Muğla Sıtkı Koçman Üniversitesi Tıp Dergisi 2025;12(3):197-203
Medical Journal of Mugla Sitki Kocman University 2025;12(3):197-203
Doi: 10.47572/muskutd.1658534

Research Article

Screening of Mutations in the Binding Domain of the *SALL2* Gene in Patients with Beta Thalassemia Major

Beta Talasemi Majör Hastalarında *SALL2* Gen Bağlanma Bölgesindeki Mutasyonların Taranması

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Received: 19.03.2025
Accepted: 19.09.2025

Abstract

Beta thalassemia (βT) is an autosomal recessive disorder that exhibit a wide clinical phenotypic spectrum, ranging from severe transfusion-dependent β-thalassemia major (β-TM) to asymptomatic β-thalassemia minor (β-Tm). This variation is primarily influenced by the effect of modifier genes, particularly those associated with fetal hemoglobin (HbF) induction. In this study, we aimed to investigate whether mutations in the binding regions of the potential modifier *SALL2* gene are associated with high fetal hemoglobin (HbF) percentages in β-TM patients. Our study included a total of 66 patients from whom blood samples were collected before blood transfusion. The second exon, containing the binding domains of the *SALL2* gene, was screened using the Sanger sequencing method, and the rs1263810 (c.2236C>G, g.18725C>G, p.Gly746Arg) variant was identified. Among the 66 patients, 6 (9.09%) had the CC genotype, 34 (51.51%) had the CG genotype, and 26 (39.39%) had the GG genotype, with the estimated frequencies of the C and G alleles being 34.85% and 65.15%, respectively. Our findings show that the p.Gly746Arg variant in the binding motif of the *SALL2* gene is reported for the first time in β-TM patients with high HbF concentrations (%) in the Turkish population. Although no statistically significant relationship was found between the genotypes of this variant and HbF percentage levels, we believe that the high G allele frequency (65.15%) found in patients with high HbF will make a significant contribution to the management of HbF in patients with beta thalassemia major when evaluated together with other epigenetic and modifying genes.

Keywords: Beta Thalassemia, HbF, Modifier Gene, rs1263810, *SALL2*.

Özet

Beta talasemi, ağır transfüzyon bağımlı β-talassemi major (β-TM) ile asemptomatik β-talassemi minor (β-Tm) arasında geniş bir klinik fenotip yelpazesi gösteren otozomal resesif geçişli hastalıklardır. Bu varyasyona, fetal hemoglobin (HbF) induksiyonu ile ilişkili modifiye edici genlerin önemli bir katkısı vardır. Bu çalışmada, potansiyel modifiye edici gen olan *SALL2* geninin bağlanma bölgelerindeki mutasyonların, β-TM hastalarında yüksek fetal hemoglobin (HbF) yüzdesi ile ilişkili olup olmadığını araştırmayı amaçladık. Çalışmamıza kan transfüzyonu öncesinde kan örnekleri alınan toplam 66 hasta dahil edilmiştir. *SALL2* geninin bağlanma bölgelerini içeren ikinci ekzon Sanger dizileme yöntemi kullanılarak tarandı ve rs1263810 (c.2236C>G, g.18725C>G, p.Gly746Arg) varyantı tespit edilmiştir. 66 hastanın 6'sı (%9.09) CC, 34'ü (%51.51) CG ve 26'sı (%39.39) GG genotipine sahipti ve C ve G alellerin tahmini frekansları sırasıyla %34.85 ve %65.15 olarak belirlendi. Bulgularımız, *SALL2* geninin bağlanma motifindeki p.Gly746Arg varyantının, Türk populasyonunda yüksek HbF konsantrasyonu (%) seviyelerine sahip β-TM hastalarında ilk kez raporlandığını göstermektedir. Bu varyantın genotipleri ile HbF yüzdesi düzeyleri arasında istatistiksel olarak anlamlı bir ilişki bulunmamasına rağmen, yüksek HbF'ye sahip hastalarda bulunan yüksek G alel frekansının (%65.15) diğer epigenetik ve modifiye edici genlerle birlikte değerlendirildiğinde beta talasemi majör hastalarında HbF'nin yönetimine önemli bir katkı sağlayacağına inanmaktayız.

