DECIPHERING THE GENETIC MODIFIERS OF SICKLE CELL ANAEMIA IN CHILDREN: THE ROLE OF *CYB5R3* GENE

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Introduction

Sickle cell anaemia is a hereditary disorder caused by a mutation in the β-globin gene that gives rise to haemoglobin S, which polymerises under low-oxygen conditions and distorts red blood cells[1]. These sickled cells impair blood flow and lead to chronic anaemia, vaso-occlusion, and organ damage, contributing to wide variability in clinical severity[2]. The *CYB5R3* gene, which encodes the enzyme responsible for reducing methaemoglobin back to its functional form, plays an important role in maintaining effective oxygen transport[3]. In individuals with sickle cell anaemia, particularly during oxidative stress, alterations in *CYB5R3* function may increase methaemoglobinaemia and worsen disease manifestations[4]. This study aimed to evaluate the potential modulatory effects of a *CYB5R3* variant, along with other well-established genetic modifiers within the globin genes, on the phenotypic variability of sickle cell anaemia patients in paediatric age.

Population and Methods

This study involved 81 children aged 3 to 17 years with confirmed sickle cell anaemia, followed in paediatric haematology of hospitals in the Greater Lisbon area. All participants were anonymised, and informed consent was obtained from their legal guardians. Each child was monitored over a three-year period, during which complete clinical history and steady-state haematological and biochemical data were collected to ensure consistency. Clinical, laboratory, and biochemical information was compiled into a structured database that supported the genetic and statistical analyses. The genes *CYB5R3*, *HBG2*, *HBA1*, and *HBA2* were genotyped using PCR, Sanger sequencing, and Gap-PCR, and association analyses were carried out using SPSS.

Results and Discussion

1. Co-inheritance of α-thalassaemia

Co-inheritance of α-thalassaemia with sickle cell anaemia was observed in 43.2% of the patients[1]. Children with sickle cell anaemia and also carrying the -α$^{3.7}$ deletion showed higher erythrocyte counts and lower bilirubin and reticulocyte levels than non-carriers[1], indicating reduced haemolysis and milder anaemia. These findings confirm a protective haematological effect of α-thalassaemia in sickle cell anaemia[1].

Table 1. Association between haematological/biochemical phenotype and α-globin genotypes

Variables	αα/αα		αα/−α$^{3.7}$ + −α$^{3.7}$/−α$^{3.7}$		p-value
	n	Mean ± SD	n	Mean ± SD	
Erythrocytes (10^{12}/L)	46	2.79 ± 0.33	34	3.38 ± 0.57	**<0.001**[2]
Haemoglobin (g/dL)	46	7.91 ± 0.97	35	8.05 ± 0.82	0.301[2]
Reticulocytes (%)	46	14.50 ± 5.35	33	9.37 ± 4.10	**<0.001**[2]
LDH (U/L)	9	796.22 ± 227.52	17	625.59 ± 197.85	0.070[1]
Total bilirubin (mg/dL)	44	3.33 ± 1.60	30	2.53 ± 1.47	**0.010**[2]
MCV (fL)	46	84.18 ± 5.97	34	72.54 ± 7.60	<0.001[1]
MCH (pg)	46	28.56 ± 2.51	34	24.37 ± 2.78	<0.001[1]
Haematocrit (%)	46	23.40 ± 2.60	34	24.15 ± 2.47	0.195[1]
RDW (%)	46	22.36 ± 3.29	34	21.80 ± 2.91	0.430[1]
Leukocytes (10^{9}/L)	46	13.27 ± 3.89	34	12.18 ± 4.86	0.171[2]
Neutrophils (10^{9}/L)	46	6.04 ± 2.56	34	6.06 ± 3.64	0.508[2]
Platelets (10^{9}/L)	46	432.94 ± 118.44	34	400.41 ± 118.91	0.229[1]
HbS (%)	13	82.73 ± 6.81	12	81.11 ± 7.89	0.586[1]
HbF (%)	27	11.13 ± 6.93	19	12.68 ± 7.35	0.482[2]

MCV – Mean corpuscular volume; MCH – Mean corpuscular haemoglobin; RDW – Red cell distribution width; HbS – Haemoglobin S; HbF – Fetal haemoglobin; LDH – Lactate dehydrogenase
[1] t-test, [2] Mann–Whitney, Statistical significance for p-value < 0.05

2. Fetal haemoglobin (HbF)

Using a 10% HbF threshold, children with HbF ≥10% had higher haemoglobin and haematocrit values, along with significantly lower reticulocyte counts and reduced LDH levels[5]. This group displayed milder anaemia and less haemolysis, showing that elevated HbF is associated with a more favourable clinical profile[5,6].

