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Interaction Between Self-Reported Environmental Sensitivity and Genetic Protective Factors in Sickle Cell Anemia

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ABSTRACT

Background: Sickle Cell Anemia (SCA) is characterized by heterogeneous clinical presentations, partly explained by endogenous fetal hemoglobin (HbF) and α -thalassemia co-inheritance. Environmental exposures may modify these genetic protective effects. We examined whether self-reported environmental sensitivity modifies the association of HbF and α -thalassemia with disease severity in patients with SCA in Yaoundé, Cameroon.

Methods: In this cross-sectional, multicenter study, 125 patients aged ≥ 5 years with confirmed hemoglobin SS genotype were recruited from five healthcare facilities in the Centre region of Cameroon. Participants completed a questionnaire assessing self-reported sensitivity to cold, humidity, wind, dust, smoke, and seasonal vaso-occlusive crisis (VOC) patterns. Latent class analysis (LCA) was used to identify subgroups with similar environmental sensitivity profiles. We tested interactions between latent class, HbF, and the α -thalassemia 3.7 kb deletion on pain episode frequency and hospitalization rates using bias-reduced regression models.

Results: Two latent classes were identified based on their conditional probabilities. Latent Class 1 (18.4%, $n=23$) was characterized by high sensitivity to dust, smoke, and year-round VOC risk. Latent Class 2 (81.6%, $n=102$) was characterized by sensitivity to cold, wind, and humid conditions with rainy-season VOC peaks. The protective effect of HbF on pain frequency (IRR 1.17, 95% CI 1.02–1.34) and hospitalization (IRR 1.12, 95% CI 1.01–1.24) differed significantly by latent class, as did the protective effect of the α -thalassemia 3.7 kb deletion on hospitalization (IRR 0.29, 95% CI 0.10–0.89).

Conclusion: The clinical benefits of HbF and α -thalassemia co-inheritance in SCA are moderated by environmental sensitivity. These findings support gene-environment interaction among SCA patients in Cameroon and support individualized care that incorporates environmental risk profiling into routine standard practice.

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Received: September 10, 2026; **Accepted:** September 15, 2026; **Published:** September 25, 2026

Keywords: Sickle Cell, Fetal Hemoglobin, A-Thalassemia, Latent Class Analysis, Gene-Environment Interaction

Abbreviations

eGFR: Estimated Glomerular Filtration Rate

HbF: Fetal Hemoglobin

HBSS: Hemoglobin SS

IRR: Incidence Rate Ratio

LCA: Latent Class Analysis

LDH: Lactate Dehydrogenase

MCHC: Mean Corpuscular Hemoglobin Concentration

OR: Odds Ratio

SCD: Sickle Cell Disease

VOC: Vaso-Occlusive Crisis

Introduction

Sickle Cell Disease (SCD) arises from a point mutation in the 6th codon of the beta-globin gene on chromosome 11, giving rise to hemoglobin S (HbS). Polymerization of deoxygenated HbS within erythrocytes is the primary molecular event underlying all subsequent pathophysiology and clinical manifestations. Although hemoglobin polymerization is reversible upon reoxygenation, repeated cycles of deoxygenation and reoxygenation cause the cells to lose their elasticity. This results in permanently damaged, rigid, dehydrated cells with a characteristic sickled morphology [1]. Polymerization is favored by a number of endogenous factors including HbS concentration, cytosolic viscosity, red cell hydration, intravascular pH, delay time, and transit time [1-5]. These factors may be influenced by environmental factors, which

partly explain differences in severity among patients [2]. Such factors may reduce the delay time, favor fiber formation, and increase the risk of intravascular hemolysis, vaso-occlusion, and inflammation. In the presence of factors that favor longer delay time, most cells will transit small vessels before fibers form [6].

HbS polymer formation is correlated with the mean corpuscular hemoglobin concentration (MCHC), as well as with genetic factors such as α -thalassemia co-inheritance and fetal hemoglobin (HbF) [7-9]. Alpha-thalassemia co-inheritance, inheritance of altered genes involved in the downregulation of HbF expression, and HBB gene haplotypes have shown significant clinical benefits [9,10]. Additionally, hemoglobin levels are generally lower in individuals with hemoglobin SS (HbSS) genotype than in those with heterozygous SCD forms such as HbSC disease [11,12]. Alpha-thalassemia (predominantly the 3.7 kb and 4.2 kb deletions) is associated with a reduced risk of clinical complications linked to hemolysis, possibly through changes in erythrocyte density and inhibition of polymerization [10-13,14].