Anahtar Kelimeler: Beta Talasemi, HbF, Modifiye Edici Gen, rs1263810, *SALL2*.

Introduction

Beta (β) thalassemia is one of the most common autosomal recessive single-gene disorders worldwide (1). It presents with a wide range of clinical phenotypes, from asymptomatic beta thalassemia minor to severe transfusion-dependent beta thalassemia major. Patients with beta thalassemia major develop severe hemolytic anemia in early childhood, requiring regular blood transfusions, iron chelation, and close medical management (2). Despite having the same beta globin gene mutations, patients may exhibit varying disease severity, ranging from mild symptoms to life-threatening complications (3). This clinical variability is influenced by genetic modifiers that affect disease expression (4-6).

Among these modifiers, increased fetal hemoglobin (HbF) levels and coexisting alpha-thalassemia are key factors in altering disease severity (7,8). Recent studies have identified additional candidate genes regulating HbF levels. Sheehan and colleagues (2014) discovered that the *SALL2* (Sal like protein) gene variant (P840R) is associated with increased HbF levels in sickle cell anemia patients treated with hydroxyurea (9). However, the role of *SALL2* in beta thalassemia remains unexplored.

SALL2, a member of the *SALL* gene family, encodes a zinc-finger transcription factor involved in embryonic development and hematopoiesis (10,11). It has been implicated in cell cycle regulation, tumor suppression, and hematopoietic differentiation (12-14). SALL2 shares structural similarities with BCL11A, a known HbF regulator, and interacts with histone deacetylases (HDAC1/HDAC2), which repress gamma-globin expression (9,15,16). Given the potential role of *SALL2* in HbF induction, we aim to investigate variations in the *SALL2* gene in beta thalassemia major patients with elevated HbF levels. This study seeks to provide novel insights into the genetic mechanisms influencing HbF expression and their potential therapeutic implications in beta thalassemia.

Material and Method

This study was approved by the Akdeniz University Faculty of Medicine Clinical Research Ethics Committee (Approval no:142, Date: 26.08.2015). A total of 66 β-thalassemia major patients with HbF levels >2% were included in the study, and blood samples were collected from the patients before blood transfusion at Antalya Training and Research Hospital. Genomic DNA was extracted from peripheral blood samples using the salting-out method (17). The target regions of interest were three binding domains located in the second exon of the *SALL2* gene. These regions were amplified by polymerase chain reaction (PCR) using specific primer pairs:

1. (Forward: 5'-GACTACTGGCAGCACAGTG-3' Reverse: 5'-AAGTGGTTGGTAAGCAGTGC-3'),
2. (Forward: 5'-GCACTGCTTACCAACCACTT-3', Reverse: 5'-GATGCCTCTTCTGAATCACC-3'),
3. (Forward: 5'-GGTGATTCAGAAGAGGCATC-3', Reverse: 5'-TCATAACTCTGGGAGGTCCT-3').

The PCR products were verified by 2% agarose gel electrophoresis and subsequently purified using the Axygen® AxyPrep™ PCR Clean-Up Kit**.** Following purification, sequencing analysis was performed using the ABI 3130 XL DNA sequencing system. The raw sequencing data were processed and analyzed with ABI Sequence Analysis v3.1 software.

Statistical Analysis

Statistical analysis was performed using version 20 of the Statistical Package for the Social Sciences (SPSS). The Kruskal-Wallis test was used to compare HbF levels among *SALL2* genotypes. A p-value of <0.05 was considered statistically significant.