Table 2. Association between haematological/haemolytic phenotype and HbF levels

Variables	HbF $_{<10}$		HbF $_{\geq 10}$		p-value
	n	Mean ± SD	n	Mean ± SD	
Erythrocytes (×10^{12}/L)	22	3.01 ± 0.44	24	3.19 ± 0.62	0.300[2]
Haemoglobin (g/dL)	22	7.76 ± 0.75	24	8.23 ± 0.91	**0.068**[2]
Reticulocytes (%)	22	12.35 ± 4.63	24	9.96 ± 5.09	**0.017**[2]
LDH (U/L)	18	808.92 ± 162.86	7	688.79 ± 160.25	0.125[1]
Total bilirubin (mg/dL)	20	2.74 ± 1.33	21	2.63 ± 1.55	0.531[2]
Haematocrit (%)	22	23.30 ± 1.88	24	24.32 ± 2.41	0.118[1]
Leukocytes (10^{9}/L)	22	14.02 ± 4.27	24	12.44 ± 3.80	0.191[1]
Neutrophils (10^{9}/L)	22	6.00 ± 3.00	24	6.01 ± 3.57	0.996[2]
Platelets (10^{9}/L)	22	449.90 ± 105.17	24	393.20 ± 111.16	0.083[1]
HbF (%)	22	6.39 ± 2.04	24	16.70 ± 6.40	<0.001[2]
HbS (%)	11	86.06 ± 6.48	14	78.73 ± 6.24	0.006[1]

LDH – Lactate dehydrogenase; HbS – Haemoglobin S; HbF – Fetal haemoglobin
[1] t-test, [2] Mann–Whitney, Statistical significance for p-value < 0.05

3. *HBG2* (rs7482144 C>T)

Carriers of the rs7482144_T allele (allele frequency = 15%) had a trend to present lower HbS percentages, higher haemoglobin levels, and reduced haemolysis compared with non-carriers[6]. These children fell within the range of moderate rather than severe anaemia and experienced fewer clinical complications, supporting a protective role for this variant[5].

Table 3. Association between haematological/haemolytic phenotype and SNP rs7482144 (*HBG2*) under a dominant model

Variables	CC		CT+TT		p-value
	n	Mean ± SD	n	Mean ± SD	
Haemoglobin (g/dL)	65	7.91 ± 0.83	16	8.23 ± 1.14	0.333[2]
Reticulocytes (%)	64	11.92 ± 5.09	15	14.26 ± 6.73	0.250[2]
LDH (U/L)	20	753.30 ± 216.24	6	683.35 ± 283.47	0.523[1]
Total bilirubin (mg/dL)	60	2.99 ± 1.55	14	3.08 ± 1.80	0.978[2]
HbS (%)	22	82.63 ± 7.20	3	76.97 ± 6.50	0.210[1]
HbF (%)	37	10.95 ± 6.57	9	15.12 ± 8.44	0.113[2]

LDH – Lactate dehydrogenase; HbS – Haemoglobin S; HbF – Fetal haemoglobin
[1] t-test, [2] Mann–Whitney, Statistical significance for p-value < 0.05

Table 4. Association between clinical phenotype and SNP rs7482144 (*HBG2*)

Clinical variables	CC			CT+TT			p-value
	n	Yes (%)	No (%)	n	Yes (%)	No (%)	
Jaundice	64	49 (76.56)	15 (23.44)	15	11 (73.33)	4 (26.67)	0.792
Gallstones	48	22 (45.83)	26 (54.17)	11	4 (36.36)	7 (63.64)	0.568
Splenomegaly	60	10 (16.67)	50 (83.33)	15	1 (6.67)	14 (93.33)	0.327
Hepatomegaly	61	22 (36.07)	39 (63.93)	13	2 (15.38)	11 (84.62)	0.148
Chronic lung disease	65	2 (3.1)	63 (96.92)	15	0 (0.00)	15 (100.00)	0.491
Pulmonary hypertension	64	0 (0.00)	64 (100.00)	15	0 (0.00)	15 (100.00)	-
Cardiopathy	65	65 (100.00)	0 (0.00)	16	16 (100.00)	0 (0.00)	-
Heart murmurs	65	65 (100.00)	0 (0.00)	16	16 (100.00)	0 (0.00)	-
Other cardiopathy	50	34 (68.00)	16 (32.00)	9	8 (88.89)	1 (11.11)	0.203
Fatal outcome	65	1 (1.50)	64 (98.46)	16	0 (0.00)	16 (100.00)	0.618