SCD is a clinically heterogeneous disease in which individuals with similar genotypes and haplotypes may present markedly different clinical severity phenotypes, so it is important to consider other contributing factors. These include environmental influences, which are often overlooked in clinical practice and differential diagnosis despite their well-documented clinical importance as triggers of episodic pain in SCD [15,16]. Stress, air pollution, and body temperature fluctuations are exogenous factors that may influence pathophysiological events in SCD through the autonomic nervous system, or a shift of the oxygen dissociation curve to the right favoring Hb polymerization [2-17]. While genetic inheritance may confer protection, the moderating influence of environmental susceptibility in individuals harboring these favorable disease-modifying genetic variants or who respond poorly to hydroxyurea is poorly understood [18]. Studying the interaction between genetic modifiers of disease and environmental factors will require quantifying individual exposures, which are hard to implement in real-life clinical practice. However, environmental exposure questionnaires can be used in clinical practice to assess exposure history to environmental factors [19,20].

Given the complex pathophysiological nature of SCD, intraindividual differences in disease-modifying and environmental factors interplay during steady state and during sickle crisis. We postulated that the protective benefits of HbF and α -thalassemia co-inheritance are attenuated in patients with high self-reported sensitivity to certain environmental influences. The findings will provide preliminary evidence for a systematic environmental-trigger risk assessment tool for SCD patients in clinical practice and will serve as the basis for a risk-stratification toolkit applicable to SCD patients in Cameroon, without requiring costly infrastructure.

Materials and Methods

Study Design

This study recruited 150 patients in a cross-sectional multicenter design conducted between January 2024 and January 2025. Patients were recruited from the Yaoundé Central Hospital, Cité Vert District Hospital (Yaoundé), the Yaoundé Gyneco-Obstetric and Pediatrics Hospital, the Centre D'animation Sociale et Sanitaire Yaoundé, and Mbalmayo District Hospital, reflecting a mix of different healthcare tiers to capture a representative sample of the study population and account for resource availability, given that healthcare in Cameroon is largely paid out-of-pocket.

Participants and Recruitment

Blood samples were collected from 125 documented hemoglobin SS patients aged ≥ 5 years, corresponding to the age at which baseline HbF levels stabilize [21]. Only participants who had resided in the Centre region for at least one year, to minimize variation in environmental exposure history, were recruited. All included patients had not experienced any painful crisis—defined as acute mild-to-severe pain or severe anemia with no underlying cause other than vaso-occlusive crisis (VOC)—in the past 4 weeks. Participants were required to have attended the same healthcare clinic for at least 12 months to facilitate retrieval of medical records. Patients who had received a blood transfusion or hydroxyurea during the past 3 months were excluded to minimize baseline bias in laboratory markers. Individuals on long-term daily steroids or non-steroidal anti-inflammatory drugs were also excluded. Each patient underwent a physical examination prior to blood collection. The study was approved by each participating hospital's institutional review board. Participants (or their parents/guardians) provided signed and dated informed consent, with assent obtained from children where applicable.

Clinical History Data Extraction

Patients' history of severe pain episodes, hospitalizations, transfusion sessions, avascular necrosis, cardiopulmonary involvement, and leg ulcers during the past 12 months were recorded. Cardiopulmonary involvement was defined as a history of systemic hypertension ($\geq 130/85$ mmHg) and/or partial oxygen saturation $<90\%$ (measured with an oximeter) during 2 or more conservative crises in the past 12 months. History of chest pain during the past 12 months was also recorded. These data were extracted from patient-held health booklets and hospital inpatient files from each participating institution. A patient-reported health questionnaire consisting of open-ended questions assessing history of illness was also administered. A hospitalization was defined as an inpatient stay greater than 20 hours due to pain episodes, VOC, anemia, or other SCD-related complications.

Self-Reported Environmental Exposure

Patients completed self-reported questionnaires on exposure to cold, heat, windy weather, humidity, outdoor/indoor dust, and indoor/outdoor smoke, as well as seasonal patterns of pain episodes, over the past 2 years. The questionnaire was a cross-sectional tool (Supplementary Table S1) intended to collect data on self-reported perceived susceptibility, to determine whether exposure consistently coincided with mild-to-severe episodic pain or other adverse effects 1–2 days post-exposure. The questions used forced-choice (yes/no) response options for exposure history items, in line with clinical practice recommendations for assessing physical environmental exposure [19,20,22]. Because individuals may experience multiple exposures simultaneously and at varying intensities, exposure clusters were derived using latent class analysis (LCA) as previously described [23].