Results

A total of 66 patients participated in this study after signing the informed consent form, including 37 women and 29 men. The average age of the patients was calculated as 28.1±7.8 years. The mean transfusion frequency was 22.5±5.4 days, and the mean transfusion volume was 1.6±0.4 units. When examining hematological parameters, the mean hemoglobin (Hb) level was found to be 8.9±0.8 g/dL, while the mean HbF percentage was 10.08±9.6%. The minimum HbF percentage was 2.1%, and the maximum was 61.4%. The mean MCV value was calculated as 80.9±5.2 fL, the MCH value as 26.7±1.9 pg/cell, the MCHC value as 32.9±1.4 g/dL, and the RDW value as 18.7±5.1%. It was known that 68.2% of the patients had undergone splenectomy. Additionally, according to patient records, the most frequently observed *HBB* (Hemoglobin beta) gene mutation was IVS.I.110 (G>A), which was known to be in the homozygous form. Demographic characteristics, beta globin gene mutations, hematological parameters, and genotype distribution of *SALL2* gene variant rs1263810 (c.2236 C>G) in 66 beta thalassemia major patients were shown in Table 1.

Table 1. Demographic Characteristics, Beta Globin Gene Mutations, Hematological Parameters, and Genotype Distribution of *SALL2* Gene Variant rs1263810 (c.2236 C>G) in 66 Beta Thalassemia Major Patients