χ^2 test, Statistical significance for p-value < 0.05

4. *CYB5R3* (rs1800457C>G)

Children carrying the G allele of rs1800457 (allele frequency = 35%) showed a trend to present lower haemoglobin levels, higher LDH and bilirubin, and more frequent clinical complications, suggesting greater haemolytic and clinical severity[3,4]. In contrast, wild-type individuals had higher HbF levels and a more moderate anaemia[5].

Table 5. Association between haematological/haemolytic phenotype and SNP rs1800457 (*CYB5R3*)

Variables	CC		CG+GG		p-value
	n	Mean ± SD	n	Mean ± SD	
Erythrocytes (×10^{12}/L)	30	3.08 ± 0.49	50	3.02 ± 0.56	0.378[2]
Haemoglobin (g/dL)	31	8.09 ± 1.03	50	7.90 ± 0.82	0.469[2]
Reticulocytes (%)	30	12.46 ± 5.34	49	12.30 ± 5.60	0.848[2]
Haematocrit (%)	30	24.03 ± 2.74	50	23.53 ± 2.45	0.404[1]
Leukocytes (10^{9}/L)	30	12.86 ± 3.80	50	12.77 ± 4.67	0.626[2]
Neutrophils (10^{9}/L)	30	5.90 ± 3.05	50	6.14 ± 3.07	0.858[2]
Platelets (10^{9}/L)	30	415.26 ± 108.00	50	421.43 ± 126.14	0.824[1]
LDH (U/L)	11	729.37 ± 273.89	15	742.87 ± 200.26	0.886[1]
Total bilirubin (mg/dL)	29	2.84 ± 1.63	45	3.12 ± 1.57	0.409[2]
HbS (%)	8	82.14 ± 8.22	17	81.87 ± 7.00	0.932[1]
HbF (%)	17	12.49 ± 7.92	29	11.35 ± 6.63	0.641[2]

LDH – Lactate dehydrogenase; HbS – Haemoglobin S; HbF – Fetal haemoglobin
[1] t-test, [2] Mann–Whitney, Statistical significance for p-value < 0.05

Table 6. Association between clinical phenotype and polymorphism rs1800457 C>G

Clinical variables	CC			CG+GG			p-value
	n	Yes (%)	No (%)	n	Yes (%)	No (%)	
Jaundice	29	22 (75.86)	7 (24.14)	50	38 (76.00)	12 (24.00)	0.989
Gallstones	24	10 (41.67)	14 (58.33)	35	16 (45.71)	19 (54.29)	0.758
Splenomegaly	29	5 (17.24)	24 (82.76)	46	6 (13.04)	40 (86.96)	0.617
Hepatomegaly	28	6 (21.43)	22 (78.57)	46	18 (39.13)	28 (60.87)	0.115
Chronic lung disease	30	1 (3.33)	29 (96.67)	50	1 (2.00)	49 (98.00)	0.712
Pulmonary hypertension	29	0 (0.00)	29 (100.00)	50	0 (0.00)	50 (100.00)	-
Cardiopathy	31	31 (100.00)	0 (0.00)	50	50 (100.00)	0 (0.00)	-
Heart murmurs	31	31 (100.00)	0 (0.00)	50	50 (100.00)	0 (0.00)	-
Other cardiopathy	21	13 (61.90)	8 (38.10)	38	29 (76.32)	9 (23.68)	0.242
Fatal outcome	31	0 (0.00)	31 (100.00)	50	1 (2.00)	49 (98.00)	0.428

χ^2 test, Statistical significance for p-value < 0.05

Conclusion

This study shows that genetic modifiers have a significant impact on the clinical expression of sickle cell anaemia in childhood. Co-inheritance of α-thalassaemia, higher HbF levels, and the presence of the *HBG2* rs7482144_T allele demonstrated clear protective effects, being associated with reduced haemolysis, milder anaemia, and a lower burden of comorbidities. In contrast, the *CYB5R3* rs1800457_G allele showed a potential deleterious effect, being linked to more pronounced haemolysis and greater clinical severity. These findings reinforce the importance of genetic characterization as part of a more personalized approach to the management and treatment of paediatric sickle cell anaemia[7].

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