Laboratory Testing

Venous blood samples were immediately placed on ice and protected from light. Serum was extracted and stored at -20°C in aliquots for later biochemical analysis. Hematologic profiles were obtained from EDTA samples using automated analyzers (HumaCount 5D, HUMAN, Germany; or DYMIND, Shenzhen, China). Hemoglobin F concentration was determined by capillary zone electrophoresis (Sebia, Norcross, USA) or agarose gel electrophoresis at Centre Pasteur Yaoundé. Hemoglobin F levels were used as a surrogate marker for the genetic polymorphisms affecting HbF expression, reflecting real-world clinical applicability, as HbF levels are easier to measure than

the underlying genetic polymorphisms. For biochemical assays, serum samples were thawed slowly on ice, mixed, centrifuged, and equilibrated to room temperature before analysis within 30 minutes [24]. All samples had undergone no more than one freeze-thaw cycle prior to analysis [25,26]. Commercial assays were used to measure total and direct bilirubin (SGM, Italy), total LDH (Wiener Laboratories, Barcelona, Spain), alanine aminotransferase and aspartate aminotransferase (SGM, Italy), and serum creatinine (SGM, Italy) according to the manufacturer's instructions. Serum nitrite was quantified using the Griess assay (Carl Roth GmbH, Germany) after deproteinization with chilled absolute ethanol [27]. Indirect bilirubin was determined as the difference between total and direct bilirubin. The estimated glomerular filtration rate (eGFR) was derived from serum creatinine concentration using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) 2021 creatinine equation for individuals aged 18 years and older [28], and the quadratic equation for those younger than 18 years [29].

PCR Assays

Genomic DNA was isolated using the Quick-DNA miniprep kit (Zymo Research, USA) according to the manufacturer's instructions. Hemoglobin genotypes were confirmed by PCR-RFLP using primers and procedures as previously described [30]. Multiplex gap-PCR was performed to detect α -3.7 and α -4.2 kb deletions using primers and procedures as previously described, with minor modifications [31]. Briefly, the reaction mixture contained five primers for the deletions, were optimized by adding 5 M betaine (Glenthams Life Sciences, UK) and 10% dimethyl sulfoxide (Glenthams Life Sciences, UK) to a final volume of 25 μ l. Analyses were conducted at LANAVET Yaoundé [31].

Statistical Analysis

LCA was used to identify subgroups of participants based on patterns of self-reported environmental sensitivity, using the approach suggested by Ko et al. (2016) [23]. LCA was performed using the poLCA (version 1.6.0.1) package in R (version 4.3.2) [32]. Models with 1 to 6 latent classes were fitted with 20 random sets to avoid local maxima, and the number of classes was selected based on BIC, entropy, and clinical interpretability as recommended by Nylund et al. (2007) [33].

Preliminary association tests between latent class and clinical and laboratory parameters were conducted using unadjusted quasibinomial logistic regression with latent class posterior probabilities as weights. The effects of the interaction between latent class and HbF levels and between latent class and the α -thalassemia 3.7 kb deletion on clinical outcomes were tested using bias-reduced regression methods with latent class posterior probabilities as weights. This approach accounted for imbalances in α -thalassemia groups, differences in latent class sizes, and overdispersion in count outcomes. Variables were selected based

on their ease of measurement in primary clinical settings and their influence on polymerization and hemolysis in SCD. They were further prescreened using Least Absolute Shrinkage and Selection Operator (LASSO) regression from a panel of hematological and biochemical markers [34]. Analyses were conducted using R, version 4.3.2 [35]. Statistical significance was set at the 0.05 level.

Results

Latent Classes of Self-reported Sensitivity to Environmental Influences

Fit statistics for models with 1 to 6 classes are presented in Supplementary Table S2. The 2-class model was retained as the final model based on its lower BIC, adequate entropy, adequate class size, and theoretical interpretability. The 2 classes were labeled based on their class-response probability profiles (Supplementary Figure S1): Latent Class 1 (elevated sensitivity to atmospheric pollutants group), comprising 18.4% of the sample ($n = 23$), was characterized by a high probability of reporting VOC year-round (particularly in the dry season) and greater sensitivity to ambient dust and smoke fumes. Latent Class 2 (elevated sensitivity to cold-weather group), comprising 81.6% ($n = 102$), showed a greater probability of VOC during rainy, windy, humid, and cold conditions.