Patient ID	Gender	Age	Beta Globin Gene Mutation	c.2236C>G	HBF	SP	HGB	MCV	MCH	MCHC	RDW
1	F	23.4	(-30T>A/IVS.I.110 (G>A))	CG	3.5	N	9.5	85.4	28.6	33.5	15.1
2	F	20.5	IVS.II.745(C>G)/IVS.II.745(C>G)	CG	7.9	N	8.5	76.7	26.6	34.7	16.9
3	M	28.5	IVS.I.110(G>A)/IVS.I.110 (G>A)	CG	11.1	Y	8.3	84.2	26.9	31.9	15.9
4	M	26.8	IVS.II.1 (G>A)/IVS.II.745 (C>G)	CG	16.7	N	7.8	77.3	26	33.6	16.5
5	M	37	IVS.I.110(G>A)/IVS.I.110 (G>A)	CG	12.3	Y	8.8	81.9	27.2	33.1	16
6	M	27.5	(-30T>A/IVS.II.745 (C>G))	GG	6.5	Y	8.6	82	27.2	33.2	16.1
7	M	22.8	IVS.I.110(G>A)/IVS.I.110 (G>A)	CG	12.5	Y	9.2	85.5	28.4	33.2	19.1
8	F	23.3	IVS.II.1 (G>A)/IVS.II.1 (G>A)	GG	7.6	N	8.5	82.4	27.5	33.4	16.7
9	M	29.4	IVS.I.110(G>A)/IVS.I.110 (G>A)	GG	20.1	Y	8.9	83.2	25.9	31.1	19.3
10	F	21.4	IVS.I.110 (G>A)/IVS.II.1 (G>A)	CG	4.9	N	8.8	83	27.5	33.2	14.6
11	M	25.5	IVS.I.110 (G>A)/IVS.I.6 (T>C)	GG	14.3	Y	8.7	85.2	25.2	29.5	19.7
12	M	31.7	IVS.I.110(G>A)/IVS.I.110 (G>A)	CC	25	Y	9.2	75.4	25.5	33.8	27.5
13	F	29.9	IVS.I.110(G>A)/IVS.I.110 (G>A)	GG	17.6	Y	8.6	83.7	27	32.2	18.5
14	M	23.2	IVS.I.1(G>A)/IVS.I.110 (G>A)	CG	16.7	N	8.9	78.3	25.6	32.7	19.4
15	F	45.1	IVS.I.110(G>A)/IVS.I.110 (G>A)	CG	10.5	Y	10.2	82.5	26.9	32.6	17.2
16	M	18.3	(-30T>A/IVS.I.110 (G>A))	GG	39	Y	9.8	81.1	24.9	30.7	25.6
17	M	30	IVS.I.110 (G>A)/IVS.I.6 (T>C)	GG	2.2	Y	7.8	81.3	26.8	32.9	15.6
18	F	40.6	IVS.I.110 (G>A)/IVS.I.6 (T>C)	CG	4.8	Y	8.1	86.9	27	31	16.7
19	M	36.9	IVS.I.110(G>A)/IVS.I.110 (G>A)	GG	17	Y	9.1	83.7	27.5	32.9	17.6
20	F	41.3	HBKNOSSOS/IVS.I.110 (G>A)	CG	19.4	Y	8.5	77.1	24.6	31.9	29.9
21	F	37.3	IVS.I.110 (G>A)/IVS.II.1 (G>A)	CG	3.1	Y	7.8	78.8	25.9	32.9	16.5
22	F	20	IVS.I.110(G>A)/IVS.I.110 (G>A)	CG	2.50	Y	9.5	84.7	28	33	15.4
23	F	63.3	(-30T>A/-30T>A)	CG	30.2	Y	8.4	76.7	22.7	29.5	28.6
24	F	23.1	IVS.I.110(G>A)/IVS.I.110 (G>A)	CG	3.8	Y	10	85.2	28.3	33.2	17.3
25	M	20.6	IVS.I.110(G>A)/IVS.I.110 (G>A)	CG	2.80	N	8.1	79.5	27.6	34.7	14.9
26	F	27.3	IVS.I.110(G>A)/IVS.I.110 (G>A)	CG	4.5	Y	10.1	86.6	28.2	32.5	14.7
27	F	38.2	IVS.I.110(G>A)/IVS.I.110 (G>A)	CG	2.1	Y	10.1	87.1	29.2	33.5	15.4
28	M	21.6	IVS.I.110(G>A)/IVS.I.110 (G>A)	GG	3.8	N	9.5	80.8	27.7	34.3	14.2
30	M	26.3	IVS.I.110(G>A)/IVS.II.745(C>G)	CG	2.20	N	9.2	78.1	26.6	34.1	14.1
31	M	28	IVS.I.110(G>A)/IVS.I.110 (G>A)	CG	7	Y	8.8	71.8	22	30.6	36
32	F	32.5	IVS.I.110(G>A)/IVS.I.110 (G>A)	CG	6.30	Y	10.5	84.7	31.4	37.1	19.3
33	F	25.2	IVS.I.110(G>A)/IVS.I.110 (G>A)	GG	61.4	Y	8	74.7	22	29.5	31
