively, of an event for the first category listed. *p*-Values in multivariate analysis were corrected including sex, age, haemoglobin levels, fetal haemoglobin levels and number of vaso-occlusive crises *per year* as covariates.

Abbreviations: AVN, avascular necrosis; CI, confidence interval; HR, hazard ratio; OR, odds ratio.

[a]Statistically significant difference ($p < 0.05$).

in patients with orthopaedic complications.[37] Altogether, these findings suggest the application of BMP6 as a potential novel direct therapy for sickle osteonecrosis.

Studies with genetic variants indicate the association of BMP6 dysregulation at the genomic level with osteonecrosis in SCA. SNPs throughout the intronic and 3'untranslated region (UTR) of the *BMP6* gene (rs270393, rs267196, rs267201, rs449853 and rs1225934) were previously associated with the development of osteonecrosis in a cohort from the Cooperative Study of Sickle Cell Disease.[8] Also, the SNPs *BMP6* rs267196 and rs267201 were confirmed as relevant risk factors for this complication in SCA patients.[9] Although we did not replicate the associations with some of the previous SNPs, we found that the *BMP6* 3'UTR variant rs3812163 A allele and AA genotype were independently associated with a lower frequency and cumulative incidence of osteonecrosis. Similar findings by Ulug et al. (2008) also confirm the relationship of *BMP6* rs3812163 with osteonecrosis in sickle cell disease patients.[7] The mechanisms by which rs3812163 might impact the *BMP6* gene and orthopaedic complications are unknown; nevertheless, these results support the relevance of BMP6 in the molecular pathophysiology of sickle AVN.

We also describe a novel association of osteonecrosis with the functional SNPs rs2228570 and rs11568820 in the *VDR* gene. VDR is a ligand-dependent nuclear transcription factor, highly expressed in multiple tissues.[38,39] Classically, the effects of VDR/1,25(OH)$_2$D interaction include the

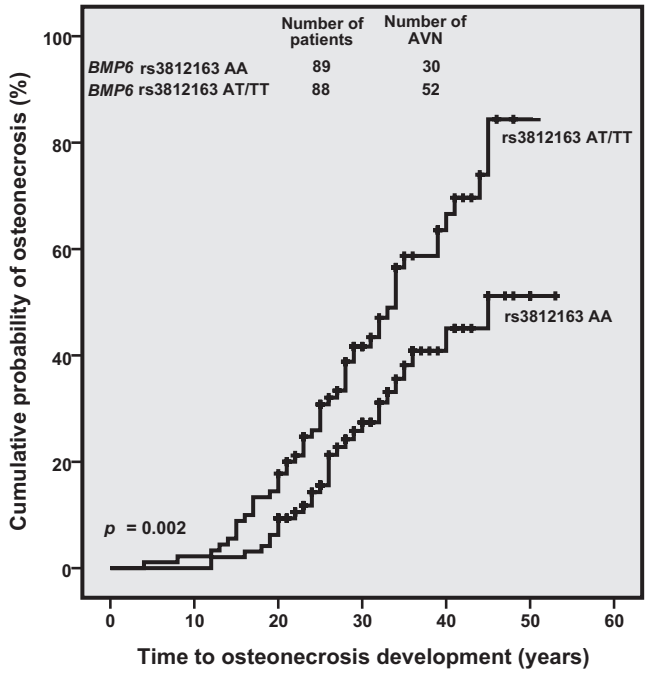

FIGURE 1 Cumulative incidence curve for osteonecrosis development. Cumulative incidence of osteonecrosis in patients with SCA according to the *BMP6* rs3812163 polymorphism.

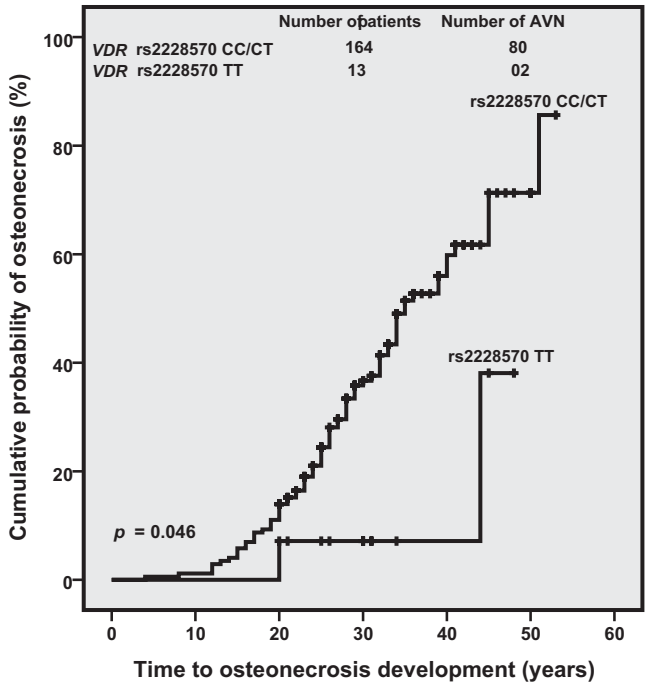

FIGURE 2 Cumulative incidence curve for osteonecrosis development. Cumulative incidence of osteonecrosis in patients with SCA according to the *VDR FokI* polymorphism (rs2228507).