Association of Latent Class Membership with Laboratory and Clinical Features

All individuals included in this study had a confirmed HbSS (sickle cell anemia-SCA) genotype, as revealed by PCR-RFLP. The mean age was 18.7 years (range, 5 to 50 years), with 63 females and 62 males. Comparisons of the latent classes across hematological, biochemical, and clinical phenotypes using unadjusted logistic regression yielded varying results (Table 1). Latent Class 1 membership was marked by higher MCHC and RDW but lower PCV and platelet LCR. Even though the hemolytic markers LDH and total/indirect bilirubin were not significantly different, they showed an increasing trend toward Class 1. Regarding clinical phenotypes, Latent Class 2 had substantially higher odds of experiencing ≥ 1 pain episode per year (OR 4.19, 95% CI 2.13–8.61, $p < .001$). The eGFR, reflecting renal function, was mildly lower in Class 2, a small but statistically significant difference. Cardiopulmonary involvement, defined here as a history of systemic hypertension ($\geq 130/85$ mmHg) and/or oximeter PaO₂ $< 90\%$ during 2 or more conservative crises in the past 12 months, showed that Class 2 had lower odds of this complication (OR 0.11, 95% CI 0.02–0.54, $p = .006$). Latent class differences in baseline polymerization marker (MCHC), pain frequency, and cardiopulmonary and renal function may suggest environmental exposure classes with partially distinct clinical phenotypes rather than purely differences in disease severity. However, these crude estimates from the bivariate analysis do not account for baseline imbalance.

Table 1: Association of Latent Class with Laboratory and Clinical Characteristics

Variable	Class 1 (n = 23)	Class 2 (n = 102)	OR (95% CI)	P
	Mean (SD)	Mean (SD)		
Hb (g/dL)	7.37 (1.0)	7.59 (1.5)	1.12 (0.89–1.42)	0.336
MCHC (g/dL)	36.61 (4.2)	34.80 (2.9)	0.86 (0.78–0.94)	0.001
MCV (fL)	78.73 (9.6)	80.01 (11)	1.01 (0.98–1.04)	0.466
PCV (%)	20.38 (3.5)	21.98 (4.6)	1.09 (1.01–1.18)	0.027
RDW (%)	22.57 (3)	20 (4.4)	0.86 (0.79–0.93)	0.001
Platelets ($\times 10^9/L$)	369.87 (150.4)	410.32 (201.2)	1.00 (0.99–1.01)	0.200
Platelets LCR (%)	24.93 (9.2)	28.83 (10.2)	1.04 (1.01–1.08)	0.019
Granulocytes ($\times 10^9/L$)	7.59 (4.9)	6.79 (4.5)	0.96 (0.90–1.03)	0.282
Hemoglobin F (%)	13.54 (6)	11.42 (7)	0.96 (0.92–1.00)	0.061
Hemoglobin A2 (%)	2.73 (0.9)	3.07 (0.8)	1.67 (1.12–2.53)	0.013
LDH (IU/L)	195.76 (88.8)	192.92 (66.4)	1.00 (0.99–1.01)	0.804
Nitrite (μM)	21.36 (12.1)	18.31 (11.7)	0.98 (0.96–1.01)	0.115
Total bilirubin (mg/dL)	1.37 (1.1)	1.13 (1)	0.82 (0.62–1.10)	0.171
Indirect bilirubin (mg/dl)	0.85 (1.1)	0.71 (0.9)	0.87 (0.64–1.22)	0.381
eGFR mL/min/1.73 m ²	118.75 (47.4)	103.44 (42.9)	0.99 (0.99–1.00)	0.035
AST (IU/L)	24.99 (5.5)	25.95 (7.6)	1.02 (0.98–1.08)	0.420
ALT (IU/L)	30.18 (7.8)	28.35 (9.8)	0.98 (0.95–1.01)	0.242
	n (%)	n (%)		
3.7 kb α-thalassemia			0.46 (0.22 – 0.97)	0.036
Normal	16 (70)	85 (83)		
Silent carrier/trait	7 (30)	17 (17)		
Pain frequency			4.19 (2.13–8.61)	<0.001
0	16 (70)	36 (35)		
≥ 1	7 (30)	66 (65)		
Hospitalization			1.00 (0.49–1.98)	0.992
0	7 (30)	31 (30)		
≥ 1	16 (70)	71 (70)		
Transfusion			1.47 (0.75–3.02)	0.270
0	16 (70)	62 (61)		
≥ 1	7 (30)	40 (39)		
Avascular necrosis				
			5.34 (0.30–94.37)	0.144*
No	23 (100)	92 (90)		
Yes	0 (0)	10 (10)		
Cardiopulmonary			0.11 (0.02–0.54)	0.006
No	19 (83)	100 (98)		
Yes	4 (17)	2 (2)		
Chest pain			0.78 (0.39–1.62)	0.494*
No	16 (70)	76 (75)		
Yes	7 (30)	26 (25)		
Ulceration			0.53 (0.07–3.77)	0.542*
No	22 (96)	99 (97)		
Yes	1 (4)	3 (3)		

OR, unadjusted odds ratio; CI, confidence interval. Estimates are from unadjusted quasibinomial logistic regression models with pseudo-class weighting based on posterior probabilities from LCA. Class 1 is the reference.