34	F	25.2	IVS.I.110(G>A)/IVS.I.110 (G>A)	CG	2.40	N	7.9	79.8	28.3	35.5	14.1
35	M	19.4	IVS.I.1 (G>A)/IVS.I.1 (G>A)	CC	10.8	N	9.3	78.3	27.2	34.8	17.4
36	M	21.8	IVS.I.110(G>A)/IVS.I.110 (G>A)	GG	12.1	N	8.5	75	25.7	34.2	27.4
37	F	36.3	IVS.I.110(G>A)/IVS.I.110 (G>A)	CG	12.8	Y	8.9	84.4	28.2	33.3	18.2
38	M	20.6	IVS.I.110(G>A)/IVS.I.110 (G>A)	GG	5.8	N	8.5	79.8	27.1	34	14.8
39	F	29.9	IVS.I.110(G>A)/IVS.I.110 (G>A)	GG	2.67	N	9.6	83	28.4	34.2	14.2
40	M	35.7	IVS.I.110 (G>A)/IVS.II.1 (G>A)	CG	25.2	Y	9.7	80	25.1	31.3	24
41	M	21.5	FSC 44(-C)/FSC 44(-C)	CG	11.4	N	8.1	78.8	26.2	33.2	15.3
42	F	21.8	IVS.I.110(G>A)/IVS.I.110 (G>A)	GG	2.70	Y	9.4	85.5	28.5	33.3	14.8
43	F	30.3	(-30T>A/IVS.II.1 (G>A))	GG	18.7	N	8.5	77.3	25.4	32.9	19.8
44	F	25.7	IVS.I.110(G>A)/IVS.I.110 (G>A)	GG	7.60	Y	9.2	82.1	26.6	32.4	17.5
45	F	38.6	IVS.I.6 (T>C)/IVS.I.6 (T>C)	CC	5.9	Y	9.2	78.6	24.3	30.9	27.2
46	M	26.3	IVS.I.110(G>A)/IVS.I.110 (G>A)	GG	6	Y	10.1	84.2	27.7	32.9	17.4
47	F	40.2	IVS.I.6 (T>C)/IVS.I.110 (G>A)	CC	8	Y	8.4	81.7	25.5	31.3	21,2
48	F	26.2	IVS.II.1 (G>>A)/IVS.II.1 (G>A)	GG	17.8	Y	10.1	81.8	27.1	33.2	22.7
49	F	24.2	IVS.I.110(G>A)/IVS.I.110 (G>A)	GG	4.6	Y	7.4	84.4	27.4	32.5	17.5
50	F	34.1	IVS.II.1 (G>>A)/IVS.II.1 (G>A)	CG	9.70	Y	8.9	83.9	27.9	33.2	15.9
51	M	27.9	IVS.I.110(G>A)/IVS I.110 (G>A)	CG	5.1	Y	8.1	84.8	27.1	31.9	14.8
52	F	35.7	IVS.I.110(G>A)/IVS.I.110 (G>A)	CC	14.1	Y	8.9	86.9	28.1	32.3	16.9
53	F	25.1	IVS.I.6 (T>C)/IVS.I.110 (G>A)	GG	4.7	Y	8.6	89.3	26.8	30.1	17.1
54	F	22	IVS.I.110(G>A)/IVS I.110 (G>A)	GG	2.3	Y	9.2	77.6	26.5	34.2	15.2
55	M	28.3	IVS.I.110(G>A)/IVS.I.110 (G>A)	CG	9.8	N	7.8	77.8	26.6	34.2	17
56	F	19.9	IVS.I.6 (T>C)/IVS.I.6 (T>C)	GG	7.5	Y	8.9	71.1	23.8	33.5	34
57	F	27	IVS.I.110 (G>A)/IVS.II.1 (G>A)	CG	8.6	Y	10	79.4	26.6	33.5	18
58	F	19.2	IVS.I.110(G>A)/IVS.I.110 (G>A)	CG	7.60	N	10.5	80.9	27.9	34.5	13.7
59	M	36.3	IVS.I.110 (G>A)/IVS.I.110 (G>A)	CG	5.6	N	10.6	83.2	27.8	33.4	16.2
60	M	18.3	IVS.I.110 (G>A)/IVS.I.110 (G>A)	CC	9.5	Y	9.1	83.5	27.6	33	18.5
61	F	22.5	IVS.II.1 (G>A)/IVS.II.745 (C>G)	GG	3.5	Y	10.2	82.7	28.1	34	14.3
62	F	24.3	IVS.I.110 (G>A)/IVS.I.110 (G>A)	GG	3.4	N	9.4	81	27.6	34.1	14.7
63	M	18.5	IVS.I.110 (G>A)/IVS.I.110 (G>A)	CG	3.6	Y	9.9	79.4	27.6	34.7	15.5
64	M	25.4	IVS.I.110 (G>A)/IVS.II.745 (C>G)	GG	4	N	6.8	81.2	28.8	35.5	18.3
65	F	31	IVS.I.110 (G>A)/IVS.I.110 (G>A)	GG	4.7	N	9.6	81.1	26.9	33.2	16.6
66	F	20.8	IVS.I.6 (T>C)/IVS.I.6 (T>C)	CG	5.6	Y	7.9	51.8	17.3	33.4	29.8