regulation of bone metabolism mainly through the control of intestinal and renal reabsorption of calcium and phosphorus, which form the hydroxyapatite crystals that mineralize and strengthen the bones.[40] Previous evidence with microarray assays demonstrated that *VDR* was upregulated and might be involved in the development of glucocorticoid-induced avascular necrosis, possibly through interaction with PTH receptor 1.[41]

Here, *VDR* rs2228570 TT genotype was associated with low osteonecrosis frequency. *VDR* rs2228570, a start codon variant, results from the transition of cytosine to thymine at the junction of intron 1 and exon 2, producing two potential translation initiation start sites.[25] The C variant results in a shortened VDR protein with 424 amino acids, while the T variant produces a full-length VDR with 427 amino acids.[42] The smaller protein is more efficient as a transcriptional activator and interacts with higher efficiency with the transcription factor 2B.[25,43] rs2228570 TT genotype was previously reported as a protector factor for discopathies and osteochondrosis development in patients with lumbar spine pathologies.[24] Moreover, in children with acute lymphoblastic leukaemia, the rs2228570 CC genotype was associated with a higher frequency of osteonecrosis.[22,44]

While functional associations have been delineated, it is important to note that the interactions of both *VDR* rs2228570 isoforms may vary significantly depending on the specific tissue and target gene in question.[45] The decreased VDR efficiency associated with the rs2228570 T allele might induce the synthesis of vitamin D levels, contributing to healthier bone metabolism. In fact, lower concentrations of vitamin D (25(OH)D) were described for the rs2228570 CC genotype compared to the TT genotype in healthy adolescents,[46] patients with systemic lupus erythematosus[47] and multiple sclerosis.[48] Furthermore, the VDR protein with C allele is more functionally active and might overexpress genes containing VDR-responsive elements, dysregulating downstream pathways involved in sickle AVN pathophysiology.

VDR rs11568820 AA genotype, a variant in the regulatory region, was also associated with higher osteonecrosis frequency, although not further confirmed in the multiple logistic regression analysis. Cdx-2 polymorphism occurs at the 5′ terminal region of the intestinal transcription factor CDX-2 binding sequence. The CDX-2 binds with greater affinity to the sequence with A allele, increasing the transcriptional activity of *VDR* promoter region in the intestine.[26,49] Although our findings require confirmation in a larger cohort, it is plausible that rs11568820 may contribute to the dysregulation of *VDR* gene expression, thereby impacting orthopaedic complications in sickle cell patients.

While our findings provide insight into novel pathophysiological mechanisms involving *VDR* polymorphisms and confirm previous associations with *BMP6* and SCA osteonecrosis, our results are confronted with some limitations. First, patients were screened for orthopaedic complications only upon the presentation of symptoms. This fact is associated with challenges related to public healthcare and socio-economic factors that limit the accessibility to and availability of diagnostic technologies, such as MRI, in Brazil. Consequently, asymptomatic patients in the control group with early-stage osteonecrosis could not be identified,

since imaging screening is not performed routinely for all patients. Under these circumstances, we recognize that a definitive categorization between osteonecrosis cases and controls could not be established. Furthermore, a substantial portion of patients within the case group received diagnoses solely through x-rays examinations. This fact greatly impacts the definition of the degree of osteonecrosis by applying standardized staging criteria such as the Ficat and Arlet classification.[50]

Second, we could not determine vitamin D levels, nor *VDR* and *BMP6* transcripts levels to better understand their impact on SCA osteonecrosis and the genetic variants associations. Moreover, the small sample size might impact the power of our association analyses. Finally, the lack of validation in independent cohorts significantly restricts the extrapolation of our data to the general population.

In summary, *BMP6* rs3812163 and *VDR* rs2228570 were independently associated with low osteonecrosis risk in a SCA cohort. These findings provide new insights into the pathogenesis of sickle-AVN and eventually offer alternatives to identify patients at high risk, improving the surveillance for chronic complications and therapy in SCA.

AUTHOR CONTRIBUTIONS

G.S.A. performed experiments and statistical analyses, interpreted data and drafted the manuscript. M.B.S. performed experiments, updated the clinical data and interpreted data. I.F.D., D.A.P.M., D.A.F., J.V.B., B.L.H., M.V.D., A.P.S. and W.L.G. updated the clinical data and reviewed the manuscript. M.F.H. and A.S.A recruited patients, assured access to patients' samples and updated the clinical data. A.F.C., S.O.S., F.F.C., A.R.L-A. and M.A.C.B. conceived and designed the study, analysed and interpreted data, and reviewed the manuscript. M.A.C.B. gave the final approval of the version to be submitted.

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CONFLICT OF INTEREST STATEMENT

The authors have no competing financial interests to declare.

ETHICS STATEMENT

The local research ethics board approved this study (#1.792.544) and following the Declaration of Helsinki, informed consent was obtained from all participants before the commencement of the study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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