*Firth's penalized logistic regression

$p < 0.05$ significance level

Interaction of α -Thalassemia 3.7 kb Co-inheritance and Fetal Hemoglobin with Environmental Exposures on Clinical Outcomes

To assess whether the protective clinical benefits of α -thalassemia co-inheritance and HbF were moderated by patients' sensitivity to environmental influences, regression models were fitted with interaction terms between latent class, HbF, and the α -thalassemia 3.7 kb deletion. Predictor selection was prioritized based on markers that are cheaper, faster, easily measurable, and clinically relevant in routine care. Thus, markers of polymerization (MCHC) and hemolysis (LDH) were selected. The results of these interaction effects on the frequency of pain episodes and hospitalizations are shown in Table 2 and Figure 1. While the main effect of both HbF and Latent Class 2 showed independent associations with baseline pain rate ratios, their interaction revealed effect modification. Specifically, the association between HbF and pain frequency significantly differed by Latent Class cluster membership (IRR 1.17, 95% CI 1.02–1.34, $p=.021$), indicating that the protective association of HbF observed in Latent Class 1 was weakened in the cold-weather-sensitive group (Latent Class 2), with a combined IRR for HbF in Class 2 of 0.98. Similarly, hospitalization

frequency differed by Latent Class. The Latent Class 2 $\times$ HbF interaction was significant (IRR 1.12, 95% CI 1.01–1.24, $p=.030$). Considering the combined effect for HbF in Latent Class 2 (IRR $\approx$ 1.03), a 1% increase in HbF was associated with a 3% higher hospitalization rate in this cluster. As shown in Figure 1, Latent Class 1 individuals with baseline HbF $<10\%$ had higher expected rates of pain episodes and hospitalization compared to those in Latent Class 2 with comparable α -thalassemia co-inheritance.

There was no evidence of pain-rate modification by latent class for the 3.7 kb α -thalassemia deletion. On the other hand, the main effect of 3.7 kb α -thalassemia silent-carrier or trait status was associated with an increased hospitalization rate ratio in the reference latent subgroup (Latent Class 1) (IRR 3.00, 95% CI 1.15–7.84, $p=.025$). The latent class $\times$ 3.7 kb α -thalassemia interaction was significant (IRR 0.29, 95% CI 0.10–0.89, $p=.031$), indicating that the strong association of 3.7 kb α -thalassemia silent-carrier or trait co-inheritance in Class 1 was reduced by 13% in Class 2 (combined IRR 0.87). The main effect of latent class on hospitalization rate was not statistically significant (IRR 0.39, 95% CI 0.11–1.40, $p=.151$).

Table 2: Effect of Environmental Influence Interactions with 3.7 kb α -Thalassemia Co-Inheritance and HbF Levels on Pain Episodes and Hospitalization Frequency During the Past 12 Months

Predictor	Pain frequency		Hospitalization	
	IRR (95% CI)	P^*	IRR (95% CI)	P^{**}
Intercept	28.30 (3.29–243.21)	0.002	0.09 (0.01–1.06)	0.056
Age (years)	1.01 (1.00–1.03)	0.019	1.02 (1.00–1.03)	0.063
MCHC (g/dL)	0.96 (0.92–1.01)	0.152	1.09 (1.03–1.15)	0.004
LDH IU/L)	1.00 (1.00–1.02)	0.576	1.01 (0.98–1.04)	0.349
Latent Class 2	0.25 (0.07–0.88)	0.030	0.39 (0.11–1.40)	0.151
HbF (%)	0.84 (0.74–0.96)	0.012	0.92 (0.83–1.02)	0.097
α -3.7 kb silent Carrier/trait	0.66 (0.23–1.89)	0.434	3.00 (1.15–7.84)	0.025
Latent Class 2 $\times$ HbF	1.17 (1.02–1.34)	0.021	1.12 (1.01–1.24)	0.030
Latent Class 2 $\times$ α -3.7 kb silent Carrier/trait	0.96 (0.31–3.01)	0.941	0.29 (0.10–0.89)	0.031
R ² Nagelkerke	0.4		0.2	

CI: Confidence Interval; **IRR:** Incidence Rate Ratio

Note: $p < 0.05$. * Calculated using Hurdle negative binomial regression; ** Calculated using mean bias-reduced negative binomial regression. Regression analyses were conducted using latent class posterior probabilities as weights.