F: Female, M: Male, HGB(g/dl): Hemoglobin, MCV(fl): Mean Corpuscular Volume, MCH(pg/cell): Mean Corpuscular Hemoglobin MCHC(g/dl): Mean Corpuscular Hemoglobin Concentration, RDW(%): Red Cell Distribution Width, HbF(%): Hemoglobin F, SP: Splenectomy Status (Y: Splenectomized, N: Not Splenectomized), c.2236 C>G: Genotype of the Variant (CC, CG, GG)

Based on the DNA sequencing results of exon 2 of the *SALL2* gene in 66 patients, a variant was identified: rs1263810 C>G (c.2236C>G, g.18725C>G, p.Gly746Arg), as a missense mutation. In the reference sequence provided by the Human Genome Organization (HUGO), the normal allele for this mutation is given as C. Based on this, the CC genotype was detected in 6 patients (9.09%), the CG genotype in 34 patients (51.51%), and the GG genotype in 26 patients (39.39%). On the other hand, when we grouped HbF levels and looked at the relationships between the genotype and allele of the variant, it was seen that there was no CC genotype in the HbF (2.0 - 4.99) group, and there were 3 patients with higher HBF in the other two groups.

When we look at all groups, the high frequency of the CG heterozygous genotype is striking in Table 2.

Table 2. The Relationship Between HbF Level (%) Groups and Genotypes of *SALL2* Gene Variant rs1263810 (c.2236 C>G) in 66 Beta Thalassemia Major Patients

HbF Level (%) Groups	**Genotypes of *SALL2* Gene Variant (n=)**			**Total (n=)**
	CC	CG	GG	
HbF (2 – 4.99)	0	13	11	24
HbF (5 – 9.99)	3	10	6	19
HbF (10 - up)	3	11	9	23
Total	6	34	26	66

The electropherograms of the normal, heterozygous, and homozygous mutant genotypes for the rs1263810 C>G variation are shown in Figure 1a, 1b, and 1c, respectively. The statistical analysis did not show a significant association between the CG and GG genotypes and HbF levels ($p = 0.311$).

Discussion

In β-thalassemia major, a critical therapeutic goal remains reducing transfusion dependency. To achieve this, various approaches such as bone marrow transplantation and gene therapy have been extensively explored (18-20). Recent studies have underscored the significance of genetic and epigenetic modifiers in fetal hemoglobin (HbF) induction, suggesting promising therapeutic strategies for β-thalassemia major (21-24). Notably, variants in genes like *BCL11A* (25), *HBS1L-MYB* (26), and *KLF1* (27) have been shown to modulate γ-globin expression, thereby influencing HbF levels. A deeper understanding of the mechanisms by which these modifiers interact with the β-globin locus could lead to the development of targeted therapies designed to reactivate fetal hemoglobin production (28-30)

SALL2, a multi-zinc finger transcription factor, has been identified as a key player in hematopoietic cell differentiation and cell cycle regulation (14). In particular, it shares a highly conserved 12-amino acid N-terminal motif with B-cell CLL / lymphoma 11(BCL11A), a well-established repressor of γ-globin expression. This shared motif facilitates the interaction of SALL2 with the nucleosome remodeling and deacetylase (NuRD) corepressor complex, which includes histone deacetylases HDAC1 and HDAC2—critical factors in the suppression of γ-globin (15,16). These interactions highlight the potential regulatory role of SALL2 in HbF production, particularly through the modulation of chromatin remodeling complexes (31).

Recent findings by Sheehan, Crosby, and colleagues have provided compelling evidence for the role of *SALL2* as a potential modifier gene in HbF induction (9). Specifically, they observed that in sickle cell anemia patients harboring the GAG>GTG base transition at codon 6 of the β-globin gene, those carrying the rs61743453 variant in *SALL2* exhibited a more pronounced increase in HbF levels in response to hydroxyurea treatment. These results further support the hypothesis that *SALL2* may function as a genetic modifier of HbF expression, particularly in disorders such as sickle cell anemia and β-thalassemia major, where elevated HbF levels can ameliorate disease severity.

In this study, we investigated the role of *SALL2* in β-thalassemia major by screening for mutations in exon 2, including the binding motif regions, which are believed to be critical for its function as a modifier gene. Our sequencing analysis revealed the missense variant rs1263810 (c.2236C>G, g.18725C>G, p.Gly746Arg). These variants were found in association with elevated HbF levels in some β-thalassemia major patients, suggesting a potential role of *SALL2* in modifying γ-globin expression and influencing disease severity.

Previously, according to the Single Nucleotide Polymorphism Database (dbSNP) of the National Center for Biotechnology Information (NCBI), the rs1263810 variant was annotated as NM_005407.3:c.2236C>G and NP_005398.2:p.Arg746Gly. However, it is now defined under the Matched Annotation from NCBI and EMBL-EBI (MANE) as NM_001364564.1:c.2230C>G and NP_001351493.1:p.Arg744Gly. This variant was

first submitted to ClinVar in December 2019 by Labcorp Genetics and classified as benign. To date, it has been submitted by this source alone. The rs1263810 variant results in a glycine-to-arginine substitution (c.2236C>G, g.18725C>G,p.Gly746Arg) and was identified in a significant proportion of patients (51.51% heterozygous and 39.39% homozygous). There are three alleles of the rs1263810 variation in the NCBI database: A, C, G. We did not observe the mutant A allele in our study. In contrast, the two alleles, G and C, were present with frequencies of 65.15% and 34.85%, respectively. According to Minor allele count was G=0.234853(1000 G).

In a study conducted by You and colleagues in 2014, a total of 105 patients were accidentally infected with hepatitis C virus genotype 1b (HCV1b) through a blood transfusion from a single blood donor (32). Exome capture and sequencing analyses identified the 20 SNPs most closely associated with HCV clearance. Among these SNPs, the rs1263810 variant identified in our study is included. As mentioned, although the rs1263810 variant is classified as benign in the ClinVar dataset, the limited number of studies in the literature means that the potential impact of this variant on clinical outcomes remains uncertain, and its contribution to clinical findings in the future continues to be unclear.

Given the structural similarities between SALL2 and BCL11A (15) it is plausible that this variant alters SALL2 protein function, thereby influencing erythroid differentiation and HbF expression. The established role of *BCL11A* in γ-globin repression, mediated through its interaction with HDAC1/HDAC2 and chromatin remodeling complexes (16), suggests that *SALL2* may exert similar regulatory effects on γ-globin expression.