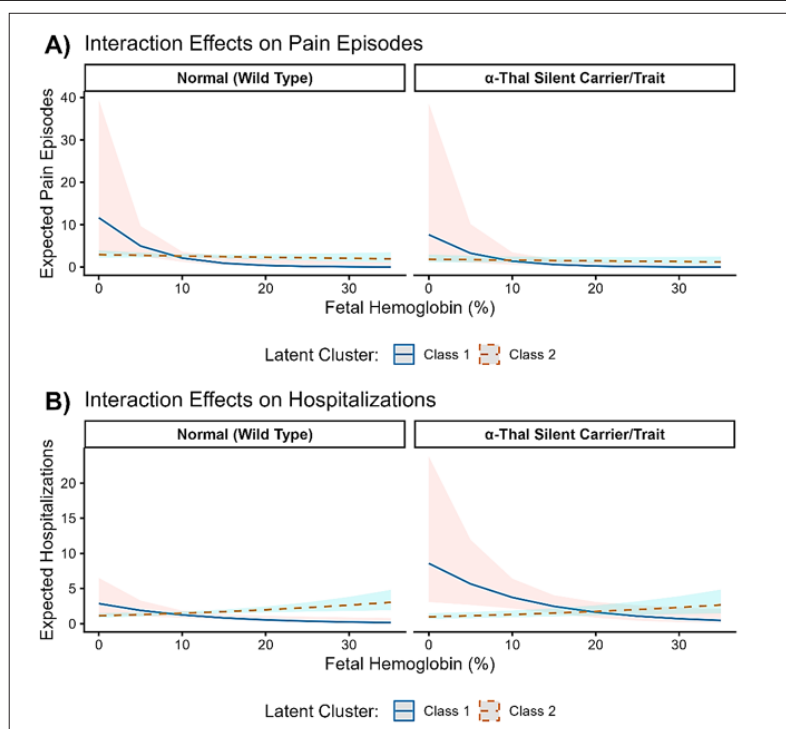


Figure 1: Interaction Plots. A) Interaction Effect on Pain Episodes Between Latent Class Membership and Fetal Hemoglobin Stratified by 3.7 Kb A-Thalassemia Silent Carrier/Trait Co-Inheritance. b) Interaction Effect on Hospitalizations between Latent Class Membership and Fetal Hemoglobin Stratified by 3.7 Kb A-Thalassemia Silent Carrier/Trait Co-Inheritance.

Analysis of a select set of clinical indicators including history of avascular necrosis, leg ulcer, chest pain, cardiopulmonary risk, and eGFR <60 mL/min/1.73 m² showed no influence of the interaction between HbF and 3.7 kb α-thalassemia with latent class (Figure S2 and Figure S3).

Discussion

Environmental Sensitivity Profiles Are Associated with Hematological Indices

In this study, we explored the moderating influence of self-reported environmental sensitivity to cold, heat, windy weather, high humidity, dust exposure, smoke exposure, and seasonal changes on the protective effects of HbF and α-thalassemia co-inheritance, focusing primarily on pain episodes and hospitalizations during the past 12 months among 125 children and adults with SCA in Yaoundé. The negative effects of environmental factors such as infection, stress, anxiety, socioeconomic factors, climate, weather, and pollution on SCD have been well documented [15,16]. Since exposure to these environmental factors seldom occurs in isolation, we used LCA to group patients with similar self-reported exposure patterns in relation to crisis episodes and VOC. The joint effect of environmental exposures provides a more realistic measure of influence on clinical outcomes than testing each factor individually [23]. We selected sensitivity to cold, heat, humidity, wind, indoor/outdoor dust, smoke, and seasonal patterns based on the fact that these exposures are easily recalled and identified by patients; they represent established environmental domains (temperature extremes, air pollution, and humidity) previously linked to SCD complications [16,36–38], and together, they form a composite surrogate for physical exposure to air pollutants (particulate matter, nitric oxide, nitrogen dioxide, and carbon monoxide) and daily environmental effects on health, without requiring quantitative monitoring devices [39–41]. This approach prioritizes clinical applicability for systematic environmental risk profiling at routine clinic visits. We identified two self-reported environmental sensitivity subgroups of patients. Latent Class

1 was made up of individuals who had a greater probability of reporting sensitivity to indoor/outdoor dust and smoke and who were prone to VOC year-round. This group of patients may be more sensitive to the adverse effects of dust and smoke particulate toxicants during the dry season. The individuals in Latent Class 2 had a high conditional probability of crisis episodes or VOC, most often during cold, windy, and humid conditions, particularly in the rainy season.

Unadjusted logistic regression analysis of the association between latent class and baseline hematological profiles revealed mild but significant differences in blood indices between Latent Class 1 and Latent Class 2. On average, MCHC and RDW were higher in Class 1, while PCV was higher in Class 2. These indices are correlated with baseline ongoing polymerization in SCD [7]. Furthermore, markers of hemolysis including LDH and indirect bilirubin were not associated with class membership. In this study, a nonsignificant trend toward higher hemolytic markers was observed in the high-pollutant-sensitivity latent subgroup (Class 1). Studies providing direct evidence of the effects of environmental factors on hematologic and hemolytic parameters in SCD are limited. One study found an inverse correlation between increased exposure to pollutants such as PM10 (from dust) and nitric oxides and both total bilirubin and LDH among children with SCA [42]. Consistent with our findings, that study found no association with WBC. However, in a recent study, higher annual WBC and absolute neutrophil counts were associated with increased annual PM2.5 exposure [43]. We found evidence of higher baseline ongoing polymerization in the high-pollutant-sensitivity latent group, as reflected in the bivariate associations between latent class and MCHC, the 3.7 kb α-thalassemia deletion, and RDW. These indices were not reported in the other studies. Methodological differences between our approach and those of other studies, namely, our reliance on patient-reported exposure and the joint effect of environmental factors rather than testing each pollutant individually may explain these differences in results.

Environmental factors are Modifiers of Fetal Hemoglobin and α -Thalassemia Protection

A natural question that arises is why some SCA patients living in the same geographical location, with similar haplotypes, or comparably high HbF, still differ markedly in their clinical manifestations. Part of this residual variability is explained by α -thalassemia co-inheritance and the loci regulating HbF expression, both established modifiers of disease severity [9]. However, genetic factors alone are insufficient to explain the phenotypic clinical heterogeneity observed among patients. This study specifically aimed to partly address this phenomenon by exploring whether the protective effects of α -thalassemia co-inheritance and HbF are uniform across patients within latent class patterns of environmental exposure, or whether environmental sensitivity constitutes a modifier of genetic protection itself.

The interaction between genetic and environmental factors has been postulated as a possible explanation for clinical variability. The interaction effects between latent class membership and 3.7 kb α -thalassemia co-inheritance and between latent class membership and HbF showed that the primary clinical outcomes (pain episodes and hospitalization) in a 12-month period were class-dependent (Table 2 and Figure 1). The significant Latent Class 2 $\times$ HbF interaction indicates heterogeneity in the HbF–pain relationship across latent subgroups. Fetal hemoglobin appears protective in Latent Class 1 compared to Class 2 when HbF was $>10\%$, irrespective of the α -3.7 kb thalassemia status, though the difference in expected pain rate appeared milder in the α -3.7 silent carrier/trait group. A similar pattern was observed with hospitalizations, although HbF required a much higher threshold ($>20\%$) to have a protective benefit among those with α -3.7 kb thalassemia in the Class 1 group. Thus, for low HbF ($<10\%$), Class 2 had few expected pain episodes and hospitalizations, even though the uncertainty in the Class 1 group at this threshold was large. This pattern suggests subgroup-specific biology or exposure profiles that modify HbF's effect. Patients characterized by a pattern of high probability of reporting VOC, particularly in the dry season, and sensitivity to ambient dust and smoke fumes were at higher risk of pain and hospitalization when their HbF was below 10%, but benefited more from the protective effects of HbF than those with a greater probability of VOC during rainy, windy, humid, and cold conditions, once their HbF rose reasonably above 10%. Since none of the individuals in this study had received hydroxyurea in the past 3 months, it is plausible that patients who appear non-responsive to hydroxyurea should be evaluated for environmental sensitivity effects before dose escalation to the maximum tolerated dose, especially if their baseline HbF is $<10\%$, or if they are prone to year-round VOC pain and sensitive to the adverse effects of dust and smoke toxicants (Latent Class 1). While the clinical benefit of treatments such as hydroxyurea is not only reflected in an increase in HbF, these findings demonstrate that high HbF, whether naturally occurring or pharmacologically induced, may not confer homogeneous protection across all environmental exposure contexts [18]. Taking the influence of extrinsic environmental factors into consideration in clinical decision-making for individual patients may offer additional benefits.