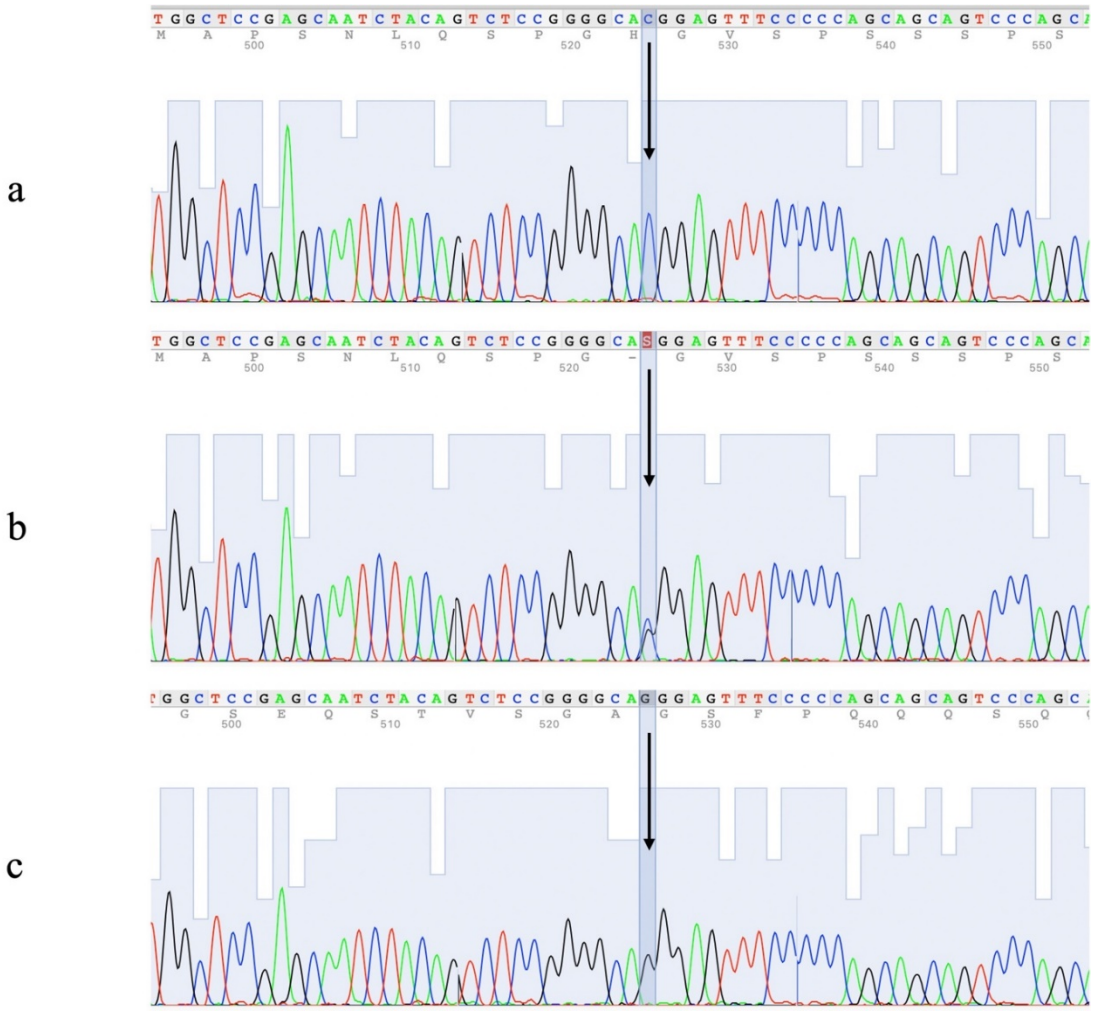

Figure 1. Sanger sequencing images of the *SALL2* gene rs1263810 (c.2236C>G) variant. Panel (a) shows the wild-type allele (homozygous C allele), panel (b) depicts the heterozygous mutation (CG), and panel (c) shows the homozygous mutation (GG). The sequence chromatograms clearly distinguish between the different genotypes based on the presence of the respective alleles.

Despite the promising findings, our study has several limitations. The study included only patients with beta thalassemia major, specifically those with an HbF level >2%. Consequently, the sample size remained relatively small (n=66). Furthermore, we did not assess the functional impact of the identified mutations on SALL2 protein activity or HbF regulation, which would be essential to establish a direct link between these variants and HbF expression. Although no statistically significant relationship was found between the genotypes of this variant and HbF percentage levels, we believe that the high G allele frequency (65.15%) found in patients with high HbF will make a significant contribution to the management of HbF in patients with beta thalassemia major when evaluated together with other epigenetic and modifying genes. Nevertheless, when the CG genotype striking is evaluated together with the GG genotype, we think that the high frequency of the G allele at high HbF levels should also be taken into consideration.

In conclusion, we plan to investigate in future research the potential impact of the variant found in the SALL2 gene, in conjunction with the genetic and epigenetic modifiers reported in the literature (22) on HbF induction in beta thalassemia patients.

Conclusion

This study provides preliminary insights into the potential role of the rs1263810 variant in the *SALL2* gene as a modifier of HbF expression in β-thalassemia major. The association between this variant and elevated HbF levels suggests that SALL2 may modulate γ-globin expression, potentially through mechanisms similar to those of BCL11A, given the structural similarities between the two. Therefore, every variant defined in the *SALL2* gene including ours will contribute to the clinical approach. Although classified as benign in the ClinVar database, the clinical significance of this variant remains uncertain due to the limited number of studies available. Therefore, further research is necessary to clarify the functional impact of this variant on SALL2 activity, its interactions with chromatin remodeling complexes, and its broader role in regulating HbF production. These findings also highlight the importance of investigating additional genetic and epigenetic modifiers that influence HbF induction in β-thalassemia major, which could pave the way for the development of targeted therapeutic strategies aimed at reducing disease severity and transfusion dependency.

Acknowledgements

The authors would like to acknowledge and thank the patients for their valuable participation in this study

Conflict of interest statement

The authors declare that they have no conflicts of interest.

Ethics Committee Approval

This study was approved by the Akdeniz University Faculty of Medicine Clinical Research Ethics Committee with approval number 142, dated 26.08.2015.

Funding

This study was supported by Akdeniz University Scientific Research Projects Coordination Unit. Project Number: TYL-2016-1232.

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