Collectively, the interaction analyses demonstrate that the magnitude of benefit from α -thalassemia co-inheritance and elevated HbF appears dependent on environmental susceptibility and may be attenuated under specific exposures. Physiologically, processes such as cold-induced vasoconstriction and the adverse effects of inhaled particulate matter may precede vaso-occlusion

independently of the ongoing HbS polymerization rate [44,45]. Therefore, reductions in HbS polymerization from higher HbF or α -thalassemia co-inheritance may be inadequate in these circumstances. Heterogeneity in environmental sensitivity may also reflect host genetic background. Variants in vascular pathways, for example α -2C adrenergic receptors may partly explain differential responses to environmental stressors such as cold [46,47]. Variants in adhesion pathways, such as CTNNA2, have been associated with SCD hospitalizations [48]. Finally, the rheologic consequences of α -thalassemia, namely increased viscosity and decreased red cell deformability, may increase risk rather than benefit in the presence of extrinsic stimuli [49].

The lack of significance in the interaction term for pain between α -thalassemia and latent class may reflect either reduced statistical power, given the relatively low frequency of α -thalassemia silent carriers and trait individuals in this study, or, alternatively, biological specificity. Therefore, the results concerning the 3.7 kb α -thalassemia deletion should be interpreted cautiously, as α -thalassemia co-inheritance was assessed as a combined group of silent carriers and trait individuals. These individuals were combined due to the small number of 3.7 kb trait individuals ($n=8$) detected. The use of self-reported categorical data also limits the precise characterization of individual exposure dosage. Residual confounding may have been introduced from other extrinsic factors such as malaria and respiratory tract infections that peak during the rainy season [50]. Other factors such as socioeconomic status, psychological factors, and disparities in healthcare access may also affect disease severity but were not included in the present model [51,52]. Despite these limitations, the LCA approach extends beyond single-exposure analysis, and the interaction results were consistent across both genetic modifiers evaluated.

Notwithstanding these limitations, the results have direct implications for patient care and treatment. Individuals with the α -3.7 kb deletion, whether in carrier or trait state, or with elevated HbF, should not be presumed to have complete protection against VOC relative to those without the deletion or with low HbF. Environmental risk assessment should therefore be incorporated into standard clinical evaluation, independent of genetic profile. The continued occurrence of pain episodes and hospital admissions in patients with high baseline HbF, whether naturally occurring or induced by hydroxyurea, indicates that HbF augmentation alone may not suffice for those with marked environmental sensitivity. For such patients, complementary measures aimed at reducing exposure to triggers should be considered before maximal hydroxyurea dosing.

Future work should emphasize longitudinal designs with precise, measured environmental data to evaluate interaction effects on outcomes. Expanding sample sizes and incorporating air pollutants, genetic factors, hemolytic markers, and hydroxyurea treatment status as exogenous variables within structural equation models, or related approaches, may help clarify whether co-exposure to correlated pollutants affects SCD morbidity. Finally, identifying modifier variants in genes governing vascular function, autonomic regulation, and sensory perception could elucidate why thresholds differ between patients, and enable truly individualized care that pairs drug therapy with environmental risk management. Latent class-based environmental sensitivity profiles could be translated into a brief, structured clinical screening instrument that captures self-reported sensitivity to environmental influences, enabling systematic environmental risk profiling at routine clinic visits.

In conclusion, this study is the first to explore the interaction of baseline HbF and α -thalassemia co-inheritance with the joint effect of environmental influences, measured as self-reported sensitivity, on pain and hospitalization frequency in SCA. The results showed that two clusters of patients with distinct exposure-sensitivity patterns exist in Yaoundé, as revealed by LCA. The frequency of pain and hospitalization rates was influenced by the interaction between latent class and both HbF and α -3.7 kb silent carrier/trait co-inheritance. Individuals in Latent Class 1 characterized by a high conditional probability of reporting sensitivity to pollutants and year-round VOC, were more prone to pain and hospitalization when their HbF was <10%. These findings provide the first evidence of gene-environment interactions among SCA patients in Cameroon that may enable truly individualized care that pairs drug therapy with environmental risk management.

Authors' Contributions

NSB, EBB and CF conceived and designed the study. CBC, ETF and PGH screened patients and performed clinical examinations. NSB and DI collected samples and performed experiments. NSB analyzed data. NSB and CF wrote the manuscript. All the authors reviewed and approved the final manuscript.

Acknowledgement

We thank the staff at the Department of Hematology, Oncology and Gastro-enterology, Yaoundé Central Hospital; and the departments of pediatrics at CASS Nkoldongo, HGOPY, HD-Cite Vert and HD-Mbalmayo for assisting with patient enrollment and data collection. We thank the staff at the Laboratory for Preclinical Animal and Pharmacotoxicology Research, and LANAVET for technical support.

Competing Interests

Authors have declared that no competing interests exist.

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